Effect of Different Infection Rates in *Galleria mellonella* Larvae on the Quality of the Produced *Heterorhabditis* Juveniles

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ABSTRACT

Effect of the initial infection densities (15,100 and 1000 IJs) on the quality of the produced juveniles of two nematode species, *Heterorhabditis bacteriophora* (H.b) and *Heterorhabditis indica* (H.i) was studied The number of the initial penetrated IJs was increased by increasing in infection density of the two species. IJs length and width measurements correlated negatively with the three levels of infection. The penetration rate of (H.b) was negatively related to the initial infection density while the opposite was evident for (H.i). Insignificant differences were found in the virulence of (H.b) juveniles produced from the three levels of infection while for (H.i) a significant increase was found between the juveniles produced from level I, II and that of level III. A significant decrease in storage ability was found after 45 days for the juveniles of (H.b). Also, a decrease was found between 15 and 30 days storage for the juveniles of 100 and 1000 levels of infection. For (H.i), a significant decrease in storage ability was observed after 15, 30 and 45 days for the juveniles produced from the three levels of infection. There was a decrease in the host finding ability of the juveniles produced from the three levels of infection of (H.b). The reverse was opposite in (H.i) with the same trend.

Key Words: *Heterorhabditis bacteriophora, Heterorhabditis indica*, Penetration efficiency, Virulence, Penetration rate, Host finding ability, Storage ability.

INTRODUCTION

Environmental concern about chemical insecticides serves as a strong impetus for the development of biological control agents or biopesticides. Biopesticides (living organisms and the naturally-occurring compounds produced by these organisms) can be safer, more biodegradable, and less expensive to develop. Entomopathogenic nematode is considered one of the most important biological control agents and numerous insect pests on many different crops are controlled by it. Entomopathogenic nematodes have several important attributes that make them excellent candidates for biological control of soil insects: 1) They are specialized to carry and introduce symbiotic bacteria into the insect haemocoel. 2) Most have a broad host range that includes the majority of insect orders and families. 3) Several species can be cultured artificially on a large scale, which makes it possible to commercially produce large quantities. 4) They have limited impact on non-target organisms and are not disruptive to the environment.

The fitness of a parasite can be adversely affected by increasing population density within the host. This has been noted in vertebrates intestinal helminthes (Keymer, 1982), mermithid nematodes in mosquito larvae (Tingley and Anderson, 1986), reduced fecundity (Williams, 1973), Density dependent effects include increased mortality (Anderson and Michel, 1977), change in sex ratio (Hominick and Tingley, 1984), reduction in adult size, increased generation time (Taylor, 1988) and reduced oviposition (Selvan and Muthukrishnan, 1988). Although reduced production of entomopathogenic nematodes progeny from cadavers exposed to high densities of infective juveniles has been reported (Woodring and Kaya, 1988), the mechanisms of their reduced fecundity are unknown. Although all entomopathogenic nematodes have the same general life history, species difference in host utilization (Selvan and Blackshaw, 1990) and reproductive (Poinar, 1991) strategies may influence the nematode response to increasing density. Entomopathogenic nematodes have a mutualistic association with *Xenorhabdus* bacteria that converts the host into a nutrient soup on which nematodes feed (Thomas and Poinar, 1979).

Finding isolates with good traits and improvement of the beneficial traits of the infective stage juveniles as pathogenicity, penetration, persistence and host finding ability are certainly excellent targets for enhancing biocontrol potential. Shamseldean *et al.* (1999) mentioned an isolate of *H. indicus* (EAS59) isolated from southern Egypt to posses a very wide thermal range (4-35°C).

In a previous paper, the effect of the initial infection density was studied on the quality of the emerging juveniles of one Egyptian and one imported *Steinernema* species (El-Assal *et al.*,

2002).

The present investigation aimed to study the effect of the initial infection density on the quality or beneficial traits of the emerging juveniles of two species of *Heterorhabditis* juveniles, *Heterorhabditis bacteriophora* and *Heterorhabditis indica*.

MATERIALS AND METHODS

Nematode Source

Two Egyptian *Heterorhabditis* species designated (TWF) and (RM1) were used in the present study. These species were defined as *H. bacteriophora* (H.b) and *H. indica* (H.i) according to the morphometric measurements and PCR (El-Assal *et al.*2002). Nematodes were reared in last instar of the great wax moth *Galleria mellonellla* L. at 25°C using methods similar to those described by Dutky *et al.* (1964).

Infection and Penetration

Last instar *G. mellonella* larvae were used as the host insect for all experiments. Hosts were exposed to nematodes in an inoculation cocoon to maximize host contact, as described by Jaworska (1986). The infection was carried out by placing one late instar *Galleria* larva in 1.5 ml Eppendorf tube lined with filter paper contaminated with nematode doses of 15, 100 and 1000 infective juveniles. Each dose and nematode combination was replicated ten times in three blocks of three replicates. After 48 hr of exposure at 25°C, the *G. mellonella* larvae were removed from the membrane filter paper in the Eppendorf tubes and rinsed with deionized water to remove any nematodes remaining externally. The larvae were then transferred to White traps (White, 1927). The emerging juveniles from each dose level (level I, II and III) were received in distilled water and stored at 15°C until used.

Ten larvae of each infection dose were washed twice and dissected in day four and the number of hermaphrodites inside each larva was counted.

Quality of the Produced Juveniles

Only infective juveniles harvested three days post emergence was used in all the following experiments:

1. Infective Juvenile Length

The juveniles emerged from the 3 levels of infection were measured at day one or two of emergence. Random selection of the individuals to be measured was done. Live IJ were killed in hot water at 80°C added to an equal amount of nematode suspension. The length of the IJ was measured using a microscope equipped with an ocular micrometer.

2. Ineffective Juvenile

The width of the infective juveniles was measured by the same procedure.

3. Infective Juvenile Virulence

Nematode infectivity of the juveniles emerged from the three levels of infection was determined using 1:1 assay according to (Woodring and Kaya, 1988) by transferring one IJs using 30 µl of water onto 1.5 ml Eppendorf tube lined with filter paper. An additional 30 µl of water was used. One last-instar *G. mellonella* larva was placed in each tube and the tubes were incubated at 25°C. Mortality records were taken after 96 hr and corrected according to Abbott's formula (Abbott, 1925) versus a control treatment contained only distilled water. Three replicates of ten larvae each were made for juveniles of each nematode population.

4. Penetration Rate

The number of penetrated juveniles was calculated in *Galleria* larvae infected with 50 IJs from each of the three populations in 1.5 ml Eppendorf tubes. Three replicates, each of ten larvae were made. After 48 hr, nematode infected larvae were washed twice with distilled water to remove any nematode juveniles that attached to them, dried and dissected on day 4 after infection. The number of hermaphrodites inside each larva was counted and penetration rate was calculated.

5. Host Finding Ability

The host finding ability was adopted by the T-tube choice test designed by Steiner (1996). Approximately 1000 infective juveniles were gently pipetted on the top of the soil in the inlet tube. The assay unit was incubated at 25C°. Host finding assessed as % IJs actually reached the bait after 48h post inoculation. Three replicates were made for each population.

6. Storage Ability

The ability of any strain to tolerate the conditions of storage with keeping its viability is an important character especially when mass production is required for large scale field application. For this purpose, the survival of infective juveniles of the tested isolates in distilled water was tested at a concentration of 100 IJs/dish. Nematode suspension was kept in 14-cm diameter Petri-dishes at 25C°. Counts of alive and dead juveniles were made at 0, 15, 30 and 45 days, for each dish and percent alive

juveniles were determined.

7. Data Analysis

The data presented in percentage values in the present study were normalized using arcsine transformation. All experiments were in 3 replicates, the values were shown as means ± standard errors. Data were subjected to analysis of variance (ANOVA), and Duncan's multiple range tests to differentiate between the means at $P< 0.05$.

RESULTS AND DISSCUSSION

Initial Penetration

For (H.b), a high significant increase in the number of penetrating juveniles (penetration efficiency) in each larva at the three levels of infection (15, 100 and 1000) was found with the increased infection density (4.56, 38.82 and 398.33), respectively, F=531.14 and the percentage of the penetrating juveniles also increased while for (H.i), although the number of the penetrating juveniles was highly significant increased (6.5, 36.2 and 110), the percentage of these juveniles exhibited significant decrease (Fig 1).

Length of the Juveniles

For (H.b) and (H.i), IJs length showed a significant negative correlation with the infection density *i.e.* smaller IJs with higher density and larger IJs with smaller density, F= 18.09 and 15.51, respectively (Table 1).

Width of the Juveniles

For (H.b) and (H.i) insignificant decrease in the width was found for the juveniles produced from the three levels of infection, F=0.75 and 1.22, respectively (Table 1).

Penetration Rate

The penetration rate of the produced juveniles from the three levels of infection of (H.b) was significantly decreased with increasing the initial infection density, F=11.79 while there was a significant positive relation between the initial infection density and the penetration rate for (H.i) , F=23.54 (Fig 2).

Virulence

Insignificant difference was obvious in the virulence (represented by LC_{50}) of the juveniles produced from the three levels of infection of H.b (2.025, 1.997 and 2.16) while for (H.i), a significant decrease in LC_{50} was found between the juveniles produced from level I, II and that of level III (3.04, 2.97 and 0.93) (Table 2).

Host finding Ability

There was a high significant decrease in host finding ability (HFA) of (H.b) with increasing the initial infection density, F=27.93. The reverse was obvious in (H.i) with the same trend i.e. a high

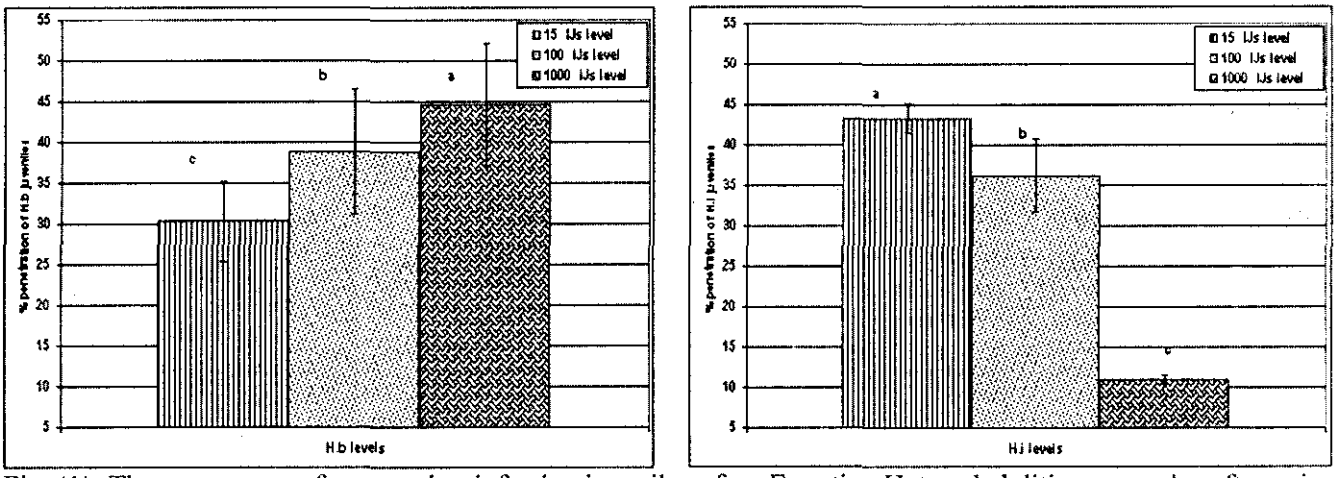

Fig. (1): The percentage of penetrating infective juveniles of an Egyptian Heterorhabditis nematodes after using three levels of infection (15, 100 and 1000 IJS/larva)

Table (1): Length and width (in µm) of infective juveniles produced from three levels of infection (15, 100 and 1000 IJS/larva) of an Egyptian *Heterorhabditis bacteriophora* and *Heterorhabditis indica* (n =20)

Species	Length (mean ±SE)			Width (mean ±SE)		
	15 IJS/larva	100 IJS/larva	1000 IJS/larva	15 IJS/larva	100 JS/larva	1000 JS/larva
H. bacteriophora	609.8±31.57a	590.0±31.37b	568.63±29.11c	22.97±1.39a	22.55±1.09a	21.44±1.40b
H. indica	537.75±26.48a	523.50±19.20b	514.75±18.88c	21.53±0.87a	20.49±0.88b	20.32±1.02b

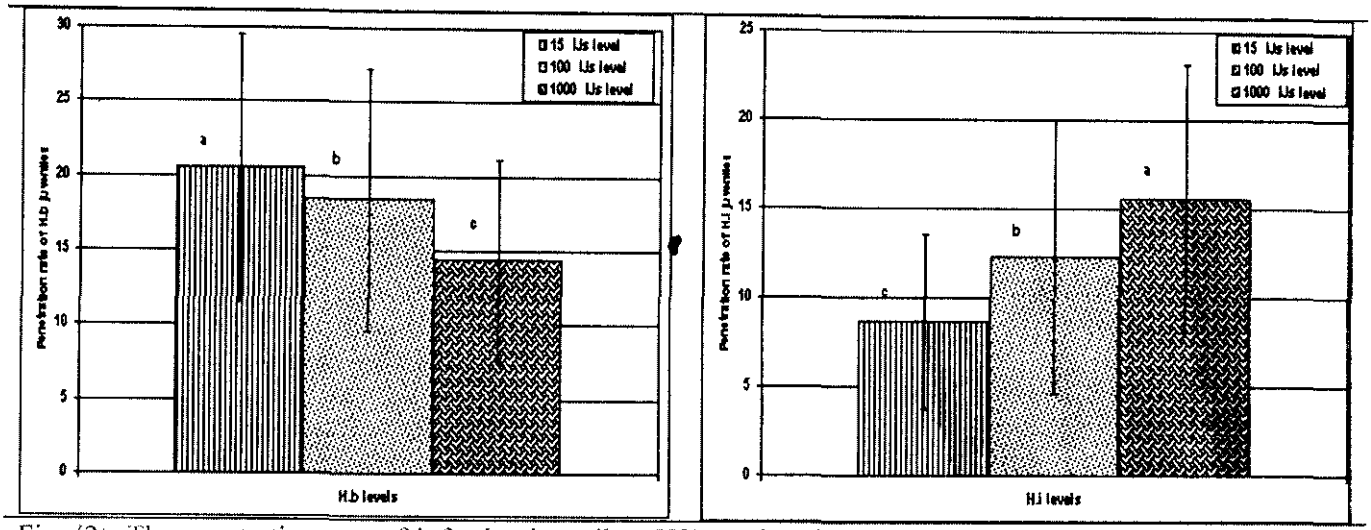

Fig. (2): The penetration rate of infective juveniles (IJS) produced from three levels of infection (15, 100 and 1000 IJS/larva) of an Egyptian Heterorhabditis nematode

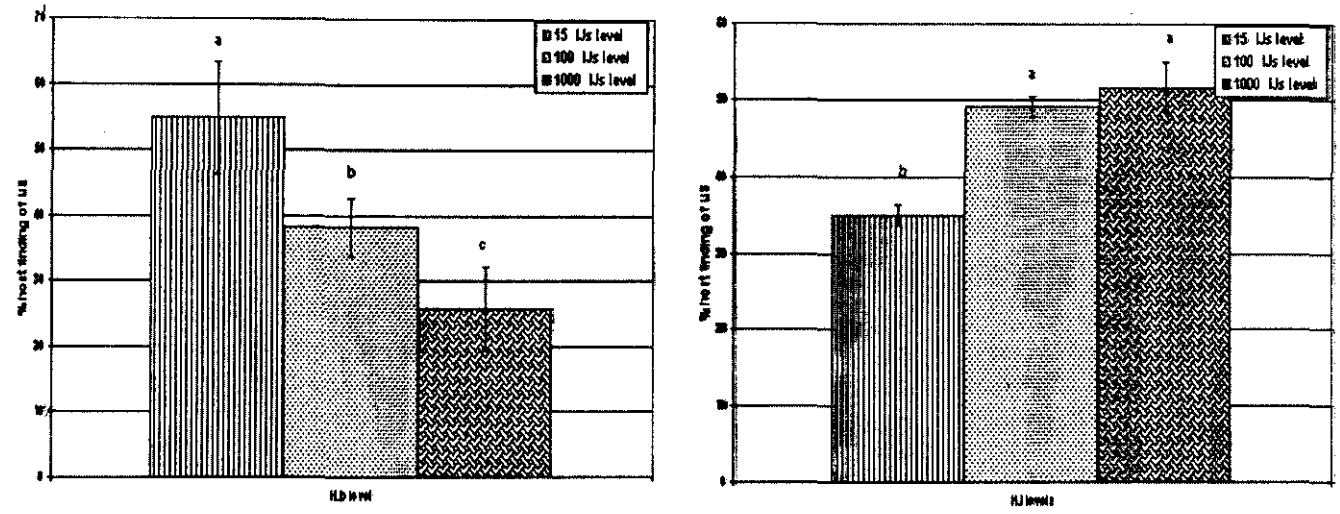

Fig. (3): The host-finding ability of the produced juveniles from three levels of infection (15, 100 and 1000 IJS/larva) of an Egyptian Heterorhabditis nematodes

Table (2): Virulence (LC_{50}value) of the produced juveniles from three levels of infection (15, 100 and 1000 IJS/larva) of Egyptian Heterorhabditis nematodes

Heterorhabditis nematodes	H.b			H.i		
	LC_{50}	Confidence limits		LC_{50}	Confidence limits	
		Lower	Upper		Lower	Upper
15 IJs/tube	2.028	1.484	2.605[a]	3.036	2.467	3.994[b]
100 IJs/tube	1.997	1.995	2.118[a]	2.972	2.375	4.016[b]
1000 IJs/tube	2.16	1.785	2.619[a]	0.934	0.266	1.442[a]

significant increase in HFA for the juveniles produced from level I and II and a significant increase between level II and III, F=12.34 (Fig 3).

Storage Ability

For (H.b) at 15 days storage in distilled water, insignificant decrease in persistence between the juveniles produced from level I and level II of infection but a significant decrease was obvious between the juveniles of level II and III, F=14.41. After 30 days of storage, a significant decrease in persistence was evident between the juveniles of level I and that of levels (II and III), F=28.27. At 45 days storage, a high significant decrease in persistence was occurred between the juveniles of the three levels of infection, F=658.57.

For (H.i) at 15 days storage in distilled water, no

decrease in persistence between the juveniles produced from the three levels of infection, (F= 0.378) but at 30 days storage, a high significant decrease in persistence was found between the juveniles produced from the three levels of infection, (F= 13.64) while at 45 days storage, a high significant decrease in persistence was occurred only in the juveniles of level I and II, (F= 808) but insignificant difference between the juveniles of level II and III was found (Fig 4).

The effect of the initial infection density was noticed clearly in the quality of the produced juveniles and had an important influence on the population dynamics of parasites. Selvan *et al.* (1993) studied the effect of increasing entomopathogenic nematode density within a *G. mellonella* larva. They found that although the number of invading nematodes increased with increasing dose, penetration percentage declined. They reported that density-dependent penetration was not sufficient to prevent the detrimental effects of overcrowding and the effects of high density appear to result from competition for limited nutrients within the host. Obtained results match with that of Selvan *et al.* (1993) for (H.i) but not with (H.b) in which the number of penetrating juveniles increased and also the penetration percentage increased. The explanation of the results of the present study relies on the previous finding of Selvan *et al.* (1993).

The effect of the initial infection density was clearly obvious in the gradual decrease in the juvenile length and width for the juveniles produced from the three levels of infection in the two tested species. For the two species, IJ average length of the first population was near the maximum value of the range reported in the original description of this species, while the juvenile length of population three was near the minimum value. The increase in the length and width at the low density may be due to the abundance of food in the cadaver. This result is supported by the finding of Hazir *et al.* (2001) who observed that infective juveniles tend to be longer when reared in wax moth larvae than in *S. exigua* and on dog-food agar. *S. exigua* larvae probably have a lower nutritional quality than wax moth larvae. Selvan *et al.* (1993) found that the longest infective juveniles were produced at the lowest densities, not at the densities that produced the largest numbers of infective juveniles. They postulated that because longer infective juveniles store more nutrients, they can be expected to survive for a longer period than shorter nematodes. This observation was also reported by Kino (1984) and coincides with the present study results of storage in distilled water for the two tested species. Alternatively, production of short-lived infective stages can increase the short-term probability of locating a new host and this is exactly happened in case of (H.i), but not matched with results of the host finding ability of (H.b), where the opposite was occurred. The explanation of this result may rely on the lipid content of the juveniles of (H.b) and (H.i). Lewis *et al.* (1995) studied the relationship between the metabolic rate, energy reserves, and foraging behavior in three species of entomopathogenic nematodes, *S. carpocapsae*, *S. glaseri*, and *H. bacteriophora*. Their studies showed that stored lipids, the major component of nematode energy reserves declined at species-specific rates.

The penetration rate of the emerging juveniles of (H.b) was negatively correlated with the initial infection dose, *i.e.*, nematodes resulted from low infection doses penetrated *Galleria* larvae better than those resulted from the higher infection doses. This pattern of penetration may be due to that, increasing penetration rate affects the carrying capacity of the larva which may exceed the optimum conditions for nematode propagation and consequently rapid host utilization by both nematode and bacteria which most likely results in inadequate nutrition and so, the resulted infective juveniles have inadequate lipid reserves that enable them to penetrate the host. On the other hand, H.b may have a high percentage of infectious IJs resulting in super parasitism at the higher rates, therefore, poor quality IJs and this was obvious in the percentage of the initial penetration.

For (H.i) the opposite was occurred, where the penetration rate and the virulence of the emerging juveniles were positively correlated with the initial infection dose. It is possible that (H.i) reached an optimal level of host penetration and the IJs stopped entering the host and this explain the better quality of the juveniles at higher density because the actual number penetrating the host may reach the optimal level. Another possible explanation for (H.i) (which follows the above thought, but from a different view), may be that only a certain proportion of IJs are infectious. The infectious ones enter and the rest were non-infectious resulting in an optimal number going in. This suggestion supported by Fan & Hominicks (1991) contention that most entomopathogenic nematodes are not infective at a given point in time. Perhaps, also, that the bacterium associated with (H.i) is better to convert host tissues into nutrient products at the higher nematode

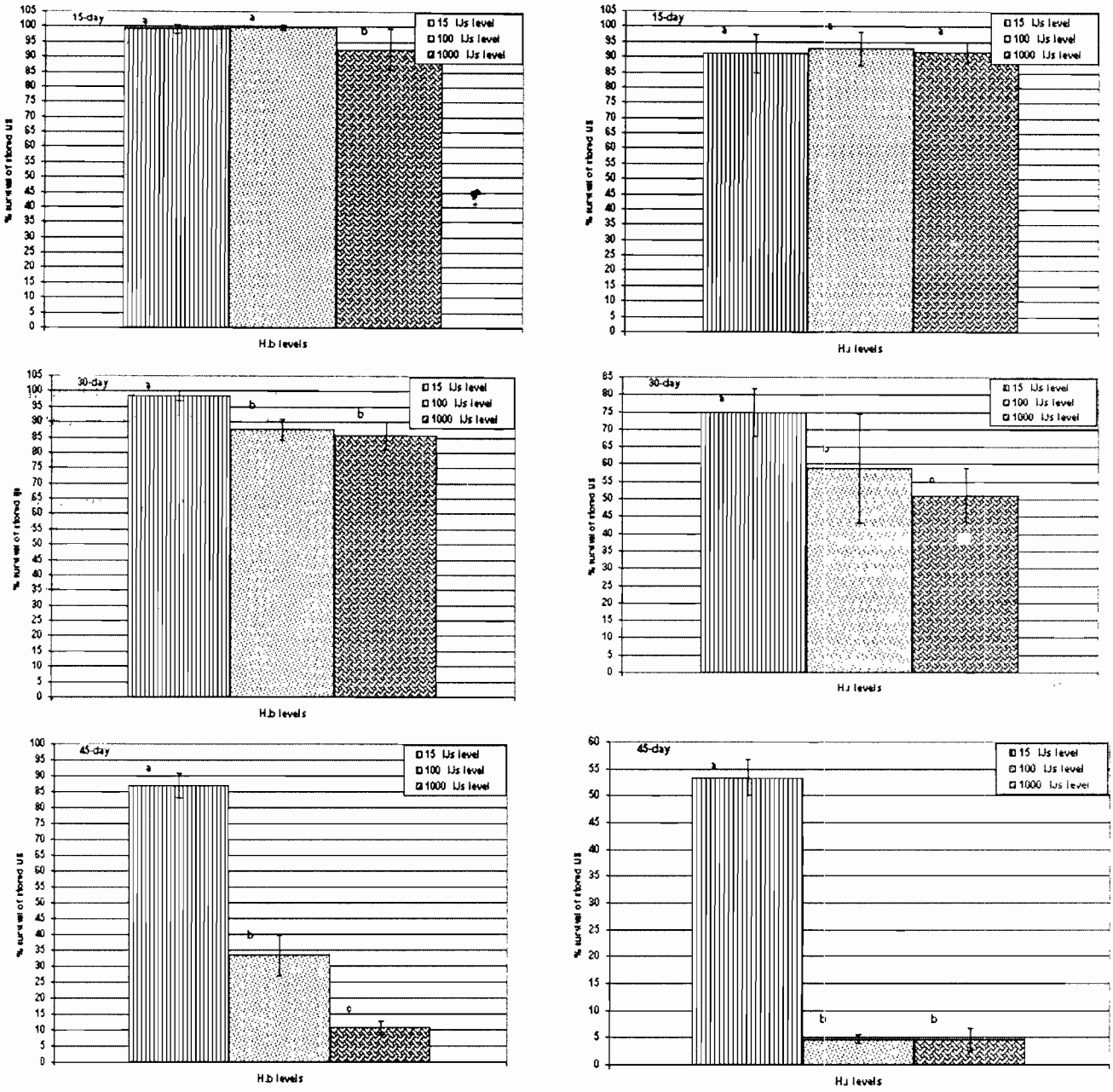

Fig. (4): The storage ability of the produced juveniles from three levels of infection (15, 100and 1000 IJS/larva) of an Egyptian Heterorhabditis nematodes

density.

Results of virulence and penetration rate can be easily correlated with each other. In other words, increasing penetration rate was accompanied by increasing virulence. Gerritsen and Smiths (1994) reported that the virulence of any nematode isolate is, most probably, due to the pathogenicity of the bacterial symbiotic. Nematode production under optimum conditions in the host's cadaver may result in better bacterial retention and hence high virulence of nematode juveniles. In the present investigation for (H.b), although the number of penetrating nematodes was decreased, a non significant difference in virulence was observed. The reasons of that may be due to that the bacterial retention in the penetrating juveniles might still enough to kill infected host (Boff *et al.*, 2000).

In conclusion, effect of infection density should be taken into account in the laboratory culture and field release of entomopathogenic nematodes to gain better quality of the produced infective stage juveniles.

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