

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 3rd instar larvae of the peach cockchafer, *Pachnoda savigny* (Coleoptera:Scarabaeidae). Bioassays indicated that both 2nd and 3rd 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 cm². 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 3rd 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

laboratory against L2 and L3 of the same insect. Laboratory experiments included the effect of soil composition and

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 10⁶ 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 3rd instar larvae of *P. savignyi* in sand. A concentration of 10,000 IJs of each strain was used for each 5 grubs kept in a plastic box (156 cm² area) containing 375 cm³ 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

boxes 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. savignyi* 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. savignyi* 3rd instar larvae for each nematode concentration initially used for the infection. The IJs produced in nematode-killed L3 of *P. savignyi* were then tested for their virulence against 3rd instar larvae of the same beetle in comparison with IJs pro-

Table (1): Mortality % 10 days after treatment, among the 3rd 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 (<i>S. carpocapsae</i>)	USA	0
E2 (<i>S. kushidai</i>)	Japan	0
J26 (<i>Steinernema</i> sp.)	Jamaica	0
K27 (<i>S. carpocapsae</i>) Breton strain	France	13±1.1
K35 (<i>Steinernema</i> sp.)	France	20±1.1
K43 (<i>Steinernema</i> sp.)	France	0
K66 (<i>S. kraussei</i>)	Czechoslovakia	0
K91 (<i>S. affinis</i>)	Australia	0
K92 (<i>S. feltiae</i>) T 319 strain	Australia	0
<i>S. glaseri</i>	USA	95±0.57

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

Soil type	Coarse sand%	Fine sand%	Silt %	Clay %	Texture	pH	EC	CaCO ₃	Soluble cations (mg/100 gm soil)				Soluble anions (mg/100 gm soil)			
									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 cuticles, frequent defaecation, wiping their mouth

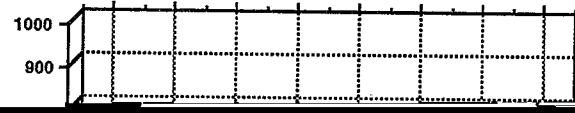
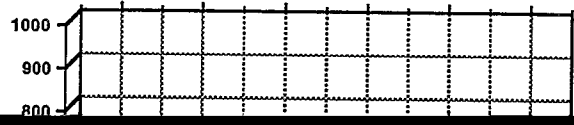
90% in L2 and 55% in L3. All L2 exposed to a concentration of 10,000 IJs died on the 4th day; the same concentration caused 95% mortality in L3 after the same period. However, a higher concentration of 15,000 IJs induced 100% mortality among the two tested larval instars after 4 days of exposure.

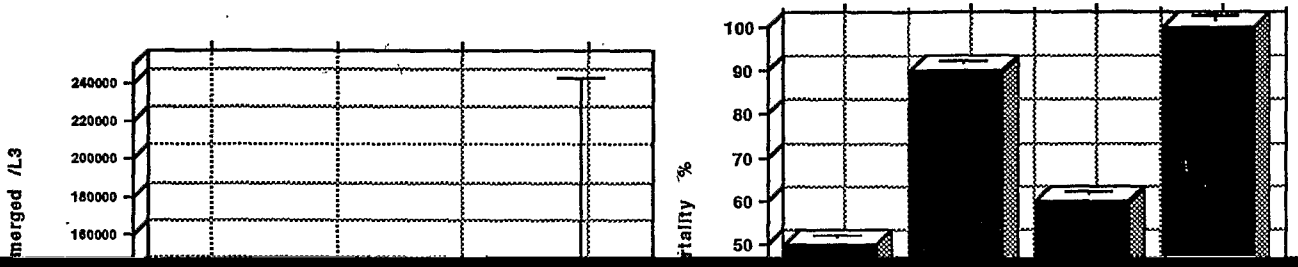
When *S. glaseri* was tested vs the 2nd and 3rd instar larvae of *P. savigny* in a soil mixture composed of equal volumes of sand, loam and cattle manure (Table 2), higher nematode concentrations and longer period of time were



these types of soils to migrate and infect the host, and they showed that the greatest dispersal and infectivity occurred

some stability (as in Fig. 5) or decline gradually or rapidly (Figs. 6, 7 & 8). This peak was reached on the 2nd day of the





the distribution and infectivity of *Neoplectana glaseri*
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