Available online at www.aappbckv.org

The Journal of Plant Protection Sciences, **6**(1) : 13-20, June, 2014

Characterization of entomopathogenic nematode, *Steinernema carpocapsae* from ginger (*Zingiber officinale* Rosc.) rhizosphere in India

Rashid Pervez, Santhosh J. Eapen, S. Devasahayam and M. Dinsha

Division of Crop Protection, Indian Institute of Spices Research, Kozhikode (Kerala) - 673 012

E-mail:rashid_pervez@rediffmail.com

(Received: 16 January 2014; Accepted: 5 May 2014)

A B S T R A C T

During a random survey, one isolate of entomopathogenic nematode from ginger rhizosphere was collected from Faizabad district of Uttar Pradesh (India). Morphological and morphometric studies identified the isolate as *Steinernema carpocapsae*. This was further confirmed by ITS-rDNA sequences analysis. Phylogenetic was constructed for studying relationship with known isolates. Pathogenic potential of the isolate of *S. carpocapsae* (IISR-EPN 06) on the larva of shoot borer (*Conogethes punctiferalis*), hairy caterpillar (*Euproctis* sp.) and greater wax moth (*Galleria mellonella*) was found under *in vitro* condition. Further this isolate displayed high virulence on above insect species. This study reported occurrence of a isolate of *S. carpocapsae* from ginger rhizosphere from India. This indigenous isolate could be investigated further for managing insect pests of ginger.

Keywords: Entomopathogenic nematodes, *Steinernema carpocapsae*, ginger, pathogenicity

Introduction

Ginger (*Zingiber officinale* Rosc.) production in India is sustained losses due to several reasons. Among them, one of the major constraints is insect pests, which causes significant yield losses (Devasahayam *et al*. 2012). The only effective method to manage this pest is the use of insecticides resulted pesticide residues in the produce affecting human health and also causing other ecological hazards. There has been a renewed interest in developing environment- friendly pest management schedules in agriculture. Entomopathogenic nematodes (EPNs) have received little attention by researchers though they have a great potential in reducing pest population, their role can be enhanced by little manipulation (Ali *et al*. 2005; Lorio *et al*. 2005). The biocontrol potential of EPNs can be exploited by isolating native EPNs tolerating local climatic condition. Some achievements have been documented for biocontrol of pests from several parts of the world (Hatting *et al*. 2009). Previous surveys revealed natural occurrence of several species/isolates of *Steinernema* and *Heterorhabditis* in Andman and Nicobar islands (Prasad *et al*. 2001), Gujarat (Vyas 2003), Kerala (Banu *et al*. 2004; 2005), New Delhi (Ganguly & Singh 2000), Tamil Nadu (Josephrajkumar & Sivakumar 1997), Meghalaya (Lalramliana & Yadav 2010) and Uttar Pradesh (Pervez & Ali 2007) of India. The study was carried out to identify of EPN isolated from ginger rhizosphere and to evaluate pathogenicity on larva of shoot borer, *Conogethes punctiferalis* (SBL), hairy caterpillar, *Euproctis* sp. (HCL) and greater wax moth, *Galleria mellonella* (GWML).

Materials and Methods

Collection of soil samples for detection of EPN

Soil samples were collected from ginger rhizosphere from Kumarganj (GPS $81^0 50^{'}$E $26^0 32^{'}$N), Faizabad districts (Uttar Pradesh). About 1 kg of soil sample was collected at a depth of 10-20cm using a hand trowel, each sample containing a composite from five random subsamples. Samples were placed in polyethylene bags to minimize dehydration, tag a label providing all necessary information and transported in to the laboratory. The hand trowel was sterilized by 70% ethanol before leaving the sampling site.

Extraction of EPN

EPN isolated from the soil using the insect baiting technique (Bedding & Akhurst 1975). About 250 g composite soil was placed in a plastic container and ten live greater wax-moth larva (GWML) used to bait the EPN. The soil sample was checked every day for 7 days for the presence of EPNs. On inspection of any dead GWML, they were placed in modified white trap (White 1927) for 2 weeks at room temperature (29±2 ^{0}C) for emergence of EPN. In case of the negative results, the isolation process was repeated two times for confirmation of the result. Emerged infective juveniles (IJs) were collected from White traps and used for infection on fresh GWML for production of EPN cultures.

Maintenance of EPN and insect cultures:

EPN cultured and maintained as per the procedure described by Kaya & Stock (1997). The IJs were surface sterilized in 0.1% Hyamine solution (Hussaini *et al.* 2000) and stored in distilled water in tissue culture flasks for identification. However, fresh nematode culture was used for bioassay studies. GWML reared on artificial diet as per the procedure described by David & Kurup (1988), while HCL and SBL procured from ginger fields of Indian Institute of Spices Research, Experimental Farm and farmers' field, district Kozhikode of Kerala. The larvae were sorted out and 3rd instar larva were used for the bioassay study.

Morphological characterization

Morphological and morphometric studies carried out based on 10 IJs and 10 first generation males (Hominick *et al*. 1997; Stock *et al*. 2000). Nematodes fixed and processed to dehydration following the method described by Seinhorst (1966). EPN isolated from the ginger rhizosphere was characterized and placed into similar species-groups using taxonomic criteria suggested by Stock & Kaya (1996) and Hominick *et al.* (1997).

Molecular characterization

Nematode-DNA was extracted following the protocol of Pastrick *et al*. (1995). Primers 18S (5' TTGATTACGTCCCTGCCCTTT 3') and 26S (5' TTTCACTCGCCG TTACTAAGG 3') as detailed by Vrain *et al*. (1992) were used for amplification of the ITS region of rDNA. After electrophoresis, the amplified products excised from 1% TAE buffered agarose gel using a QIA-quick PCR purification kit (QIAGEN), cloned and purified DNA se-

quenced at Xleres Biotechnology Pvt. Ltd., Bangalore.

Phylogenetic analysis

Phylogenetic tree was constructed with a Maximum Likelihood method (MLM). The evolutionary history was inferred by using the MLM based on the Tamura-Nei model (Tamura & Nei 1993). The tree with the highest log likelihood(-1022.2340) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree (s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The analysis involved 13 nucleotide sequences. Codon positions included were 1st+2nd+3rd+ Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 319 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 (Tamura *et al*. 2013).

Entomopathogenicity

Pathogenicity of *S. carpocapsae* (IISR-EPN 06) on SBL, HCL and GWML was tested in Petriplate. For this, ten larva of tested insect were kept in Petriplate and 100 IJs of *S. carpocapsae* (IISR-EPN 06) were inoculated and their mortality was recorded at 24h interval. The experiment was conducted at room temperature (33 ± 2^0C) and replicated ten times along with control. The mortality was calculated using following formula (Pervez & Ali 2009):

$$\text{Mortality (\%)} = D \times 100 / N$$

Where, D- Number of dead larvae; N – Total number of larvae

Results

Morphological characterization

Morphological characters of *Steinernema* sp. resembles that of "*carpocapsae"* group. Key diagnostic traits of the third-stage IJs and males were similar to a member of "*carpocapsae*" group. First generation males have small mucro at the end of the tail, yellow spicules with prominent capitulum, rostrum, vellum and a pointed terminus. The total length (infective juveniles (IJ) - 598.5µm & first generation male (FGM) - 1097 µm); width (IJ- 26.17 µm and FGM - 89.23 µm); EP (IJ- 43.71 & FGM- 61.69%), NR (IJ- 73.8 & FGM- 114.4%), ES (IJ- 131.8 & FGM- 141.6%); D (IJ- 33.1 & FGM- 44.4%); E (IJ- 82.1 & FGM- 217.7%) and tail length (IJ- 53.3 µm & FGM- 29.1 µm) observed were within the characteristics of the species (Nguyen & Smart 1995).

Molecular characterization

Nematode DNA sequence deposited in Gen Bank under accession number KM 212952 was compared to sequence of *Steinernema* spp. available in Gen Bank. The BLAST search indicated a 99% similarity in sequence of PCR product of our isolate with the isolates of *S. carpocapsae* available in the Genbank with accession number *viz*., KC571265,

GQ421605, HM140694, FJ860033, AY230164 and HQ406729. Other quite similar sequences were those of *S. eapokense* (AY487921) with 98%, *S. sasonense* (AY487919) with 97%, *S. siamkayai* (JN571085) and *S. tami* (AY171280) with 95% similarity. Some *Steinernema* spp. available in GenBank showed less than 95 % similarity.

Phylogenetic analysis

Phylogenetic analysis of ITS- rDNA sequence data placed this species in a clade with other isolates of *S. carpocapsae*. The ITS regions are much more variable and provide most of the base differences for species diagnosis (Nguyen *et al*. 2001). The isolates *S. carpocapsae* (IISR-EPN 06) aligned clearly and without gaps, with those of other *S. carpocapsae* isolates. In phylogram based on ITS sequences, *S. carpocapsae* (IISR-EPN 06) was close to *S. carpocapsae* (isolate IS 34) from Israel followed by *S. carpocapsae* (strain Caba 02) from Mexico (Fig. 1).

Entomopathogenicity

Results indicated *S. carpocapsae* (IISR-EPN 06) was found pathogenic to all tested insect pests; it caused cent percent mortality within 72h. The *S. carpocapsae* (IISR-EPN 06) isolate was found as quick killer; they start killing insect larvae within 24h. No mortality was found in control treatment (Fig. 2).

Discussion

The *S. carpocapsae* previously described as *Neoaplectana carpocapsae* from infected *Laspeyrasia pomonella* in Chehoslovakia (Weiser 1955) and later redescribed by Wouts *et al.* (1982). However, Karimi *et al.* (2010) summarized the occurrence of different isolates of *S. carpocapsae* across the world. PCR amplification of the ITS regions followed by DNA sequencing of the PCR product provided reliable data for diagnosing *Steinernema* species. However, the use of ITS region for species delimitation and phylogenetic reconstruction should be restricted to only those nucleotide positive for which character polarization and homology statements are robust. *S. carpocapsae* is well known species among the EPNs. The most important attributes include ease of mass production and ability to survive in a partially desiccated state allows them to store several months of room-temperature shelf-life. Rao & Manjunath (1966) used DD 136 (*S. carpocapsae*) for the control of insect pests of rice, sugarcane and apple. *S. carpocapsae* has been used against many insect pests like *Psendaletia separeta* and *Spodoptera litura* in pulses (Abdel-Razek *et al*. 2007; Pervez & Ali 2009), *Amsacta albisting* in groundnut (Bhaskaran *et al*. 1994), *Agrotis ipsilon* and *A. segatum* (Hussaini *et al*. 2000), *Athalia proxima* in mustard (Pervez *et al*. 2007), *Helicoverpa armigera* in pigeonpea and chickpea (Ali *et al*. 2008), and *Maruca vitrata* in pulses (Pervez 2012).

Our study revealed an EPN species identified as *S. carpocapsae* based on morphometrics and molecular characterization from ginger rhizosphere. The EPN isolate is capable of

killing *G. mellonella*, *C. punctiferalis* and *Euproctis* sp. within 24-72h under laboratory conditions. This indigenous strain could be investigated further for the control insect pests of ginger in the similar ecological conditions of India.

Acknowledgement

The authors express their gratitude to Director, Indian Institute of Spices Research(IISR), Kozhikode for providing all the facilities. We are also thankful to Bioinformatics Centre, IISR, Kozhikode, India for necessary help.

Literature Cited

Abdel-Razek AS Abd-Elgawad MM. 2007 Investigations on the efficacy of entomopathogenic nematodes against *Spodoptera littoralis* (Biosd.) and *Galleria mellonella* (L.). *Archives of Phytopathology and Plant Protection* **40** (6): 414–22.

Ali SS Ahmad R Hussain M A Pervez R 2005 *Pest management of pulses through entomopathogenic nematodes.* Indian Institute of Pulses Research, Kanpur, Army press, Lucknow (India), 59pp.

Ali SS Pervez R Hussain MA Ahmad R. 2008 Susceptibility of three lepidopteran pest to five entomopathogenic nematodes and *in vivo* mass production of these nematodes. *Archieves of Phytopathology and Plant Protection* **41**(4): 300– 04.

Banu GJ Nguyen KB Rajendran G. 2005 Occurrence and distribution of entomopathogenic nematodes in Kerala, India. *International Journal of Nematology* **15**(1): 9–16.

Banu GJ Subahasan K Iyer R. 2004 Occurrence and distribution of entomopathogenic nematodes in white grub endemic areas of Kerala. *Journal of Plantation Crops* **32**: 333–34.

Bedding RA Akhurst RJ. 1975 A simple technique for the detection of insect parasitic rhabditid nematodes in soil. *Nematologica* **21**: 109–10.

Bhaskaran RKM Sivakumar CV Venugopal MS. 1994 Biocontrol potential of entomopathogenic nematode in controlling red hairy caterpillar, *Amsacta albistriga* (Lepidoptera: Arctiidae) of groundnut. *Indian Journal of Agricultural Sciences* **64**: 655–57.

David H Kurup NK. 1988 Biocontrol Technology for Sugarcane Pest Management, In: *Techniques for mass production of Sturmiopsis inferens Tns.* (Eds David H Easwaramoorthy E) Sugarcane Breeding Institute, Coimbatore, India, 87–92pp.

Devasahayam S Eapen SJ Jacob TK Pervez R. 2012 Zingiberaceous crops (present and future cardamom, ginger, turmeric and others). In: *Pests* (Singh HP Parthasarthy VP Kandianan K Krishnamurthy KS) Westville publishing house, New Delhi, 332–47pp.

Ganguly S Singh LK. 2000 Entomopathogenic nematodes distributed in Delhi and adjoining areas. In: *Proceeding of National Congress on Centenary of Nematology in India, Appraisal and future plans*. IARI, New Delhi, 118 pp.

Hatting J Stock SP Hazir S. 2009 Diversity and distribution of entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) in South Africa. *Journal of Invertebrate Pathology* **102**: 120–28.

Hominick WM Briscoe BR Garcı´a del Pino F Heng J Hunt D J Kozodoy E Mra´cˇek Z Nguyen KB Reid AP Spiridonov S Stock SP Sturhan D Waturu C Yoshida M. 1997 Biosystematics of entomopathogenic nematodes: currents status, protocols and definitions. *Journal of Helminthology* **71**: 271–98.

Hussaini SS Singh SP Parthasarathy R Shakeela V. 2000 Virulence of native entomopathogenic nematodes against black cutworms, *Agrotis ipsilon* (Hufnagel) and *A. segetum* (Noctuidae : Lepidoptera), *Indian Journal of Nematology* **30** (1): 103–05.

Josephrajkumar A Sivakumar CV. 1997 A survey for entomopathogenic nematodes in Kanyakumari district, Tamil Nadu, India. *Indian Journal of Entomology* **59**: 45–50.

Karimi J Kharazi-Pakdel A Yoshiga T Koohi-Habibi M. 2010 Introduction of *Steinernema carpocapsae* Weiser, 1955 (Rhabditida: Steinernematidae) from natural population of white grub, *Polyphylla olivieri* (Coleoptera: Melolonthidae) from Iran. *Journal of Agricultural Faculty of Uludag University* **24** (1): 47– 54.

Kaya HK Stock SP. 1997 Manual of techniques in insect pathology. In: *Techniques in insect nematology* (Ed Lacey LA) Academic Press, San Diego, CA., 281–24 pp.

Lalramliana Yadav AK. 2010 Occurrence of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) in Meghalaya, NE India. *Science Vision* **10** (3): 89–100.

Lorío LU Mora M Stock SP. 2005 First record of entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) in Costa Rica. *Journal of Invertebrate Pathology* **88:** 226–231.

Nguyen KB Smart G C Jr. 1995 Morphometrics of infective juveniles of *Steinernema* spp. and *Heterorhabditis bacteriophora* (Nemata: Rhabditida). *Journal of Nematology* **27**(2): 206–12.

Nguyen KB Maruniak J Adams BJ. 2001 Diagnostic and phylogenetic utility of the rDNA internal transcribed spacer sequences of *Steinernema*. *Journal of Nematology* **33** (2-3): 73–82.

Pastrik KH Rumpenhorst HJ Burgermeister W. 1995 Random amplified polymorphic DNA analysis of a Globodera pallida population selected for virulence. *Fundamental and Applied Nematology* **18**: 109–14.

Pervez R. 2012 Efficacy of entomopathogenic nematodes against legume pod borer, *Maruca vitrata* Fabricius (Lepidoptera: Pyralidae). *Current Nematology* **23** (1&2): 17–21.

Pervez R Ali SS. 2007 Natural occurrence of entomopathogenic nematodes associated with chickpea ecosystem. *Current Nematology* **18**(2): 19–22.

Pervez R Ali S S. 2009 Infectivity of *Spodoptera litura* (F.) (Lepidoptera: Noctuidae) by certain native entomopathogenic nematodes and their penetration in test insect and *in vivo* production. *Trends in Biosciences* **2** (2): 70–73.

Pervez R Ali SS Ahmad R. 2007 Efficacy of some entomopathogenic nematodes against mustard saw fly and *in vivo* production of these nematodes. *International Journal of Nematology* **17** (1): 55–58.

Prasad GS Ranganath HR Singh PK. 2001 Occurrence of the entomopathogenic nematode in parts of south Andamans. *Current Science* **80** (4): 501–02.

Rao VP Manjunath TM. 1966 DD-136 nematode that can kill many insect pests. *Indian Journal of Entomology* **33**: 215–17.

Steinhorst JW. 1966 Killing nematodes for taxonomic study with hot FA 4:1. *Nematologica* **12**: 178.

Stock SP Kaya HK. 1996 A multivariate analysis of morphometric characters of *Heterorhabditis* species and the role of morphometrics in the taxonomy of the species of the genus. *Journal of Parasitology* **82**: 806–13.

Stock S P Mracek Z Webster M. 2000 Morphological variation between allopatric populations of *Steinernema kraussei* (Steiner, 1923) (Rhabditida: Steinernematidae). *Nematology* **2**: 143–52.

Tamura K Nei M. 1993 Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution* **10**: 512–26.

Tamura K Stecher G Peterson D Filipski A Kumar S. 2013 MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* **30**: 2725–29.

Vrain TC Wakarchuk DA Lévesque AC Hamilton RI. 1992 Intraspecific rDNA restriction fragment length polymorphism in the *Xiphinema americanum* group. *Fundamental and Applied Nematology* **15** (6): 563–73.

Vyas RV. 2003 Current Status of Research on Entomopathogenic nematodes in India., In: *Entomopathogenic nematodes- a new tool for management of insect pests of crops* (Hussaini SS Rabindra RJ Nagesh M) Project Directorate of Biological Control, Bangalore, 69–108pp.

Weiser J. 1955 *Neoaplectana carpocapsae* n. sp. (Anguillulata, Steinernematidae) novy cizopasnic housenik obalece jablecneho, *Carpocapsa pomonella* L. *Vestnik Cesk*. Zoologicke *Spolecnosti* **19**: 44–52.

White GF. 1927 A method for obtaining infective nematode larvae from cultures. *Science* **66**: 302–03.

Wouts WM Mracek Z Gerdin S Bedding RA. 1982 *Neoaplectana* Steiner, 1929 a junior synonym of *Steinernema* Travassos, 1927 (Nematoda: Rhabditida). *Systematic Parasitology* **4** (2): 147–54.

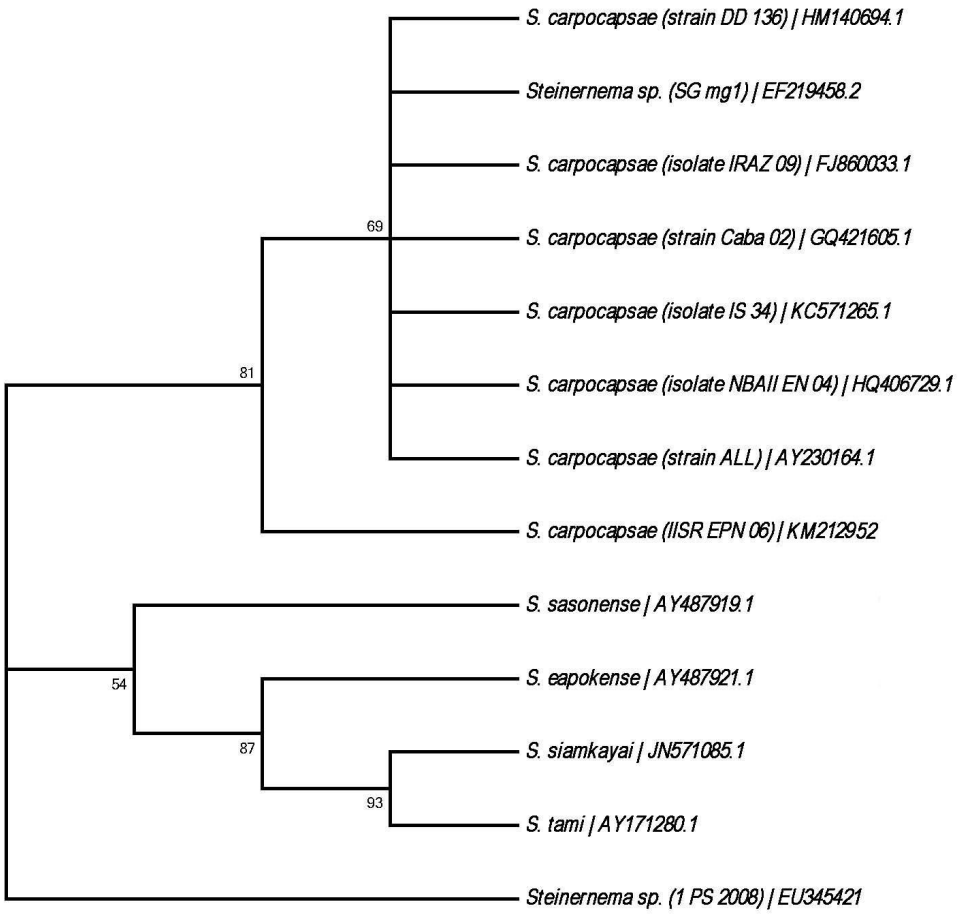

Fig 1. Phylogenetic relationship of *S. carpocapsae* (IISR-EPN 06) among closely related species based on the sequences of the ITS region.

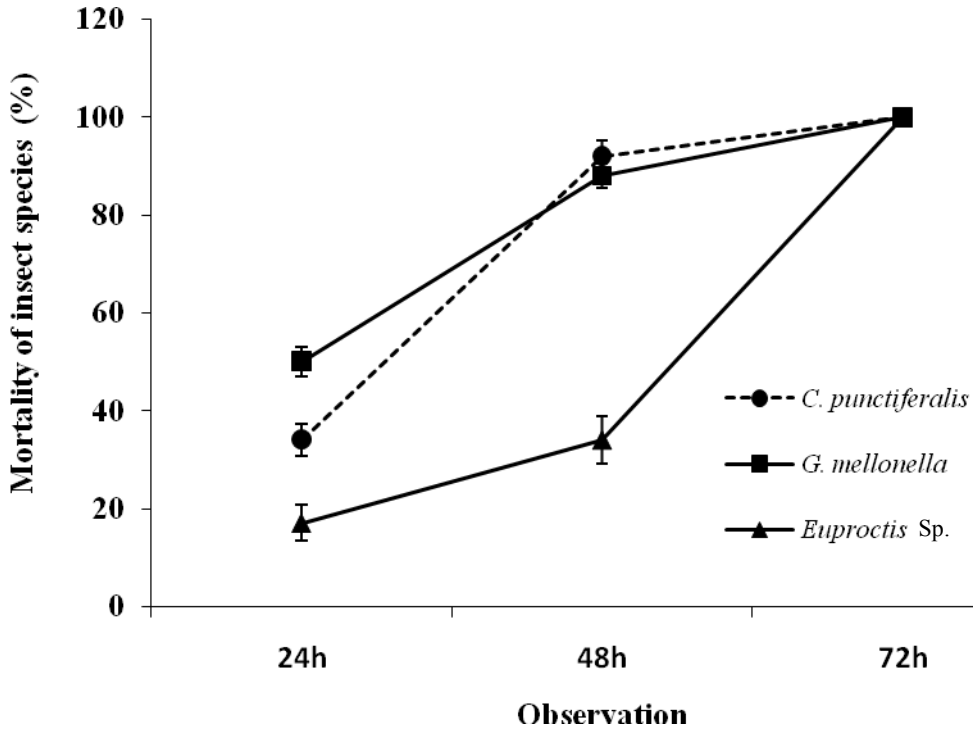

Fig 2. Insect mortality for *Steinernema carpocapsae* (IISR-EPN 06)