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EVALUATION AND STANDARDIZATION OF MASS PRODUCTION OF NATIVE STEINERNEMA SP. (AAU St-1)

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Abstract

The present investigation evaluated the in vivo multiplication of the native entomopathogenic nematode Steinernema sp. (AAU St-1) using larvae of Corcyra cephalonica as the insect host. Larvae belonging to the second, third, fourth, and fifth instars were exposed to infective juveniles(IJs) of Steinernema sp. (AAU St-1) to determine the most suitable developmental stage for nematode production. Among the tested instars, the fifth instar larvae produced the highest nematode yield, followed by the fourth and third instars. The greatest mean production of infective juveniles was 90,307 IJs per larva from the fifth instar, whereas the fourth and third instars yielded 78,674 and 47,771 IJs per larva, respectively. C. cephalonica, particularly its fifth instar larvae, is a highly suitable host for mass multiplication of Steinernema sp. (AAU St-1). The study further demonstrates the native entomopathogenic nematode as a promising biological control agent for agricultural pests.

***Keywords**: Entomopathogenic nematode, EPNs, Steinernema sp., Mass production.*

I. INTRODUCTION

Entomopathogenic nematodes (EPNs) are insect-parasitic roundworms that serve as effective biological control agents against a wide range of insect pests. They maintain a mutualistic relationship with symbiotic bacteria belonging to the genera *Xenorhabdus* and *Photorhabdus*, which are carried in the intestine of the infective juveniles. After entering a susceptible insect host, they release bacteria into the haemocoel, where they rapidly multiply and cause septicaemia, resulting in host mortality within 24–48 hours. The bacteria also create

favorable conditions for nematode growth and reproduction inside the insect cadaver. (Shapiro *et al*., 2002) Extensive research on the biology, ecology, and host interactions of EPNs has enhanced our understanding of their role in sustainable pest management and their practical use in biological control programmes. (Maru *et al*., 2007)

The study of EPNs began with the discovery of *Steinernema glaseri* by Glaser in 1932 from the Japanese beetle, *Popillia japonica* (Newman) (Chand *et al*., 2016). Since that time, numerous investigations have demonstrated the effectiveness of EPNs against several economically important insect pests, particularly those belonging to the orders Lepidoptera and Coleoptera. Their successful performance under different agro-ecological conditions, including those of Rajasthan, has highlighted their value as an important component of integrated pest management (IPM). By suppressing pest populations below the economic injury level, EPNs provide an environment friendly and sustainable alternative to conventional chemical insecticides (Chand *et al*., 2016) (Tavivad *et al*., 2024) (Suman *et al*., 2024).

Large-scale production of EPNs is essential for their successful commercialization and widespread use in biological pest management. EPNs can be propagated either through *in vivo* or *in vitro* production methods, with each approach offering distinct benefits in terms of production cost, technical requirements, and scalability (Ehlers, 2001). Recent studies have highlighted the effectiveness of native isolates, including *Steinernema* sp. (AAU St-1), against economically important insect pests such as *Spodoptera* sp. (Maru *et al*., 2007)

Suitability of rice moth *(Corcyra cephalonica*) larvae as a host for the *in vivo* multiplication of *Steinernema* sp. (AAU St-1). The study aimed to develop an efficient mass-production system by building upon earlier research that demonstrated the nematode's effectiveness as a biological control agent and its adaptability to regional agro-ecological conditions (Prajapati *et al*., 2022).

II. METHODOLOGY

The present investigation was carried out at the Department of Nematology, B.A. College of Agriculture, Anand Agricultural University (AAU), Anand, Gujarat, during the Kharif and Rabi seasons of 2020–21. The experimental materials and methodologies adopted for the study are described in the following sections.

Isolation of Entomopathogenic Nematodes (EPNs):

Rearing of the Bait Insect, (*Corcyra cephalonica):*

In the present study, *Corcyra cephalonica* was utilized as the bait insect for the mass multiplication of EPNs isolates collected from the AAU campus, Anand. Eggs of *C. cephalonica* were procured from the AICRP on Biological Control of Crop Pests, ICAR Unit-9, Anand Agricultural University, Anand, and the culture was maintained according to the procedure described by (Gautam, 2008). The larvae were reared on an artificial diet prepared using the following ingredients:

I. Sorghum whole grains: 2.5 kg
II. Broken sorghum grains: 0.5 kg
III. Streptomycin: 0.5 g
IV. Yeast extract powder: 1 g

Rearing of *Corcyra cephalonica*:

An artificial diet consisting of sorghum grains, yeast extract, and streptomycin was prepared and filled into round aluminium containers (33 × 12 cm). About 0.5 cc of *C. cephalonica* eggs (1 cc ≈ 16,000 eggs) was uniformly spread over the diet. The containers were covered with a black cloth and secured with thread to facilitate hatching. The eggs hatched within four days, and the larvae initially fed on broken grains before shifting to whole grains after approximately 20 days. Mature larvae spun silken cocoons among the grains and entered the pupal stage, which lasted about 10 days. Adult moths emerged around the 35th day and the freshly laid eggs were collected, cleaned and used to maintain the culture.

Mass Multiplication, Isolation and Extraction of EPNs:

EPNs isolated from the AAU campus were multiplied *in vivo* using third, fourth, and fifth instar larvae of *C. cephalonica*. Before inoculation, the nematodes were washed three times with 0.1% formaldehyde solution to minimize contamination. Each Petri dish containing 20 larvae was inoculated with 500 infective juveniles (IJs) of *Steinernema* sp. (AAU St-1). The nematodes infected and killed the larvae within 48 hrs and new IJs began emerging from the cadavers after 3–4 days. The dead larvae were surface sterilized with 0.1% formalin, rinsed with sterile distilled water and transferred to modified White traps lined with moist Whatman No. 1 filter paper for nematode collection. The harvested IJs were washed repeatedly with sterile distilled water, allowed to settle and the supernatant was discarded several times to

obtain a clean suspension. The final nematode suspension was stored at room temperature and monitored periodically until further use (Chitara *et al*., 2019).

III. RESULTS AND DISCUSSION

Mass production of *Steinernema* sp. (AAU St-1) was carried out on the larvae of the rice moth, *Corcyra cephalonica*, in the laboratory. Fresh suspensions of *Steinernema* sp. (AAU St-1) were inoculated on different stages (2^{nd}, 3^{rd}, 4^{th}, and 5^{th} instars) of larvae, with 500 IJs of *Steinernema* sp. (AAU St-1) introduced into petri plates containing twenty larvae. It was observed that the IJs were capable of invading and killing the larvae of *C. cephalonica* within two days. The emergence of EPNs from the insect cadavers began after 3–4 days.

Table-1: Yield of *Steinernema* sp. (AAU St-1) from the larvae of rice moth *C. cephalonica*.

Sr.no	**Larval instar (Size in mm)**	**IJs harvested/ larva**
1	Second (3.12 mm)	33397
2	Third (5.60 mm)	47771
3	Fourth(7.52 mm)	78674
4	Fifth (10.1 mm)	90317

Note: Number of larvae = 20, Values in mean

The results presented in Table 1 indicated that the yield of infective juveniles (IJs) of *Steinernema* sp. (AAU St-1) varied significantly among the larval instars of *C. cephalonica*. The maximum recovery of IJs (90,307 per larva) was obtained from fifth instar larvae, followed by fourth instar larvae (78,674 IJs) and third instar larvae (47,771 IJs). The increase in nematode yield with advancing larval instar suggests that larger and heavier larvae provide more favourable conditions for nematode development and reproduction.

The present findings are in agreement with earlier reports (Chitara *et al*., 2022), which demonstrated that the production of *Steinernema* spp. and *Heterorhabditis* spp. in *C. cephalonica* is largely influenced by host size and body weight. Similar observations were also recorded by (Dhaliwal, 2006). Who reported higher *in vivo* multiplication of EPNs in larger insect larvae. Likewise, (Mehraj *et al*., 2018) obtained yields ranging from 0.5 to 2.0 lakh IJs from *Galleria mellonella* and *C. cephalonica* using *Steinernema* and *Heterorhabditis* species. Further support for these results is provided by studies, which emphasized that larger host

larvae produce greater numbers of IJs. Overall, the study confirms that *C. cephalonica*, particularly the fifth instar larvae, is a suitable host for efficient *in vivo* multiplication of *Steinernema* sp. (AAU St-1), making it a promising option for large-scale production of EPNs for biological pest management.

IV. CONCLUSION AND FUTURE SCOPE

The present study confirmed the successful *in vivo* multiplication of *Steinernema* sp. (AAU St-1) using *C. cephalonica* larvae as the host insect. Among the different larval stages, the fifth instar produced the highest number of IJs, indicating its superiority for nematode propagation. The results suggest that *C. cephalonica* is a suitable and economical host for the large-scale production of EPNs.

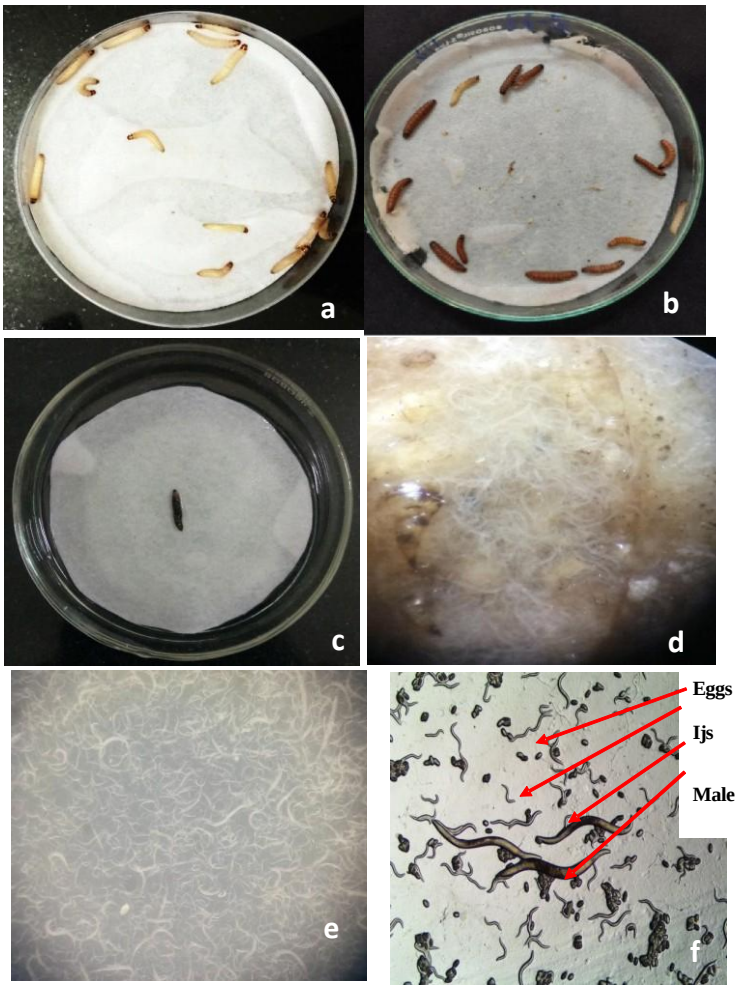

Plate-1: White trap method for *C. cephalonica*

a= Baiting of *C. cephalonica*,

b= Infected dead larvae by EPNs, c= White trap,

d= EPNs feeding on dead larvae

e= Photomicrograph of mass multiplied EPNs,

f= Photomicrograph of different stages of *Steinernema* sp. (AAU St-1)

The findings of this study provide valuable information for improving the mass production of native EPNs and support their wider use in integrated pest management programmes. Optimized production of *Steinernema* sp. (AAU St-1) can enhance the availability of biological control agents for sustainable and eco-friendly management of agricultural insect pests.

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