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Influence of Level of Inoculum and Temperature on the Infectivity and Multiplication of the Entomopathogenic Nematode, *Steinernema feltiae* Filipjev (DD-136 strain) on *Corcyra cephalonica* (Staint.) Larva*

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ABSTRACT

Multiplication rate of *Steinernema feltiae* Filipjev (DD-136 strain) on *Corcyra cephelonica* (Staint.) larva was significantly influenced by the inoculum level with ten dauers per larva being the optimum for maximum multiplication. The relationship between the inoculum level and multiplication was curvilinear. The infectivity of the nematode to *C. cephalonica* was on a par at 25 and 30°C, significantly less at 33°C and nil at 35°C. Multiplication of the nematode was maximum at 25°C, signifincantly less at 30°C and absent at 33°C.

Key words: *Steinernema feltiae*, infectivity, multiplication, *Corcyra cephalonica*.

The entomopathogenic nematode, *Steinernema feltiae* Filipjev is mass cultured *in vivo* by using lepidopteran larvae like *Galleria mellonella* L. (Zhilyaeva *et al.*, 1973), *Corcyra cephalonica* (Staint.) (Rajeswari *et al.*, 1984) and *Spodoptera litura* Fab. (Kondo and Ishibashi, 1986). Twenty dauers is optimal inoculum level for obtaining maximum production in *G. mellonella* larva (Woodring and Kaya, 1988).

Experiments conducted to determine the optimum inoculum level and temperature for maximum *S. feltiae* production on *C. cephalonica* larva are reported in this paper.

MATERIALS AND METHODS

The stock suspension of *S. feltiae* obtained from the Biological Control Centre, Bangalore was continuously subscultured on rice moth *C. cephalonica* larvae which were reared on broken pearl millet grains in plastic basins and the nematodes were recovered from cadavers as per the method of White (1929).

Four inoculum levels *viz.*, 5, 10, 20 and 40 infective juveniles (IJs) per larvae were used on full grown *C. cephalonica* larvae of almost equal size, for fixing the optimum level of inoculum for mass multiplication. Four replications were maintained for each level of inoculum. Each replication consisted of a 10 cm dia Petri dish containing a filter paper inoculated with the required number of nematodes contained in one ml of suspension and to which 10 insect larvae had been added and confined for 10 days. The inoculated Petri dishes were incubated at 25 ± 1°C. The nematodes were recovered 10 days after inoculation by using White's trap at room temperature and counted.

Influence of temperature on infectivity and multiplication rate of *S. feltiae* were tested using *C. cephalonica* larvae as host at four different levels of temperatures *viz.*, 25, 30, 33 and 35°C. The insect larvae were infected using a Petri dish as infection chamber as per the method described earlier. Each treatment was replicated eight times and each replication contained ten last instar insect larvae with the inoculation chamber containing 200 infective juveniles.

The infectivity was determined 72h after exposure to the nematode at each temperature by dissecting five insect larvae in normal saline. The dissected insect larvae were left undisturbed for one h to enable the nematodes to escape from the tissues and then counted sexwise.

Ten days after inoculation, the remaining five insect larvae in each replicate was used for extraction of nematodes in 0.1% formalin using a White's trap. The nematodes extracted were collected and stored on alternate days and the trap was replaced with fresh 0.1 per cent formalin. Extraction was continued until no more nematodes collected in the trap.

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Table 1. Influence of level of nematode inoculum on the rate of multiplication

Inoculum level IJs/insect larva	Nematodes harvested/insect larva (n = 4)
5	26242[b]
10	42756[a]
20	38811[a]
40	29717[b]

Means followed by a common letter are not significantly different from each other at 5 per cent level by DMRT

RESULTS AND DISCUSSION

The highest number of nematodes (42,756 per larva) was harvested at an inoculum level of ten infective juveniles and it was found to be on par with immediate higher inoculum level of 20 infective juvniles per insect larva (Table 1). Nematode multiplication at the lowest level of incoulum of 5 nematodes per insect larva and highest level of 40 nematodes per insect larva were on a par, but significantly different from the two intermediate inoculum levles. A significant curvilinear relationship existed between the inoculum level and multiplication indicating that the optimum level of inoculum for *in vivo* multiplication of the nematode on *C. cephalonica* is 10 dauers per larva (Fig.1).

The nematode infected the host at 25, 30 and 33°C, the highest number being at 25°C. At the three levels of temperature in which the nematode infected the insects, more number of individuals became females irrespective of the total number of infective juveniles that infected the insect (Table 2). Thus the sex ratios (female:male) were 1.78 : 1, 1.68 : 1 and 1.67 : 1 at 25, 30 and 33°C respectively.

The nematode reproduced at a significantly higher rate at 25°C than at 30°C. Even though the infective juveniles developed into first generation males and females at 33°C, there was no reproduction. Thus the data showed that the rate of infection, sex ratio and the rate of reproduction were influenced by temperature.

The mortality caused by the nematode was cent per cent at 25 and 30°C, 80 per cent at 33°C and no mortality at 35°C. Host mortality occured in less than one day at 25 and 30°C, while it took about three days at 33°C.

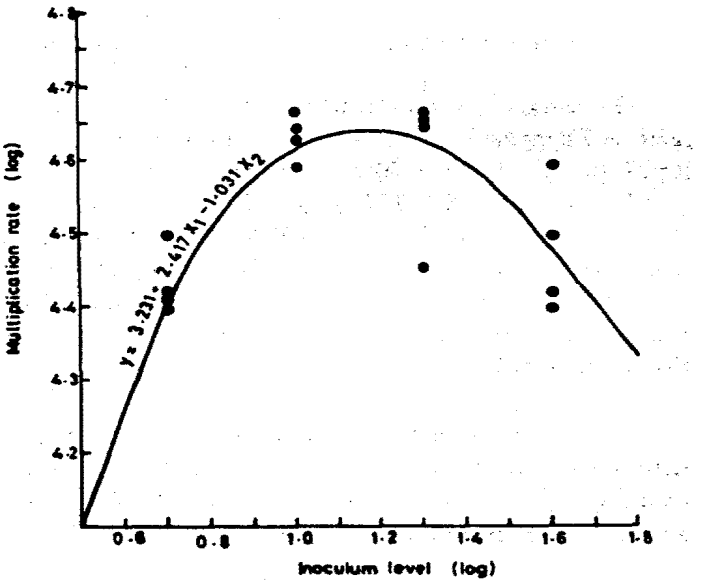

Fig. 1. Influence of Level of Nematode Inoculum on the rate of multiplication

The multiplcation of nematode in large numbers at 30°C is contradictory to the finding of Kaya (1977). However, it must be stated that the experimental conditions between the two experiments were different. Another possible reason could be

Table 2. Influence of temperature on infectivity and multiplication of *S. feltiae* on *C. cephalonica*

Incubation temperature	% Nematode inoculum infecting host* (n = 8)			Nematodes multiplied per insect larva
	Males	Females	Total	
25°C	14.5[a]	25.75[a]	39.75[a]	15,488.17[a]
30°C	11.60[a]	19.50[b]	30.63[a]	13,182.57[b]
33°C	0.75[b]	1.75[c]	3.00[b]	0.00
35°C	0.00	0.00	0.00	0.00

* Inoculum = 20 infective juveniles per insect larva.
Inoculum, means followed by a common letter are not significantly different from each other at 5 per cent level by DMRT

that the isolate available in India which was brought from USA, several years back has probably marginally acclimatized to a higher temperature. It has been proved that the behaviour of different strains of *S. feltiae* is influenced by temperature differently. Thus the Agriotos strain infected even at temperatures beyond 33° C (Hackett and Poinar, 1973). The nematode has also been proved to acclimitize itself to lower range of temperatures when repeatedly cultured at those temperatures (Burman and Pye, 1986) and quite possibly it would adapt itself to temperatures beyond 30°C.

From a single *C. cephalonica* larva, about 42,756 ± 2970 nematodes could be harvested at the optimum level of inoculum and temperature. Generally for field application, a dosage of 200 L of spray fluid containing 3000 infective juveniles per ml has been found effective. In other words, for spraying one acre, a dosage of about 14,000 *C. cephalonica larval equivalent is necessary*. The cost of in *vivo* multiplication of the nematode required for one acre, using *C. cephalonica* would be about Rs. 40/- as per the costing schedule worked out by Solayappan (1988). However, it must be stated that *in vitro* methods like the use of wheat bran media (Yoshihiko, 1987) would be more economical compared to the *in vivo* method.

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