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Reproductive Potential of Entomopathogenic Nematode, *Steinernema feltiae* Against Cabbage Butterfly, *Pieris brassicae* (L.)

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ABSTRACT

The reproductive potential of native strain of *Steinernema feltiae* MN044868 at different concentrations (50, 100, 150 and 200%) was evaluated against 4th and 5th instar larvae of cabbage butterfly (*P.brassicae).* The results revealed that reproduction rate was highest at concentration of 50 IJs as compared to 100, 150 and 200IJs. Application of *S. feltiae* MN044868 @ 50 IJs/larvae resulted in the highest progeny production (0.403 x 10^6) in 5th instar larva followed by 100 IJs/larvae (0.390 x 10^6), 150 IJs/larvae(0.320 x 10^6) and 200 IJs/larvae (0.317 x 10^6) whereas in case of 4th instar larvae, lowest progeny production0.365 x 10^6 was recorded at 50IJs/larvae, followed by100 IJs/larvae (0.334 x 10^6), 150 IJs/larvae(0.310 x 10^6) and 200 IJs/larvae (0.304 x 10^6), respectively.

Key words *: Reproductive potential, Entomopathogenic nematode, Steinernema feltiae, Pieris brassicae.*

Introduction

Entomopathogenic nematodes (EPNs) are beneficial nematodes that parasitize insect pests and are used as a biopesticide to control a wide range of insect pests. Nematodes are considered as potential alternative to pesticides in integrated pest management (IPM) programmes because of the outstanding properties of EPN, which have sparked significant economic interest in them as biological insecticides (Ahmad *et al.*, 2005). Askary and Abd-Elgawad (2017) reported entomopathogenic nematodes (EPNs) as fatal parasites of insect pests that are harmless for people, other vertebrates, and non-target creatures, hence exempt from registration requirements in the majority of the Nations. Additionally, they can easily be multiplied using *in vivo* and *in vitro* procedures, have a short life cycle of 3–7 days and are resistant to unfavourable environmental circumstances. The only infective juvenile (IJ), which is a free-living, non-feeding active stage capable of withstanding unfavourable environmental circumstances and being unavailable to the host for a longer amount of time, is the third stage juvenile of these worms. *Steinernema* is genera of entomopathogenic nematodes (EPNs), symbiotically associated with the gram-positive bacteria *Xenorhabdus* (Boemare *et al.*, 1993). Infective juve-

niles enter into the insect body through natural openings such as mouth, anus or spiracles or intersegmental membrane of the cuticle (Bedding and Molyneux, 1982). The insect killed by *Steinernema* species become pale yellow, creamy or tan in colour. The colour of the insect cadaver is indicative of the pigments produced by monoculture of mutualistic bacteria growing in the body of host insect (Muthulakshmi and Subramanian, 2016).

Materials and Methods

Preparation of entomopathogenic nematode culture under *in vivo* conditions

S. feltiae used in the study was obtained from its clay powder formulation supplied by National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru, India. It was maintained in the laboratory at Division of Entomology, SKUAST-K, wadura campus, Srinagar, India. The nematode was cultured in the last instar larvae of *C. cephalonica*. Hundred larvae were kept in 50 cm diameter petri dish, lined with a filter paper and inoculated with approximately 10,000 IJs of *S. feltiae* contained in 1 ml of sterilized distilled water. The petri dishes were placed in BOD incubator at 20 °C (Dutky *et al.*, 1964) upto 3 days for incubation and the infected *C. cephalonica* larvae were taken out from the petri dishes and the cadavers were placed on white traps (White 1927) again in BOD incubator at 20 °C. After 7-10 days, IJs moved from the *C. cephalonica* cadavers to the water reservoir of white trap were collected in a clean beaker, allowed to settle for one hour and the supernatant was decanted. The beaker was refilled by distilled water and the process was repeated 3 times until a clean suspension was obtained.

Rearing of *P. brassicae* of cabbage butterfly in laboratory

Larvae of the cabbage butterfly, *P. brassicae* collected from the cabbage growing fields at SKUAST-K, Wadura campus were brought to the laboratory and kept in rearing cages (20 cm × 12 cm) at room temperature and fresh unsprayed leaves of cabbage were used for rearing of larvae. The feed was changed at every 24 hours and the cages were simultaneously cleaned thoroughly to prevent any contamination. The appropriate instar of *P. brassicae* was harvested and placed in sterilized plastic containers with a mesh for ventilation ready to be used in the bioassay. Dead *P. brassicae* larvae were removed from the rearing cage daily and the fecal pellets were cleaned with moist cotton wool. IJs of *S. feltiae* were evaluated against the 4th and 5th instar larvae but prior to the evaluation, viability of the IJs were ascertained after examining them under a stereoscopic microscope. Only live and active IJs were considered for the experiment. Four different concentrations (50, 100, 150 and 200) of IJs were prepared. Bioassay was performed in 6-well plates. Each well was lined by a Whatman no. 1 filter paper. One surface sterilized *P. brassicae* was placed in each well of a single 6-well plate and required concentration of IJs was added into each well. Each treatment was replicated 6 times. After placing the larva and adding IJs into it, the 6-well plates were covered by their respective lids, labeled and incubated into BOD at 20 ± 2 °C. The infected larvae were lightly rinsed in sterilized distilled water and placed on a white trap for the emergence of IJs.

Statistical analysis

The design used was CRD and production of IJs/larvae was analysed by ANOVA and CD ($P < 0.05$) was calculated to compare the treatments.

Results and Discussion

The results revealed that S. *feltiae* MN044868 at 50 IJs/larvae resulted in highest yield production (0.403 x 10^6) in 5th instar larva followed by 100 IJs/larvae (0.390 x 10^6) and 150 IJs/larvae(0.320 x 10^6). However the lowest yield (0.317x 10^6) was obtained with 200 IJs/larvae. In case of 4th instar larva maximum yield (0.365x 10^6) was obtained at 50 IJs/larvae and it was significantly different from 100 (0.334x

Table 1. Reproductive capacity (10^6) of entomopathogenic nematode strain, *Steinernema feltiae* MN044868 in 4th and 5th instar larvae of *Pieris brassicae* at different inoculum levels

Inoculum	Instars		Mean
	4th	5th	
50	0.365^{c}	0.403^{a}	0.384^{a}
100	0.334^{d}	0.390^{b}	0.362^{b}
150	0.310^{ef}	0.320^{e}	0.315^{c}
200	0.304^{f}	0.317^{e}	0.310^{c}
Mean	0.328^{b}	0.358a	
CD (p<0.05)	Inoculum (Inn) = 0.008		
	Instar (I) = 0.006		
	Inoculum*Instar (T*I) = 0.012		

10^6), 150 (0.310x 10^6) and 200 IJs (0.304x 10^6) (Table 1). It was observed that yield of IJs was directly proportion to the size of larva. Average yield obtained from 5th stage larva was highest (0.403 x 10^6) followed by 4th instar larva (0.365x 10^6). In case of each treatment the yield obtained from 5th and 4th instar larva was significantly different from each other. However, among the treatments 50 IJs resulted in maximum yield from 5thinstar larva followed by 4th instar larva.

From the results it is clear thatproduction of IJs within the insect cadaver was found directly proportional to the size of the insect cadaver. IJs obtained from 5th instar larva were more than 4th instar of *P. brassicae*. The increased production may also be the result of other factors such as bacterial infection initiating the breakdown of complex food storage molecules, allowing quicker utilization by the nematode and thereby contributing to enhanced nematode development (Askary and Ahmad, 2020). In the present study, for both 4th and 5th instar larva of *P. brassicae*, progeny productionat inoculum level of 50 IJs was highest and with the increase in inoculum level, the production of IJs decreased. This observation confirms the findings of Selvan *et al*. (1993) who reported that the proportion of *S. carpocapsae* and *H. bacteriophora* infecting *G. mellonella* larvae declined with the increase in inoculum level of IJs.

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