DOSE- AND TIME- RESPONSE ASSESSMENTS OF HETERORHABDITIS HELIOTHIDIS AND STEINERNEMA FELTIAE [NEM. : RHABITIDA] AGAINST AEDES AEGYPTI LARVAE

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Steinernema feltiae (= Neoaplectana carpocapsae) and Heterorhabditis heliothidis were tested against 3rd instar Aedes aegypti larvae in the laboratory. Different dosages of the nematodes and varying durations of exposure were assessed. H. heliothidis was more effective than S. feltiae. Larval mortality showed a positive linear correlation with both nematode dosage and the duration of exposure. The number of nematodes of both species that gained access to the haemocoel of larvae was always low, but increased with dosage and exposure time. The rate of melanization of the nematodes in the larvae was correlated with dosage, but was not affected by the duration of exposure.


Under laboratory conditions, rhabditoid nematodes belonging to the genera Steinernema (= Neoaplectana, Wouts et al., 1982) and Heterorhabditis infect late-instar mosquito larvae. Welch & Bronskill (1962) infected Aedes and Culex species with S. feltiae (= N. carpocapsae), Poinar & Kaul (1982) reported that H. bacteriophora would infect Culex pipiens larvae and Finney & Harding (1981) investigated factors affecting use of steinernematids for mosquito control. Bedding et al. (1983) and Molyneux et al. (1983) demonstrated interspecific and intraspecific differences in infectivity of entomophilic rhabditids for terrestrial insect species and stressed the need for testing more than one nematode species or strain against any potential target insect. It is also important that tests provide quantitative information, and the slopes of dose-response curves are widely used to indicate the potency of a control agent or measure the susceptibility of a pest (Hewlett & Plackett, 1979; Bedding et al., 1983; Molyneux et al., 1983; Hughes et al., 1984). Such information is essential for selecting control agents for possible field application against specific insects (Bedding et al., 1983) and for commercial interests (Hughes et al., 1984), but is not available for rhabditoids parasitizing mosquito larvae. Hence, this paper describes bioassays which measured the mortality of 3rd instar A. aegypti larvae exposed to varying dosages of, and times of exposure to, infective H. heliothidis and S. feltiae juveniles.

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MATERIALS AND METHODS

Nematodes used were the DD-136 strain of *S. feltiae* and the New Zealand strain of *H. heliothidis*, cultured in vivo, in *Galleria mellonella* larvae. Third-instar larvae of *A. aegypti* were used throughout, because earlier experiments had shown that this instar was the most susceptible. The nematodes and mosquitoes were reared at 25 ± 2 °C, the same temperature used for the bioassays.

DOSE-RESPONSE BIOASSAY

Each test utilized 24, 10 cm diameter plastic dishes containing 15 3rd-instar *A. aegypti* larvae in dechlorinated tap water (total volume 100 ml, approximate depth 1.3 cm). Nematode dosages were 15, 150, 300, 750, 1,500, 3,000, and 15,000 infective juveniles per dish (i.e. host-parasite ratios of 1 : 1, 1 : 10, 1 : 20, 1 : 50, 1 : 100, 1 : 200, and 1 : 1,000 respectively). Each dosage had 3 replicates and 3 dishes without nematodes served as controls.

Test dishes were inoculated with infective nematodes from suspensions of known concentration. For the lowest dosage, nematodes were hand-picked from a shallow watchglass. Then, the batch of larvae was introduced into each dish together with 40 mg powdered mixture of Tetramin, crumbled bread and yeast extract. After 24 h, the larvae were separated from any residual nematodes and kept singly in the depressions of multiwell tissue culture plates. Each larva was fed on a pinch of food mixture for a post-exposure period of 24 h. Dead larvae were dissected in Ringer’s saline between 48 and 72 h after the start of the experiment. The test was repeated 3 times for both nematode species. Data obtained for each species were pooled and statistically analysed using probit analysis (Hewlett & Plackett, 1979), after a test for correlation between dosage and mortality.

TIME-RESPONSE BIOASSAY

Third instar *A. aegypti* larvae were exposed to nematodes for periods ranging from 15 to 240 min. In each experiment, 18 plastic dishes (10 cm in diameter) were used, each with 100 ml of dechlorinated tap water and 15 larvae. The dishes were divided into 3 groups of six, and 1 in each group served as a nematode-free control. In Group I, 1 inoculum of 3,000 (+ 80) infective nematodes was added to each of the 5 test dishes and exposures were terminated 15, 30, 60, 120 and 240 min later. This procedure was repeated in Group II. However, after 30 min, a 2nd dose of 3,000 nematodes was added to each of the remaining 3 dishes. Exposures in these 3 dishes were terminated 60, 120 and 240 min respectively after the start of the trial. This gave 2 dishes in which mosquitoes had been exposed to 3,000 nematodes for 15 or 30 min and 3 dishes in which they had been exposed to 3,000 nematodes for 30 min followed by an additional 3,000 for 30, 90 or 210 min. Group III was treated as Group II, but all exposures were allowed to run for 240 min. This gave 2 dishes in which mosquitoes had been exposed to 3,000 nematodes for 240 min and 3 dishes in which they had been exposed to 3,000 nematodes for 30 min followed by the additional dose of 3,000 for 180 min. This experiment was repeated twice for *H. heliothidis* and 5 times for *S. feltiae*.

Since the longest exposure period did not cause mortality by starvation, no food was provided during these experiments. Post-exposure treatments of the larvae were the same as those described for the dose-response experiments. The water in each exposure dish was examined for residual nematodes whose numbers were counted if less than 200 or estimated by counting 3-4 aliquots. Parasitism of the larvae was assessed between 60 and 96 h after