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Entomopathogenic nematode (EPN), *Heterorhabditis indica* proved effective against mango stem borer, *Batocera rufomaculata* De Geer

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ABSTRACT: Laboratory and field studies were conducted at ICAR- Indian Institute of Horticultural Research, Bengaluru, India during 2020-23 to evaluate the efficacy of native strains of two species of entomopathogenic nematodes (EPN), *viz*., *Heterorhabditis indica* and *Steinernema carpocapsae* against larvae of mango stem borer, *Batocera rufomaculata* De Geer (Coleoptera: Cerambycidae). Bioassay studies revealed the virulence of all five strains tested and based on the LC_{50} value (10.72), *H. indica* (IIHR-2) was selected to be the most effective one and further evaluated under *in vivo* and field conditions. Similar efficacy levels were recorded when it was tested against larvae reared on drumstick twigs to simulate natural habitat of stem borer larvae. Finally, IIHR-2 strain of *H. indica* was evaluated under field conditions by injecting the EPN suspension into the trunks of mango trees infested with stem borer. An insecticide treatment was maintained as a standard check. The EPN has resulted in 81.72% reduction in stem borer damage by larvae of *B. rufomaculata* compared to 90.80% with insecticide. Though it was marginally lower that chemical treatment, considering the merits of non-chemical treatments to the environment and sustainable crop management, EPN can be recommended as an effective component of stem borer management. This is the first insight into establishing the efficacy of EPN against mango stem borer, *B. rufomaculata* under laboratory and field conditions.

Keywords: Entomopathogenic nematode, *Heterorhabditis*, mango, stem borer, *Batocera rufomaculata*

INTRODUCTION

Stem borers of the genus *Batocera* (Coleoptera: Cerambycidae) are one of the serious pests of mango in India (Veeresh, 1989). Among different species reported, *Batocera rufomaculata* De Geer is the most destructive and frequently found borer in mango orchards. Besides mango, it attacks fig, jackfruit, mulberry, papaya, apple, etc. (Butani, 1979; Tandon and Verghese, 1985). Generally the older trees of more than fifteen year old or those already weakened from other causes, either pathological or environmental, are more vulnerable to attack by stem borers. The damage in mango ranges from 5-25% and up to 40% damage was also recorded under high density planting. If not managed in time, stem borer can kill an entire productive plant thus causing huge economic losses. In the recent past, stem borer infestation has been increasing in all major mango belts across the country (Reddy and Rashmi, 2022). Female beetle lays eggs singly on the main trunk of relatively older mango trees. After hatching from the egg, the neonate larva initially feeds under the bark. The larvae feed through the sapwood and make tunnels of about 2-3 cm width which interfere with sap flow and affect foliage and production. Normally the attack goes unnoticed till a branch or two start shedding leaves and drying up. A hole with dripping sap, and chewed plant tissues and frass either extruding from bark or fallen on the ground around trunk are symptoms visible in advanced stages of infestation. The damage results in yellowing of branches followed by drying and die back of terminal shoots and branches ultimately leading to the death of whole tree (Butani, 1979).

In order to kill the grub inside the stem/trunk, injecting an insecticide into stem is a widely followed practice. Insecticide is either injected directly or a cotton dipped in insecticide solution is inserted into active holes and plugged with mud (Reddy and Shivananda, 2021). Dichlorvos was the most widely used chemical for stem injection. However its use was prohibited by the Government of India with effect from 31 December 2020 for its adverse effects on non target organisms and environment (vide Notification S.O. 3951(E) dated 08.08.2018 of the Gazette of India). Being cryptic in nature, managing trunk borers is a challenging task. Keeping in view the growing interest in organic or chemical free crop production, it was felt that identifying a safer and sustainable alternative would be ideal and entomopathogenic nematodes are an option.

Entomopathogenic nematodes (EPN) have considerable potential as biological control agents of a number of cryptic insect pests (Kaya, 1985; Arthers *et al*., 2004) and several strains of *Heterorhabditis* spp.

showed activity against Coleopteran insects (Fallon *et al.*, 2004; Chandel *et al.,* 2005; Nagesh *et al.,* 2006). Rapid host mortality is the most desirable feature of EPN thus reducing the extent of insect damage to crops (Kaya and Gaugler, 1993). The EPNs have symbiotic relationships with bacteria that are species specific. On locating a host insect, they enter through the natural openings as well as by rupturing the insect cuticle to finally reach the haemocoel (Gaur and Mohan 2005). Infected insects are often flaccid, and turn colour to orange, yellow or brown or a brownish-red to brick red. The EPNs have a potential in inundative and inoculative releases and with little adverse effects on environment and non-target organisms (Bathon, 1996). Considering the need of a safer means to manage stem borer in mango, present study was undertaken to evaluate native strains of EPN against mango stem borer.

MATERIALS AND METHODS

Laboratory and field studies were conducted at ICAR-Indian Institute of Horticultural Research, Bengaluru during 2020-23 to evaluate the efficacy of native strains of EPN against larvae of mango stem borer, *B. rufomaculata*.

EPN Cultures

Four native strains of *Heterorhabditis indica* (IIHR 1,2,3 and 4) and one strain of *Steinernema carpocapsae* (IIHR-1) isolated from the fields of ICAR-IIHR, Bengaluru were maintained at the Nematology Laboratory, ICAR – IIHR, Bengaluru. The EPN strains were cultured on the final instar larvae of greater wax moth, *Galleria mellonella* as per Woodring and Kaya (1998). Using white traps, the emerging infective juveniles (IJ) were harvested within three days of first emergence and viable EPN were tested under laboratory conditions against the grubs of mango stem borer. Based on the efficacy recorded under laboratory bioassay studies, they were taken forward for further testing on lab host and ultimately in the field.

In vitro bioassay of EPN strains against mango stem borer:

The EPN suspension containing infective juveniles (IJ) of five strains including four of *H. indica* and one strain of *S. carpocapsae*) (Table 1) was applied @ 0, 10, 50 and 100 IJs per larva on double layer of moistened filter paper kept in a petridish. Second instar larvae of *B. rufomaculata* from the lab culture being maintained at the Entomology laboratory of ICAR-IIHR were used for testing. Ten larvae were exposed to each concentration by releasing one larva in each petri plate with EPN treated filter paper. Larvae were observed for mortality after 72h of exposure. The LC_{50} was calculated for all strains based on per cent mortality in different concentrations.

In vivo evaluation against stem borer larvae reared on lab host

The strain-2 of *H. indica* selected based on bioassay studies was evaluated for its bioefficacy against third and fourth instar larvae of *B. rufomaculata*. The stem borer, *B. rufomaculata* was reared on the laboratory host, drumstick, as per the procedure standardized by Reddy and Varun Rajan (2021). The solution of EPN was injected into drumstick twigs @ 10^4 IJs per hole containing lab cultured larvae of stem borer. Generally twigs with stem borer grubs exhibit symptoms of damage through droppings of excreta and chewed plant tissues around twig. Efficacy of EPN was ascertained by observing the feeding symptoms daily till they were stopped or otherwise. When dropping of chewed stem tissues in powder form were completely stopped, it was considered that larvae died and hence feeding was stopped. These were compared with untreated twigs (Fig. 1B). There were five twigs with one larva inside each for treated and untreated conditions.

Field Evaluation

After ascertaining the efficacy under laboratory conditions, the strains were tested under field conditions. For this, 12 mango trees of about thirty year old (cv. Alphonso) with active stem borer infestation were selected in 2021-22 and 10 trees in the following year 2022-23. This variation was due to limited availability of borer infested trees. The suspension of EPN (10 ml) was injected into stem borer holes on mango tree trunks @ 10^4 IJs/hole (Fig. 2A). After injecting EPN suspension, holes were plugged with mud. The treatment was done two times at five days interval. Though only one application was given, it was repeated in the field as precise location of larvae inside trunk is not known and to increase the chance of insect coming in contact with the EPN suspension. Insecticide solution (imidacloprid 17.8 SL diluted with water @ 10 ml/L) was also injected into other 10 trees as standard check. Six infested trees were maintained as untreated control. Two trees were considered as one replication. Borer chewed plant tissues and excreta dropped on the ground around the trunk of all trees under testing were removed daily from the day treatment was imposed and were observed for one week. In case of EPN treated trees, they were observed for one week after second application. Wherever fresh droppings were stopped, it was considered as borer larvae were

Table 1. Virulence of different strains of EPN against mango stem borer, *B. rufomaculata* recorded in bioassay

EPN Strain	$X^2_{(n-2)}$	b	SE	LC_{50}	Lower limits	Upper limits
H. indica (IIHR strain 1)	1.86	7.13	0.60	16.72	13.92	19.24
H. indica (IIHR strain 2)	1.33	4.28	0.87	10.72	7.55	12.97
H. indica (IIHR strain 3)	0.19	5.76	1.13	12.05	9.82	13.79
H. indica (IIHR strain 4)	2.84	2.74	0.38	18.20	10.32	30.79
Steinernema carpocapsae (IIHR strain 1)	6.32	3.66	0.54	13.41	10.34	15.99

dead due to treatment. The number of trees where fresh damage was stopped out of total treated was recorded and per cent protection was calculated. The significance of difference was tested through ANOVA at 5% level of significance.

RESULTS AND DISCUSSION

Bioassay studies

All the five tested strains of EPNs had caused 100 per cent mortality of grubs of *B. rufomaculata* under *in vitro* conditions. Completely deformed and colour changed larvae were observed after 72 h of exposure (Fig. 1A). This gave an indication that the strains of both the species of EPN used were effective against stem borer grubs. However their virulence varied and the LC_{50} values of five strains ranged from the lowest 10.72 with *H. indica* (IIHR-2) to the highest 18.20 of H. indica (strain 4) (Table 1). Based on these values, *H. indica* (IIHR-2) was identified as the most virulent one and taken forward for *in vivo* and field evaluation. Earlier, Fallen *et al.* (2006) reported up to 58% mortality of larvae of a Cerambycid, Cottonwood borer, *Plectrodera scalator* (Fabricius) caused by EPN in filter paper bioassays.

In vivo evaluation of EPN using a lab host of stem borer

When the suspension of EPN *H. indica* (IIHR-2) was injected into drumstick twigs containing stem borer larvae, there was complete cessation of feeding in eight twigs which accounted to 80% control of borer damage. It was ascertained by cut opening and observing the larvae inside the drumstick twig after a week. On contrary, larvae continued feeding and developed into adult in untreated twigs. Efficacy of EPNs *viz*., *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* against longicorn beetle was established through similar studies by Sharifi et al. (2014) against the rosaceae longhorned beetle, *Osphranteria coerulescens* who found that, EPNs were able to penetrate and reproduce within *O. coerulescens* larvae. They used apricot tree branches, where both species of EPN penetrated into the larval galleries and located and killed the larvae of *O. coerulescens* in their natural habitat deep inside the branches. Present findings are also on similar lines where strain of *H. indica* was capable of reaching to larvae of *B. rufomaculata* inside drumstick twigs. This outcome has led to further field studies to evaluate their potential under the environmental conditions in which *B. rufomaculata* larvae are found on mango.

A. Dead larvae in bioassay

B. Treated and untreated drumstick twigs with larvae feeding inside

Fig. 1. Laboratory evaluation of EPN against stem borer, *B. rufomaculata*

Field evaluation

Data presented in table 2 reveal that both EPN and insecticide treatment had significantly reduced the borer infestation. Out of 12 trees treated with EPN suspension, larval feeding inside the trunk was stopped in 10 trees in 2021-22, and eight trees out of 10 trees in 2022-23. This equates to a mean reduction of 81.72 per cent in borer damage. Effect of EPN on borer larvae was further confirmed by extracting larvae and observing for symptoms of EPN infection (Fig. 2B). In case of insecticide treatment, borer feeding was stopped in 11 trees out of 12 and 9 trees out of 10 in 2021-22 and 2022-23 respectively which accounts for 90.80 per cent reduction. Though the efficacy of insecticide treatment was marginally but significantly higher (90.80%) than EPN treatment (81.72%), considering the merits of non-chemical treatments to the environment and sustainable crop management, EPN can be recommended as an effective component of stem borer management. This is an encouraging result as in some cases, the efficacy of EPNs recorded under bioassay tests could not be sustained under field conditions (Fallen *et al.,*2006).

Based on the findings of our study, it can be summerized that stem injection of EPN suspension of *H. indica* (IIHR-2) is effective in significantly bringing down stem borer, *B. rufomaculata* damage in mango. Further large scale multiplication and multilocation testing would help in taking forward this technology as a safe and sustainable component of integrated pest management of mango. This is the first insight into establishing the efficacy of EPN against mango stem borer, *B. rufomaculata* under laboratory and field conditions.

A. Stem injection of EPN suspension **B. Dead larvae infected by EPN**

Fig. 2. Field evaluation of EPN against stem borer, *B. rufomaculata* in mango

Table 2. Effect of stem injection of entomopathogenic nematode, *H. indica* on damage caused by stem borer, *B. rufomaculata* in mango

S. No.	Treatment	2021-22		2022-23		Mean per cent protection
		No. of trees treated	No. trees where larval feeding stopped	No. of trees treated	No. trees where larval feeding stopped	
1.	EPN (*H. indica*)	12	10	10	8	81.72
2.	Imidacloprid 17.8 SL (Std. check)	12	11	10	9	90.80
3.	Untreated Control	6	0	6	0	0.00
	S. Em ±					1.71
	CD (p = 0.05)					5.36

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