Journal of Biological Control, 34(1): 66-72, 2020, DOI: 10.18311/jbc/2020/23012

Research Article

Eco-friendly management of lepidopteran insect pests through entomopathogenic nematodes

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ABSTRACT: In a recent survey, we have isolated six entomopathogenic nematodes from Hapur, Shamli, Saharanpur and Meerut districts of western Uttar Pradesh. Preliminary identification at genus level classified one isolate as *Heterorhabditis* sp., two isolates as *Steinernema* spp. and three as *Oscheius* spp. These six newly isolated EPNs have been designated as *Oscheius* sp. (IARI-EPN RP 01), *Oscheius* sp. (IARI-EPN RP 02), *Steinernema* sp. (IARI-EPN RP 03), *Oscheius* sp. (IARI-EPN RP 04), *Steinernema* sp. (IARI-EPN RP 05) and *Heterorhabditis* sp. (IARI-EPN RP 06). All these were evaluated for infectivity against lepidopteran larvae, *Maruca vitrata* infesting pigeonpea, *Pieris brassicae* infesting mustard and *Spodoptera litura* infesting chickpea. Among the tested EPNs, *Steinernema* sp. (IARI-EPN RP 03), *Oscheius* sp. (IARI-EPN RP 04), *Heterorhabditis* sp. (IARI-EPN RP 06) were found to be promising against *M. vitrata* as they showed 100% mortality within 48 h followed by *Oscheius* sp. (IARI-EPN RP 01), *Oscheius* sp. (IARI-EPN RP 02) which took 72 h, while *Steinernema* sp. (IARI-EPN RP 05) took about 120 h to kill the insects. *Oscheius* sp. (IARI-EPN RP 02), *Steinernema* sp. (IARI-EPN RP 03) and *Heterorhabditis* sp. (IARI-EPN RP 06) gave 100% mortality of *P. braissicae* within 48 h followed by *Oscheius* sp. (IARI-EPN 04) and *Steinernema* sp. (IARI-EPN RP 05) that killed larvae after 72 h, while *Oscheius* sp. (IARI-EPN RP 01) took about 120 h. Lastly, *Steinernema* sp. (IARI-EPN RP 03) and *Heterorhabditis* sp. (IARI-EPN 06) were found promising for *S. litura* as both of them caused 100% mortality within 48 h followed by *Oscheius* sp. (IARI-EPN RP 01), *Oscheius* sp. (IARI-EPN RP 02) and *Steinernema* sp. (IARI-EPN RP 05) that took 72 h, while, *Oscheius* sp. (IARI-EPN RP 04) was effective at about 120 h. The present results indicated that both *Steinernema* sp. (IARI-EPN RP 03) and *Heterorhabditis* sp. (IARI-EPN RP 06) were highly virulent against the tested lepidopteran insect pests. Further evaluation of these new EPN isolates under field conditions will indicate their utility in integrated pest management.

KEY WORDS: Biological control, entomopathogenic nematodes, insects, Lepidoptera

(Article chronicle: Received: 08-10-2019; Revised: 18-02-2020; Accepted: 15-03-2020)

INTRODUCTION

Agriculture production in India is affected by losses due to several reasons including many biotic stresses. Insect pests are one of the major constraints and one or more insect pests are always associated with every crop causing economic losses. Hence, insect management is a major requirement to increase the crop production. Pesticides are widely used to manage these pests that can lead to environmental and health concerns and also suppress other naturally occurring biocontrol agents (Gaugler and Kaya, 1990). In addition, development of resistance in insect pests to several insecticides is a major concern (Georgis, 2004). Hence, there is a need to develop alternative methods for the management of these insect pests. Entomopathogenic nematodes (EPN) have emerged as potent biocontrol agents against several insect pests (Simoes and Rosa, 1996; Ali *et al*., 2008; Pervez *et al*., 2012, 2014, 2016). They have a great potential as biological control agents against insect pests of crops due to their wide host range, easy to handle, short life cycle, economically produced at large scale and environmentally safe. These nematodes are symbiotically associated with bacteria which are released into the insect hemocoel, causing septicemia and death of the insect (Gaugler and Kaya, 1990).

There is a significant specificity between EPN and the insect group. In addition, proper match of the EPN to the host entails virulence, host finding and ecological factors. If EPN does not possess a high level of virulence towards the target pest, there is little hope of success (Georgis, 2004). Bedding *et al*. (1983) indicated the importance of screening several nematode species against the target insect in the laboratory before commencing field evaluations. Generally, EPNs have been found effective against lepidopteran insect pest infesting various crops (Karunakar, 1999). EPN will be an ideal alternative to chemicals, economical and will fit in long term pest control system without risk to non-target organisms.

They can also be a component of integrated pest management (Gaugler and Kaya, 1990).

EPNs have been reported to occur in tropical, subtropical and temperate countries (Gaugler and Kaya, 1990) except Antarctica (Griffin, 1990). Several surveys conducted and new species or strains of EPNs have been isolated from abroad such as Europe (Steiner, 1996; Midituri *et al*., 1997; Constant *et al*., 1998; Shishiniova *et al*., 1998; Sturhan and Liskova, 1999; Rosa *et al*., 2000; Mracek *et al.,* 2005; Chhetri *et al*., 2010; Herrera *et al.*, 2010), Costa Rica, California, USA, South America, South Africa and Asia (Mason *et al*., 1996; Tangchitsomkid and Sontirat, 1998; Rosales and Suarez, 1998; Yoshida *et al*., 1998; Stock *et al*., 1999; Griffin *et al*., 2000; Iraki *et al*., 2000; Luc *et al*., 2000; Liao *et al*., 2001; Lorio *et al*., 2005; Hatting *et al*., 2009), whereas in India such as, Meghalalaya (Lalramlina and Yadav, 2010), Andaman and Nicobar islands (Prasad *et al*., 2001), Gujarat (Vyas, 2003), Kerala (Banu *et al*., 2004; 2005; Pervez *et al*., 2014), Uttar Pradesh (Kaushal *et al*., 2000; Pervez and Ali, 2007), New Delhi (Ganguly and Singh, 2000), and Tamil Nadu (Poinar *et al*., 1992; Josephrajkumar and Sivakumar, 1997). However, no such information is available about the EPNs associated from Hapur, Shamli, Saharanpur and Meerut districts of western Uttar Pradesh.

Hence, objective of the present study was to isolate, identify and screen the performance of EPNs from Hapur, Shamli, Saharanpur and Meerut districts of western Uttar Pradesh against lepidopteran larvae, *Maruca vitrata* infesting pigeonpea, *Pieris brassicae* infesting mustard and *Spodoptera litura* infesting chickpea.

MATERIAL AND METHODS

Collection of soil samples

Soil samples were collected from tuberose rhizosphere from different locations of Hapur, Shamli, Saharanpur and Meerut districts of western Uttar Pradesh. Within collection site, about 1 kg of soil sample was collected at a depth of 10-20 cm using a hand trowel, each sample containing a composite from five random subsamples. The hand trowel was sterilized with 70% ethanol before leaving the sampling site. Samples were placed in polyethylene bags to minimize dehydration, tag a label providing all necessary information and transported in to the laboratory.

Insect sources

Greater wax moth, *Gallaria mellonella* was used for baiting and multiplication of EPNs. Larvae were reared on artificial diet as per the procedure described by David and Kurup (1988). Test insects, *Maruca vitrata and Spodoptera litura* were collected from pigeonpea and chickpea field of ICAR-Indian Agricultural Research Institute, Experimental Farm, respectively; whereas *P. brassicae* were collected from mustard field, ICAR-National Bureau of Plant Genetic Resources, Experimental Farm, New Delhi. The larvae were sorted out and those of same size were taken for the present study.

Isolation of EPNs from soil

EPNs were isolated from the soil using the insect baiting technique (Bedding and Akhurst, 1975). About 250 g composite soil was placed in a plastic container and ten 4th instar *Galleria mellonella* larvae were released. The soil sample was checked every day up to 7-10 days for mortality of *Galleria larvae*. If larvae found dead, those were placed on modified White trap (White, 1927) for 2 weeks at room temperature for emergence of Infective Juveniles (IJ). In case of the negative results, the isolation process in the soil was repeated two times for the confirmation of the result. Emerged IJs collected from White traps was used to infect fresh wax moth larvae to harvest sufficient IJs for further screening against insect pests.

Identification of EPNs

EPNs were fixed and processed to dehydration following the method described by Steinhorst (1966). EPNs identified upto generic level on the basis of morphological characteristics.

Screening of EPNs against lepidopteran insect pests

Infectivity of newly isolated native EPNs against lepidopteran larvae, *M. vitrata, P. brassicae* and *S. litura* were tested in a Petri plate (5.5 cm diameter), 100 IJs were released in each plate and larva of test insect was kept per plate. Observations on mortality of insects were recorded at 24 h intervals. All experiments were conducted at room temperature and replicated 6 times along with control.

Collection of soil samples

One hundred and twenty-seven soil samples were collected from Tuberose (*Polianthes tuberose*) rhizosphere from different locations of five villages of each block viz., Hapur, Dhaulana and Garhmukteshwar of district Hapur; Shamli and Kairana block of district Shamli, Saharanpur and Deoband block of Saharanpur district and Meerut block of Meerut district of western Uttar Pradesh (Table 1).

RESULTS AND DISCUSSION

Isolation of EPNs from soil

Out of 127 soil samples, only six samples (4.7%) were found to be positive to EPNSs. Among these EPN strains, three EPNs were found from Hapur district, two from Shamli

district and one from Saharanpur districts (Table 1). EPNs recovery from various soil surveys conducted throughout the world have summarized by Rosa *et al*. (2000) followed by Pervez *et al*. (2014). Most surveys showed their recovery rate from soils between 6-35%. Other surveys with 5% or less recovery of EPNs includes, 2.0% in Turkey by Hazir *et al*. (2003), 2.20% in Scotland by Boag *et al*. (1992), 3.8% in Northern Ireland by Blackshaw (1998), 4.6% in Korea by Choo *et al*. (1995), 4.7% in Turkey by Ozer *et al*., (1995) and 5.0% in Italy by Ehlers *et al.* (1991). Further, our findings were comparatively average to studies in India, where Rajkumar *et al*. (2001) showed that out of 105 soil samples collected from Rajasthan, only 5 (4.76%) were found to be positive for steinernematids and heterorhabditids. Subsequently, Parihar *et al*. (2002) undertook another survey in Rajasthan and reported the presence of EPN in 8 samples out of 477 samples (1.68%) studied. They further mentioned that out of 8 positive samples, 5 (62.5%) were positive with *Heterorhabditis* spp. and the other 3 (37.5%) constituted *Steinernema* spp. Josephrajkumar and Sivakumar (1997) in their study in Tamil Nadu reported the prevalence of steinernematids (94.44%) and heterorhabditids (5.55%). In contrast to this, Singh *et al*. (1992) reported a very low prevalence (1.82%) of *Steinernema* sp. at ICRISAT centre, Hyderabad. Kaushal *et al*. (2000) examined 207 soil samples from diverse areas of India (Uttar Pradesh, Himachal Pradesh, Gujarat), of these 17 (8.21%) were found EPN positive, and 10 (58.82%) comprised steinernematids, while 7 (41.18%) samples constituted heterorhabditids. Diversity among the species of *Oscheius* could not be compared due to lack of information but it may be assumed that diversity vary considerably with habitat, area and the number of individuals.

Identification of EPNs

Among the six isolated EPNs, one isolate have been identified as *Heterorhabditis* sp., two as *Steinernema* spp. and three as *Oscheius* spp. on the basis of morphological characteristics. These newly isolated EPNs have been designated as *Oscheius* sp. (IARI-EPN RP 01), *Oscheius* sp. (IARI-EPN RP 02), *Steinernema* sp. (IARI-EPN RP 03), *Oscheius* sp. (IARI-EPN RP 04), *Steinernema* sp. (IARI-EPN RP 05) and *Heterorhabditis* sp. (IARI-EPN RP 06) (Table 1).

Screening of EPNs against lepidopteran insect pests

All the test EPNs were found pathogenic against *Maruca vitrata, Pieris brassicae* and *Spodoptera litura* but the time of mortality was varying. Among the tested EPNs, *Steinernema* sp. (IARI-EPN 03), *Oscheius* sp. (IARI-EPN 04) and *Heterorhabditis* sp. (IARI-EPN RP 06) were found to be promising against *M. vitrata* as they showed 100% mortality within 48 h followed by *Oscheius* sp. (IARI-EPN RP 01), *Oscheius* sp. (IARI-EPN RP 02) which took 72 h, while *Steinernema* sp. (IARI-EPN RP 05) took about 120 h to kill the insects. In case of *P. brassicae*, *Oscheius* sp. (IARI-EPN RP 02), *Steinernema* sp. (IARI-EPN RP 03) and *Heterorhabditis* sp. (IARI-EPN RP 06) gave 100% mortality within 48 h followed by *Oscheius* sp. (IARI-EPN RP 04), *Steinernema* sp. (IARI-EPN 05) that killed larvae after 72 h and *Oscheius* sp. (IARI-EPN RP 01) took about 120 h to kill

Table 1. Soil sample analysis for detection of entomopathogenic nematodes

Districts	Blocks	GPS		Soil Profile		No. of Samples	Positive Samples	EPN Baited out	Name of EPN
		Altitude (ft)	Latitude; Longitude	pH	Soil texture				
Hapur	Hapur Kshetra	702	77° 41' E 28° 49' N	7.2	Sandy loam	13	-	-	
	Dhaulana	710	77° 39' E 28° 37' N	6.9	Loam	24	02	IARI-EPN RP 02 IARI-EPN RP 06	*Oscheius* sp. *Heterorhabditis* sp.
	Garhmukteshwar	716	78° 04' E 28° 56' N	7.1	Sandy loam	17	01	IARI-EPN RP 01	*Oscheius* sp.
Shamli	Shamli	799	81° 18' E 29° 26' N	7.8	Sandy loam	15	01	IARI-EPN RP 04	*Oscheius* sp.
	Kairana	778	81° 07' E 29° 37' N	5.6	Clay loam	15	01	IARI-EPN RP 05	*Steinernema* sp.
Saharanpur	Saharanpur	912	77° 33' E 29° 58' N	6.8	Sandy	18	01	IARI-EPN RP 03	*Steinernema* sp.
	Deoband	842	77° 40' E 29° 41' N	5.4	Clay loam	10	-	-	
Meerut	Meerut	747	78° 42' E 28° 59' N	6.2	Silt loam	15	-	-	
Total						127	06		

the insect. Lastly, *Steinernema* sp. (IARI-EPN RP 03) and *Heterorhabditis* sp. (IARI-EPN RP 06) were found promising for *S. litura* as both of them caused 100% mortality within 48 h followed by *Oscheius* sp. (IARI-EPN RP 01), *Oscheius* sp. (IARI-EPN RP 02) and *Steinernema* sp. (IARI-EPN RP 05) that took 72 h, while, *Oscheius* sp. (IARI-EPN RP 04) was effective at about 120 h (Fig. 1).

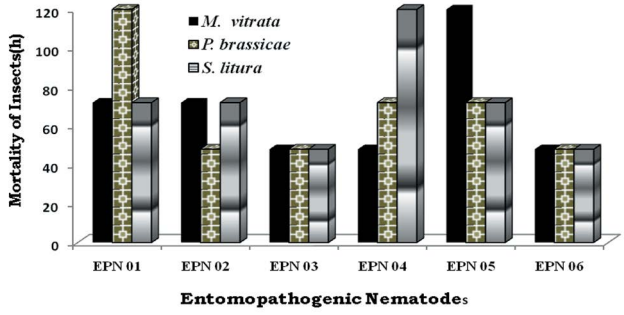

Fig. 1. Mortality of lepidopteran insect pests through entomopathogenic nematodes [EPN 01 - *Oscheius* sp. (IARI-EPN RP 01); EPN 02 - *Oscheius* sp. (IARI-EPN RP 02); EPN 03 - *Steinernema* sp. (IARI-EPN RP 03); EPN 04 - *Oscheius* sp. (IARI-EPN RP 04); EPN 05 - *Steinernema* sp. (IARI-EPN RP 05); EPN 06 - *Heterorhabditis* sp. (IARI-EPN RP 06)]

Screening of EPNs infectivity can be an important component of developing a biological control programme for a particular pest (Ricci *et al*., 1996). In past, one of the basic reasons for failure of EPNs for biological control of insect pests is because of the wrong choice of nematode species or strain. Efficacy studies have shown considerable inter and intra specific variations in infectivity of different isolates of EPNs (Menti *et al*., 2000; Pervez *et al*., 2012) which have been attributed to the variation in the ability of the IJ to find and enter a host (Sankaranarayanan *et al*., 2011) as well as the different host susceptibility among various insects (Ali *et al*., 2008; Pervez *et al*., 2012) or insect stages (Premchandra *et al*., 2007; Pervez, 2010). However, there is considerable variation in the infectivity of EPNs and no single species or strain is suitable for controlling all or even most insect species (Simoes and Rosa, 1996).

CONCLUSIONS

Our survey revealed that *Oscheius* spp. and *Steinernema* spp. occur widely. These indigenous strains will be suitable for managing the insect pests. The results of this survey extend the knowledge on EPNs from Hapur, Shamli, Saharanpur and Meerut districts of western Uttar Pradesh. The present results indicated that *Steinernema* sp. (IARI-EPN 03) and *Heterorhabditis* sp. (IARI-EPN 06) were highly virulent against the tested lepidopteran insect pests. Therefore, further evaluation of these new EPN isolates undere field conditions will indicate their utility in integrated pest management.

ACKNOWLEDGEMENTS

The authors express their gratitude to Director and Joint Director (Research), ICAR-Indian Agricultural Research Institute, New Delhi, India, for providing all the facilities.

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