NEW ESTIMATION METHOD FOR THE DENSITY OF ENTOMOGENOUS NEMATODES (RHABDITIDA : STEINERNEMATIDAE) IN THE SOIL

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The use of entomogenous nematodes (Steinernematidae) as biological control agents against soil-inhabiting pests requires a simple method for calculation of the infestive juvenile density in the soil. With this estimation one can examine the persistence of biological agents in the environment and the growth and dispersion of their populations. The knowledge of these factors is important in using insect enemies in integrated pest management (Fuxa, 1987).

Saunders and All (1982), and Poinar and Hom (1986) used methods that have generally been applied by nematologists (Baerman funnel, flotation, sieving), and were able to recover 5-71 % of the infective juveniles from soil samples. It appears that techniques used for the estimation of entomogenous nematodes in ecosystems contain significant error. Relatively small numbers of entomogenous juveniles in the sample with respect to the total number of the soil-inhabiting nematodes makes the process of identification of entomogenous nematodes difficult. On the other hand, Bednarek and Nowicki (1986) have shown that only a small fraction of the nematodes introduced into the soil are able the find an insect host. That fact shows that the above methods are insufficiently selective to investigate the biological potential of the agents in the soil, because they detect all entomogenous nematodes, not only those able to infect the insect. Another method of quantitatively assessing the concentration of nematodes in the soil was reported by Mracek (1982). It is based on the determination of insect mortality caused by infective juveniles from the soil (Bedding & Arkhurst, 1975); a recovery rate of 10-20 % of the juveniles was reported. Using this method, it was possible to estimate the biological potential of the agent in the ecosystem. However, this method can best be applied when the population density of juveniles in the soil is quite high.

The method presented here is based on the determination of the number of infective juveniles recovered from dead trap insects. The use of *Galleria* larvae is suggested based on the *Galleria* trap method described by Bedding and Akhurst (1975). Using the soil drill with a length of 25 cm and a diameter of 1.5 cm, soil samples are taken from an area of approximately 5 000 m² (Bednarek, 1987). Fifty samples were mixed carefully, and half of the volume (in order to reduce labor) was divided over a number of pots each containing 200-250 g of soil and two *Galleria* larvae (IV-V instar). Then the pots were kept at 21-26 °C for 16 days. Every 4 days, all larvae were replaced and the dead insects were dissected immediately to detect the number of nematodes they contained. For accuracy only first generation (preimaginal stage of giant generation) should be considered. Development of the nematodes can be delayed by keeping the dead insects at temperatures of 4-6 °C.

The density of entomogenous nematodes in the soil per square meter (N) can be calculated according to the following formule :

$$N = 2\frac{P}{M\pi r^2}\sum_{k=1}^{n} I_k$$

where :

P = the area (recommended area 5 000 m²) on which M soil samples are taken;
n = number of *Galleria* larvae infected by nematodes;
I_k = number of first generation nematodes recovered from one *Galleria* larva;
M = number of soil samples that were taken (recommended number 50 per 5 000 m²);
r = radius of soil drill.

The formule should be multiplied by 2 when only half of the soil sample is used.

It was of interest to know the number of nematodes that could be detected by this method using soil samples containing different numbers of juveniles. Using the method above described, 10, 100 and 1000 infective juveniles of *Steinernema feltiae* were placed with two *Galleria* larvae in two separate pots containing sterile, damp soil. The total number of nematodes recovered from *Galleria* larvae varied between 40 and 53 % (Table 1). The number of nematodes obtained from dead larvae appeared to be lower when the dissected larvae were dead for 16 and 20 days than for 4 and 8 days. It was shown that the number of nematodes detected in dead *Galleria* larvae after 16 days was not important for the estimation procedure.

Using this method, Bednarek and Mraček (1986) showed that up to 1600 juveniles entomogenous nematodes per square meter could be present in the forest soil in the Beskid Sądecki Mountains (South Poland).

This method also was used to study the density of entomogenous nematodes in crop fields and meadows in different regions of Poland. The density of *Steinernema* juveniles in meadow soil varied from 82 to 650 individuals per m², and from 1220 to 11 980 in crop soil. The population density of this method was relatively low as compared with densities of free living nematodes (Wasilewska, 1981).

Table 1

Number of *Steinernema feltiae* nematodes recovered from dissected *Galleria* larvae in potted soil

Number of nematodes placed in pots	Period of nematode exposure to *Galleria* larvae (days)					Recovered nematodes (%)
	0-4	4-8	8-12	12-16	16-20	
150*	43	19	10	4	4	53.3
1 500	376	135	124	63	13	47.6
15 000	3 662	1 247	797	279	33	40.2

* in fifteen pots

An important question is what percentage of the nematode population occuring in the soil may infect a host? This question deals with the biological potential of the applied agent. The method described above may be useful for estimation of the number of juveniles able to infect the insect. Also, the biological potential of entomogenous nematodes applied as a factor of integrated pest management could be determined.

Some nematodes species do not infect *Galleria* larvae readily. Beside of this, we suppose that this method may be better than the extraction method used to determine the biological potential of entomogenous nematodes. The extraction method is indiscriminate and is unable to indicate those individuals actually capable of infecting a host.

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