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# SUSCEPTIBILITY OF *PACHNODA SAVIGNY* G.&P. LARVAE (COLEOPTERA: SCARABAEIDAE) TO THE ENTOMOPATHOGENIC NEMATODE *STEINERNEMA GLASERI* (STEINER) (RHABDITIDA:STEINERNEMATIDAE).

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ABSTRACT

A laboratory screening of 10 strains of entomopathogenic nematodes belonging to *Steinernema* spp. revealed that *S. glaseri* is the most effective steinernematid against the 3*rd* instar larvae of the peach cockchafer, *Pachnoda savigny* (Coleoptera:Scarabaeidae). Bioassays indicated that both 2*nd* and 3*rd* instar grubs are susceptible to *S. glaseri* using nematode concentrations ranging between 8,000 and 35,000 infective juveniles (IJs) per a surface area of 156 $cm^2$. Higher mortalities occurred when bioassay was carried out in sandy soil than in a soil mixture composed of equal volumes of sand, loam and cattle manure. The 3*rd* instar larvae of *P. savigny* killed with *S. glaseri* proved to be a suitable environment for the multiplication of *S. glaseri* infective juveniles. The number of emerging IJs ranged between 30,000 and 125,000 IJs/grub depending on the infective inoculum. Preliminary trials indicated that infectivity of *S. glaseri* to *P. savigny* larvae could be enhanced by the passage of nematode through the larvae of the target host.

**Key Words:** Entomopathogenic nematodes, *Steinernema glaseri*, Scarabaeidae, *Pachnoda savigny*, efficacy, screening, *in vivo* production, infectivity improvement.

## INTRODUCTION

The peach cockchafer, *Pachnoda savigny* Gory & Percheron (Rigout, 1989), which was previously known in Egypt as *P. fasciata* F. (Alfieri, 1976), is a common scarabaeid beetle in the country. The adults appear in late June and early July and last up to September where they attack flowers and ripe fruits of the summer orchards as well as some ornamental plants (Abou Bakr *et al.*, 1989a). As eggs are laid in the soil, larval development takes place in soil rich in organic matter upon which the grubs feed (Abou Bark *et al.*, 1989b; Helmi *et al.*, 1989). The populations of *P. savigny* have been steadily increasing in different parts of Egypt during the recent decade. In order to reduce the use of chemical insecticides, efforts have been directed to biological and agricultural control methods, among which the entomopathogenic nematodes of the families Steinernematidae and Heterorhabditidae possess many qualities that make them excellent biological control agents (Gaugler, 1988). They have a broad host range, kill their host rapidly and are environmentally safe. Isolation of *S. glaseri* from Japanese beetle larvae in New Jersey in 1930s resulted in the first effort to use nematodes against insect pests (Klein, 1993). Recent field tests with nematode in the genus *Steinernema* (=*Neoaplectana*) have shown that they can be effective biological control agents against scarabaeid insects (Klein, 1990). *Steinernema glaseri* is known as particularly efficient against various scarabaeids, *e.g.* *Anomala* (Mamiya, 1989), *Ligyrus* (Sosa and Hall, 1989), *Phyllophaga* (Kard *et al.*, 1988; Forschler and Gardner, 1991a,b), *Popillia japonica* (Shetlar *et al.*, 1988), and *Rhizotrogus majalis* (Villani and Wright, 1988). In the present study a comparison was made between the infectivity of 10 strains belonging to different *Steinernema* species, isolated in different parts of the world, against the 3*rd* instar larvae of *P. savigny*. Then, *S. glaseri* was tested n the laboratory against L2 and L3 of the same insect. Laboratory experiments included the effect of soil composition and nematode concentration on nematode-induced larval mortality. Nematode multiplication inside *P. savigny* dead larvae was also examined. The infectivity of the IJs produced in *P. savigny* larvae against L3 of the same insect was compared to that of IJs produced in *Galleria mellonella* larvae.

## MATERIALS AND METHODS

### Laboratory Maintenance of *P. savigny*

*P. savigny* adults were collected by hand picking and insect net in the field during the period of June-September, 1992 and 1993. In the laboratory, beetles were held in 60x60x75 cm metal wire- mesh, wooden-framed cages provided with pieces of banana for feeding the insects, and plastic boxes containing a moistened autoclaved mixture of equal parts of sand, loam and cattle manure as oviposition media (Abou Bakr *et al.*, 1989a). Food was renewed daily, and soil moisture was continuously kept at a suitable level. The boxes were inspected every two days where eggs were picked up and kept in plastic boxes (14 cm diameter, 4.5 high) filled with the previously mentioned autoclaved soil with suitable moisture. The boxes were covered with perforated plastic lids. Eggs were kept for hatching at 28°C where larval development took place. Under such conditions, *P. savigny* grubs reach the 2*nd* larval instar within about 20 days; the 3*rd* larval instar may last for up to 256 days.

### Nematode Production and Storage

*Steinernema* strains used in this work were provided by Dr. C. Laumond, "Laboratoire d'Entomonématologie", INRA, Antibes, France. The nematodes were produced *in vivo* by infecting last instar larvae of the greater wax moth,

diameter plaster disc (modified after Woodring and Kaya, 1988) kept inside an extraction chamber, a rounded plastic box with a thin layer of 0.1% formalin in distilled water. The boxes were tightly covered with plastic lids to avoid the entry of *Drosophila* flies. After four days of incubation at 28°C the new IJs individuals started emerging from the *Galleria* cadavers taking their way down to water around the plaster disc. IJs were collected daily and finally stored in 100 ml d.w. with 0.1% formalin in transparent plastic boxes 8 cm diameter, 5 cm high) in a refrigerator (9.0±1°C) at density of $10^6$ IJs/100 ml water. Bioassay testes were carried out using nematodes stored for 3-4 weeks.

**Screening Tests**

Ten strains of entomopathogenic nematodes, all belonging to the genus *Steinernema*, isolated in different parts of the world (Table 1) were screened for their virulence against the 3*rd* instar larvae of *P. savigny* in sand. A concentration of 10,000 IJs of each strain was used for each 5 grubs kept in a plastic box (156 $cm^2$ area) containing 375 $cm^3$ of sand. Four replicates were used for each nematode strain. Similar 4 boxes containing a sum of 20 L3 larvae served as control where sand was moistened with tap water. Larval mortality was recorded up to 10 days after nematode exposure at a temperature of 28°C.

**Soil Preparation**

Moderately fine sand, loam taken from autoclaved soil in the Experimental Farm at the Faculty of Agriculture, Giza and dry cattle manure taken from animal husbandry section in the same farm, were used for preparing the soil mixture. Sand was washed and dried; both loam and cattle manure were separately autoclaved. Two types of soil were experimentally prepared for testing the effect of soil composition on the infectivity of *S. glaseri* on the 2*nd* and 3*rd* instar larvae of *P. savigny*. Type 1 soil consisted of plain sand; and type 2 was made of equal volumes of sand, loam and cattle manure. Samples of the two types of soil were subjected to physical and chemical analysis (Table 2).

**Infectivity Tests with *S. glaseri***

Three-week old IJs of *S. glaseri* were taken out of their storage in the refrigerator and left at room temperature for about one hour for reactivation. Then they were microscopically examined for viability, and numerically adjusted to the wanted concentrations as number of IJs/ml water using Hawskley counting slide. Nematode concentrations generally ranged between 8,000 and 35,000 IJs/box.

The previously described plastic boxes (of 156 $cm^2$ area) were filled with fixed volumes (375 $cm^3$) of the tested soils. Accordingly, nematode concentrations were calculated as the number of IJs/box, or IJs/$cm^2$ area as indiboxes served as control where the soil was moistened with tap water. Boxes were covered with perforated lids and kept at a temperature of 28°C. Boxes were inspected each two days for larval mortality. Dead larvae were removed, washed with distilled water and individually placed on the previously described plaster discs to assure that death was because of the nematode infection.

**Nematode Production in Nematode-Killed Grubs**

Nematode-killed L3 of *P. savigny* were kept individually in the extraction chambers at room temperature of 28°C until the emergence of the new generation of the IJs which were collected and counted daily throughout the production period. Then distilled water was added to the daily harvest of IJs to reach a standard volume of 100 ml. One ml of the suspension was pipetted and spread onto the Hawskley slide and counted. This was repeated six times and the average of six readings was used to express the number of IJs/ml. Both daily and total productions were based on the average IJs numbers produced from 5 *P. savigny* 3*rd* instar larvae for each nematode concentration initially used for the infection. The IJs produced in nematode-killed L3 of *P. savigny* were then tested for their virulence against 3*rd* instar larvae of the same beetle in comparison with IJs produced in *G. mellonella* larvae.

**Statistical Analysis**

Data were subjected to analysis of variance (ANOVA) and means (±SE) were compared at $P<0.05$ level significance.

## RESULTS AND DISCUSSION

**Screening of 10 *Steinernema* strains *vs* 3*rd* Instar Larvae of *P. savigny***

A list of the tested nematode strains and their sources is given in Table (1). As shown in this table, no larval mortality was recorded 10 days after larval exposure to the nematodes of strains: All (*S. carpocapsae*), E2 (*S. kushidai*), J26 (*Steinernema* sp.), K43 (*Steinernema* sp.); K66 (*S. kraussei*), K91 (*S. affinis*), or K92 (*S. feltiae*). Mortalities of 20% and 13% were recorded among larvae exposed to strains K35 (*Steinernema* sp.) and K27 (*S. carpocapsae*). On the other hand, a remarkable mortality of 95% were recorded among larvae treated with *S. glaseri*.

These results indicate that *S. glaseri* is the most effective among the 10 tested steinernematid strains against *P. savigny* grubs. The lack of infectivity of some entomopathogenic steinernematids towards scarabs was a point of concern among many researchers. Klein (1993) concluded that *S. glaseri* and *H. bacteriophora* are the most infectious nematodes for use against scarab larvae. Wang *et al.* (1994)

Table (1): Mortality % 10 days after treatment, among the 3*rd* instar larvae of *P. savigny* treated with 10 *Steinernema* strains in sandy soil (nematode concentration: 10,000 IJs/5 larvae).

| Nematode strain | Source | Mortality % |
|---|---|---|
| All strain (*S. carpocapsae*) | USA | 0 |
| E2 (*S. kushidai*) | Japan | 0 |
| J26 (*Steinernema* sp.) | Jamaica | 0 |
| K27 (*S. carpocapsae*)Breton strain | France | 13±1.1 |
| K35 (*Steinernema* sp.) | France | 20±1.1 |
| K43 (*Steinernema* sp.) | France | 0 |
| K66 (*S. kraussei*) | Czechoslovakia | 0 |
| K91 (*S. affinis*) | Australia | 0 |
| K92 (*S. feltiae*) T 319 strain | Australia | 0 |
| *S. glaseri* | USA | 95±0.57 |

Table (2): Physical and chemical properties of two soil types used in nematode bioassay.

| Soil | Coarse | Fine | Silt | Clay | Texture | pH | EC | $CaCo_3$ | Soluble cations (mg/100 gm soil) | | | | Soluble anions (mg/100 gm soil) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| type | sand% | sand% | % | % | | | | | $Ca^{++}$ | $Mg^{++}$ | $Na^{+}$ | $K^{+}$ | $CO_3^{--}$ | $HCO_3^{-}$ | $Cl^{-}$ | $SO_4^{--}$ |
| 1* | 84.910 | 10.663 | 2.848 | 1.579 | Sandy | 7.89 | 2.20 | 3.280 | 0.20 | 0.08 | 0.09 | 0.01 | - | 0.08 | 0.05 | 0.26 |
| 2** | 32.894 | 34.201 | 29.287 | 3.618 | Sandy loam | 7.69 | 2.90 | 3.108 | 0.74 | 0.22 | 0.14 | 1.12 | - | 0.09 | 0.11 | 0.91 |

* Sand

** Mixture of equal volumes of sand, loam and cattle manure.

compared the infectivity of *S. carpocapsae, S. glaseri, S. scapterisci* and *H. bacteriophora* to *P. japonica* larvae using external exposure and haemocoelic injection. They reported that only *H. bacteriophora* and *S. glaseri* caused high mortality after external exposure to 10,000 IJs, while *S. carpocapsae* had a low level of infectivity.

Since the nematodes and white grubs have co-evolved in the soil environment, larvae possess a number of defence mechanisms such as low carbon oxide output, sieve-plates on their spiracles, frequent defecation, wiping their mouth parts to remove nematodes and possession of peritrophic membrane that serves as a barrier once nematode enter the insect gut. All of these defences prevent scarabs from being highly susceptible to nematode parasites (Bedding and Molyneux, 1982; Froschler and Gardner, 1991c; Klein, 1993). Our results find supports by previous field studies of various researchers indicating that *S. glaseri* can provide acceptable levels of *P. japonica* control (Wright *et al.*, 1988; Villani and Wright, 1988; Klein, 1990; Klein and Georgis, 1992; Selvan *et al.*, 1993), whereas other species such as *S. carpocapsae* were less effective (Georgis and Gaugler, 1991; Smits, 1994). Georgis and Gaugler (1991) attributed differences in nematode efficacy to differences in levels of host-parasite adaptation. Cui *et al.* (1993) reported that infective juveniles of *S. glaseri* are highly mobile "cruisers" that actively search for hosts whereas *S. carpocapsae* mostly wait for hosts to come to them as "ambushers". Gaugler (1993) attributed the superior performance of *S. glaseri* against scarabs in large part to their cruiser host-finding strategy. On the other hand, lack of scarab susceptibility to entomopathogenic nematodes could be attributed to encapsulation and melanization inside the host. Studies of Wang and his co-workers (1994) on *P. japonica* showed that few *S. glaseri* (2.8%) were encapsulated and melanized, whereas nearly all infective juveniles of other tested species became encapsulated and melanized. *S. glaseri* in *P. japonica* can evade the immune response because the two organisms, the nematode and the grub, associate under natural conditions. According to Wang *et al.* (1994) the mechanism of this avoidance is unclear and may differ among nematode species. Therefore, screening for nematode ability to overcome or evade the host defence response may provide an important first step in narrowing the list of suitable nematode species.

**Susceptibility of *P. savigny* Larvae to *S. glaseri***

As *Steinernema glaseri* proved to be the most virulent steinernematid amongst the 10 tested strains, susceptibility of both *2nd* and *3rd* instar larvae of *P. savigny* was tested using nematode concentrations ranged between 8,000 and 15,000 IJs/plastic box. Mortality % induced by the different nematode concentrations among L2 and L3 is graphically illustrated in Figs. (1&2), respectively. Two days after grub exposure to the infective juveniles, much higher mortalities were recorded amongst L2 than L3 in all the tested nematode concentrations. For instance, the lowest concentration of 8,000 IJs induced 70% mortality among L2 and about 10% mortality in L3. Four days after exposure, larval mortality induced by this concentration (8,000 IJs) reached 90% in L2 and 55% in L3. All L2 exposed to a concentration of 10,000 IJs died on the *4th* day; the same concentration caused 95% mortality in L3 after the same period. However, a higher concentration of 15,000 IJs induced 100% mortality among the two tested larval instars after 4 days of exposure.

When *S. glaseri* was tested *vs* the *2nd* and *3rd* instar larvae of *P. savigny* in a soil mixture composed of equal volumes of sand, loam and cattle manure (Table 2), higher nematode concentrations and longer period of time were needed to obtain considerable larval mortalities than those previously obtained in the sandy soil. The results of this bioassay are shown in Figs. (3&4). Nevertheless, according to these figures, the observed larval mortality was generally inconsistent. No mortality was recorded among L2 up to the *6th* day of exposure, while mortalities between 5% and 30% were recorded among L3 during the first 4 days following the nematode treatment (Fig. 4). Ten days after treatment, larval mortality among L2 did not exceed 55% (conc. 30,000 IJs/box) and this mortality did not change during the following days (not illustrated in the histogram). On the other hand, the maximum mortality recorded among L3 was 70% in the nematode concentration of 30,000 IJs after 3 days. A comparison between mortality values in the two tested soils clearly indicates that the presence of loam and organic matter in the soil mixture causes an obvious decrease in larval mortality among the two tested larval instars in terms of both nematode concentration and the time needed to obtain high larval death.

Statistical analysis of the obtained results showed no significant differences in the susceptibility of L2 and L3 when they were exposed to the infective juveniles in sand, regardless of the nematode concentration used, although the initial mortality among L2 was higher than in L3. But when the exposure to *S. glaseri* took place in the soil mixture of sand, loam and cattle manure, L3 was significantly more susceptible than L2 regardless of the nematode concentration. Similar findings were recorded by Smits (1994) who stated that grubs become more sensitive to nematode infection with increasing age. However, he added that the data may still toll sporadic to allow such a general conclusion. He suggested that the natural openings are usually larger in the older larvae and therefore may provide a better opportunity for nematodes to enter the body. Physiological defence mechanisms may also show differences between the instars. Nevertheless, since *P. savigny* grubs do not feed upon living plant material, and since the *3rd* larval instar represents the longest stage in the insect life cycle (Abou Bakr *et al.*, 1989a), the increased susceptibility of the L3 should be considered as an advantage from the point of view of pest control. The results also showed a significant decrease in nematode infectivity when bioassays were carried out in a soil mixture containing silty soil and organic matter. Glaser *et al.* (1940) mentioned that parasitism by insect nematodes varied depending on soil moisture, nematode dosage, soil temperature, and host density. Georgis and Poinar (1983) attributed the decreased nematode infectivity in soils containing increased proportion of clay and silt to the difficult movement of the juvenile nematodes in

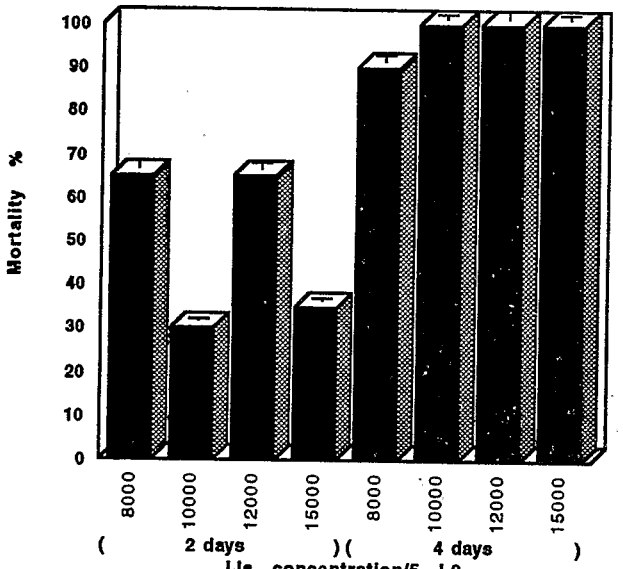


Fig.(1): Mortality % among L2 of P. savigny treated with S.glaseri in sand.

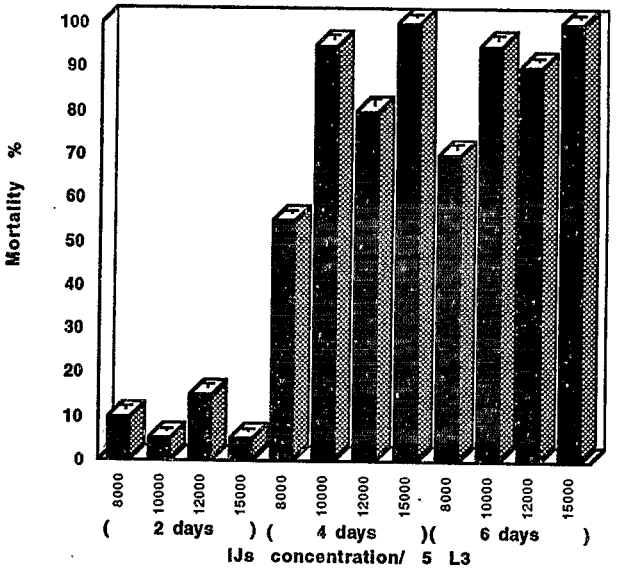


Fig.(2) : Mortality % among L3 of P.savigny treated with S.glaseri in sand.

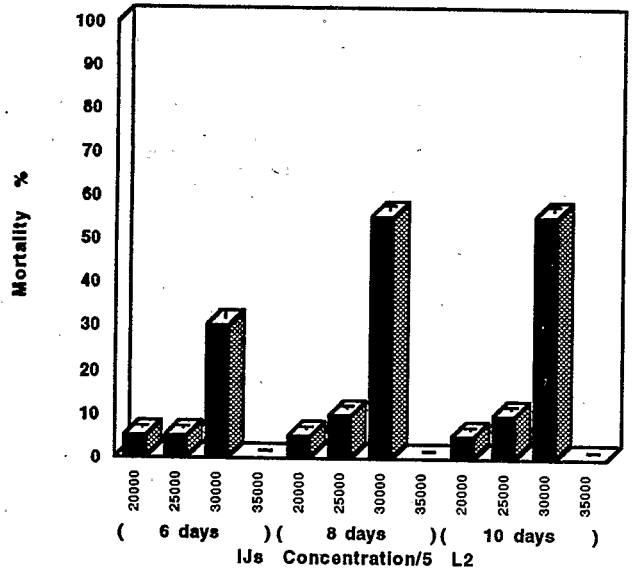


Fig. (3) : Mortality % among L2 of P.savigny treated with S.glaseri in soil mixture.

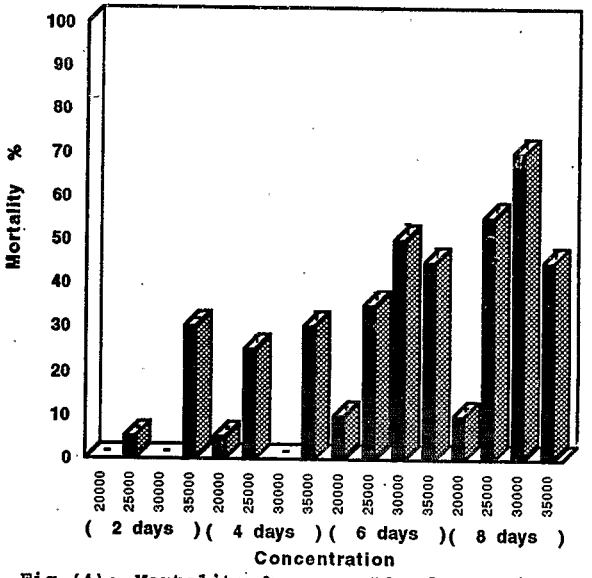


Fig.(4): Mortality % among L3 of P.savigny treated with S.glaseri in soil mixture.

these types of soils to migrate and infect the host; and they observed that the greatest dispersal and infectivity occurred in pure silica sand and coarse sandy loam. More explanations were given by Molyneux and Bedding (1984), they reported that nematode movement within the soil requires water filled pores or films of sufficient thickness and continuity to allow migration. They added that the higher clay content of the clay loam is associated with more pores of small diameter and greater tortuosity which limits nematode movement, reflected in lower larval mortality. Meanwhile, the levels of parasitism occurring in the sandy soils at high moisture potentials reflect the amount of aeration in these soils at high moisture contents.

The soil mixture in which the bioassay was carried out contained a great part of organic matter (cattle manure) to which the decreased nematode infectivity is attributed. The effect of manure and increased organic matter in the soil was a point of concern among several workers. Mullens *et al.* (1987) reported that the application of *S. carpocapsae* and *H. bacteriophora* to control maggots in poultry houses produced conflicting results. The failure was attributed to poor nematode survival in manure because of the high ambient temperature, toxic ammonia and salts (Georgis *et al.*, 1987), or possibly predatory mites (Wicht and Rodriguez, 1970).

In the present work our data show that grub mortality does not necessarily increase as the nematode dosage increases. That was more obvious when nematode was applied in the soil mixture (Figs. 3&4). No clear explanation is available so far, and several previous works pointed to the inconsistency of the results of nematode applications. However Selvan *et al.* (1993) observed that although the number of invading nematodes increased with increasing dose, percentage penetration declined. This density-dependent penetration was not sufficient to prevent the detrimental effects of overcrowding. Although the exposure method followed by Selvan and his co-workers assured host contact, no more than half of the nematode population initiated host infection. They reported that their results bolstered Fan and Hominick's (1991) contention that most entomopathogenic nematode infective juveniles are not infective at a given point in time. In addition, Bohan and Hominick (1994) mentioned that there is a proportion of dauers that are infectious at any given time and that this proportion changes through the time.

**Multiplication of *S. glaseri* in the Cadavers of *P. savigny***

Nematode-killed 3*rd* instar larvae of *P. savigny* were examined for their suitability for the multiplication of *S. glaseri*, taking into consideration the initial nematode concentration used for larval infection. Results shown in Figs. (5-8) indicate that at a temperature of 28°C the IJs began to emerge from the cadavers 14- 19 days after death of the infected larvae. The longest period (19 days) was related to the least nematode concentration used for infection, *i.e.* 1,000 IJs/5 L3 (= 200 IJs/L3). The rate of IJs emergence seems to follow a general pattern regardless of the initial infective dose: a gradual increase of emerging IJs until reaching a peak, then nematode emergence may show some stability (as in Fig. 5) or decline gradually or rapidly (Figs. 6,7&8). This peak was reached on the 2*nd* day of the initial emergence in all cases, except in case of using 5,000 IJs/5 L3 (1,000 IJs/L3) as initial infective dose (Fig. 6). The total period of active emergence ranged between 4 days (when very high infective dose of 2,400 IJs/L3 was used, Fig. 8) and 7 days when 1,600 IJs/L3 was used. However, the initial infective dose seems to have a slight effect on the total active period of nematode production; rather it affects the level of the peak reached in each case: the highest infective dose (2,400 IJs/L3) resulted in the highest peak (Fig. 8). Moreover, a proportional relationship can be detected between the initial infective dose and the total number of IJs produced in the dead grub. As shown in Fig. (9) a gradual increase in nematode production is recorded by the increase of the infective dose. Number of emerging IJs ranged between ca 30,000 IJs and 125,000 IJs using infective doses of 200 and 2,400 IJs/L3, respectively. According to our experiments (unpublished) a full mature *Galleria mellonella* larva may produce about 43,000 IJs when an initial infective dose of 100 IJs of *S. glaseri* was used. It is noteworthy that the weight of a 3*rd* instar larva of *P. savigny* ranges between 1.8-2.6 gm with an average of 2.22±0.24 gm, while the weight of *G. mellonella* larva ranges between 0.112- 0.184 gm with an average of 0.156±0.02 gm. Although we did not examine the size of individual juveniles produced in each infective dose, Selvan *et al.* (1993) reported that the longest infective juveniles were produced at the lowest nematode densities, indicating a trade off between size and number of progeny. They also stated that effects of high density appear to result from competition for limited nutrients within the host.

**Virulence of *S. glaseri* IJs Produced in Different Hosts**

A comparison was made between mortalities among the 3*rd* instar larvae of *P. savigny* when they were infected with IJs of *S. glaseri* produced in two different hosts, *i.e. G. mellonella* and *P. savigny*, using an infective dose of 10,000 IJs/5 L3 *P. savigny*. As shown in Fig. (10), the IJs produced in *P. savigny* larvae were more virulent than those produced in *G. mellonella*. Four days after nematode exposure 60% mortality was recorded among *P. savigny* infected with *S. glaseri* produced in *G. mellonella*, while 100% mortality was recorded after the same period among the *P. savigny* L3 infected with nematode produced in nematode-killed grubs. These results indicate that virulence of *S. glaseri vs P. savigny* larvae could be enhanced by passing the nematode into the larvae of the target insect. The present results are of one-passage nematodes and more passages may result in more virulence. Another trial to improve the activity of a nematode strain against grubs was recently made by Smits (1994) who passed a strain of *S. glaseri* four times through *M. melolontha* larvae and found a 10- fold increase in the biological activity.

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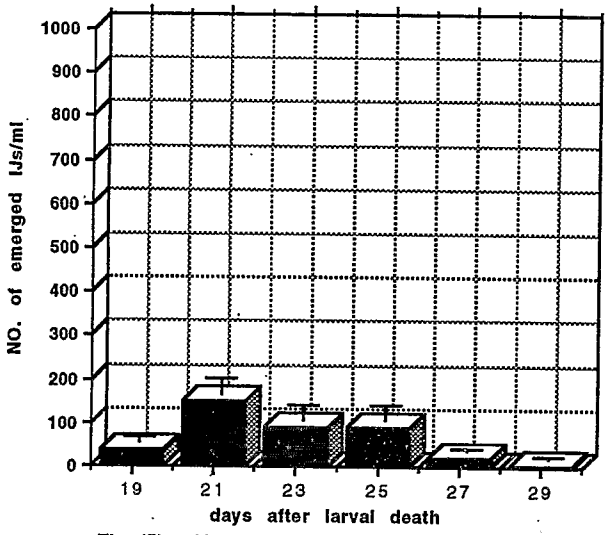


Fig (5) : Number of S. glaseri IJs emerged from L3 cadavers of P.savigny infected with 1000 IJs/5 larvae in sand.

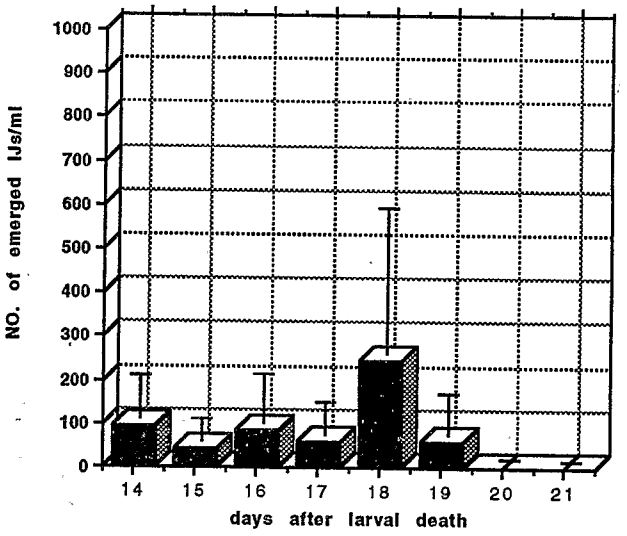


Fig (6) : Number of S.glaseri IJs emerged from L3 cadavers of P. savigny infected with 5000 IJs/5 larvae in sand.

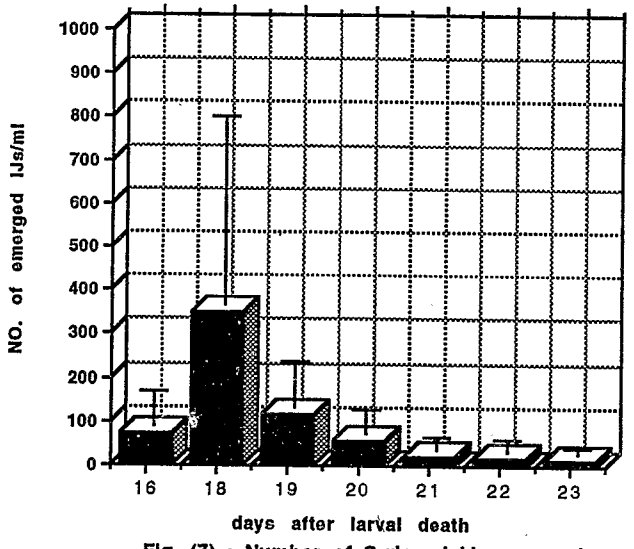


Fig. (7) : Number of S.glaseri IJs emerged from L3 cadavers of P.savigny infected with 8000 IJs/5 larvae in sand.

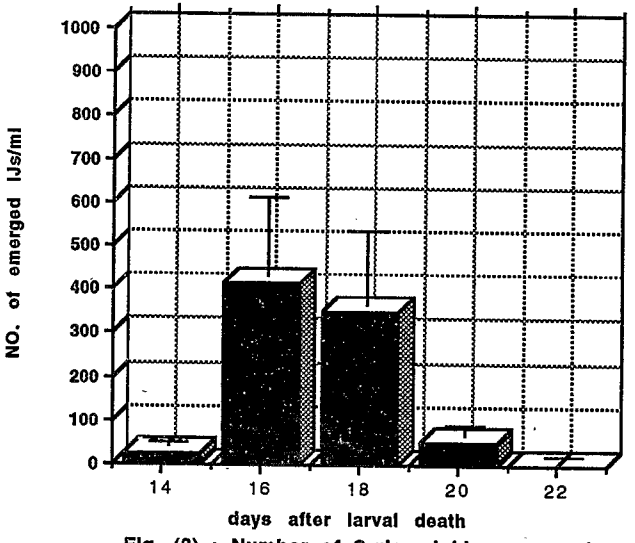


Fig. (8) : Number of S.glaseri IJs emerged from L3 cadavers of P.savigny treated with 12 000 IJs/ 5 larvae in sand.

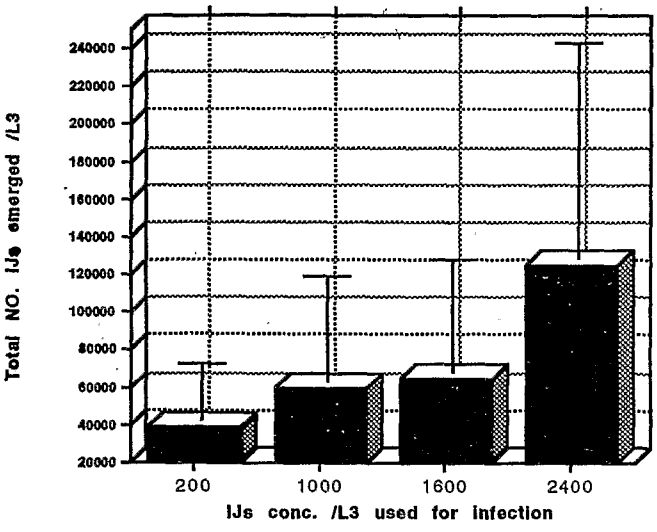


**Fig. (9) : Total number of S.glaseri IJs produced in P. savigny L3 infected with different nematode concentrations.**

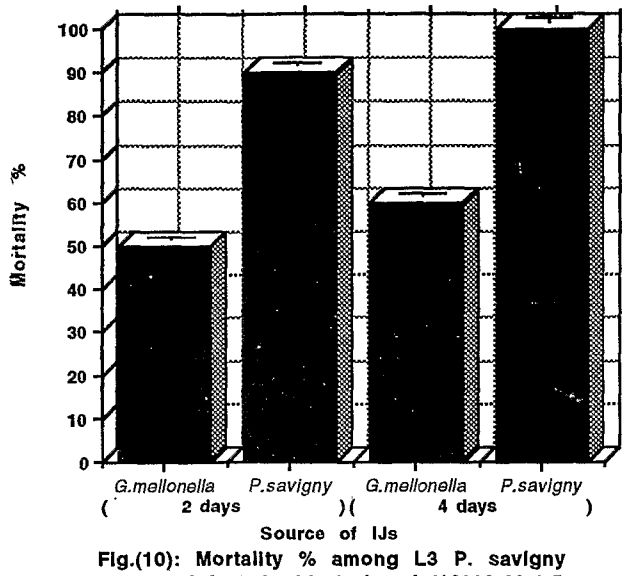


**Fig.(10): Mortality % among L3 P. savigny infected with S.glaseri (10000 IJs/ 5 larvae) produced in two different hosts.**

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