# ISOLATION AND AUTHENTICATION OF ENTOMOPATHOGENIC NEMATODES FROM ALLAHABAD REGION

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**Abstract:** Random surveys were carried out for the detection of entomopathogenic nematodes from cultivated areas in different villages of Allahabad district, Uttar Pradesh, India. A total of 60 soil samples were processed for baiting using larvae of cabbage semi-looper, *Thysanopulsia orichalcea* (Lepidoptera: Noctuidae). Of these, only ten soil samples (16.67%) yielded EPNs. *Heterorhabditis sp.* was yielded from six soil samples (10%) collected from different villages, while *Steinernema sp*. was yielded from four samples (6.7%).However, no EPNs were recovered from soil samples of twelve villages. The nematodes were recovered from sandy, sandy loam and alluvial soils with soil pH ranging from 6.50 to 8.00. The isolated entomopathogenic nematodes were found to be *Steinernema sp*. and *Heterorhabditis sp*. The bioassay of the isolated EPNs was studied under laboratory condition on *Corcyra cephalonica* larvae in different inoculums level of 50, 100, 150, 200 and 250 IJs/ml. After 120 hours of inoculation the % mortality of the test insect with *Steinernema sp*. was found to be 97.5% with 250 IJs/ml while that of *Heterorhabditis sp*. was found to be 100%. And also the % net mortality after 120 hours of inoculation with 250 IJs/ml of *Steinernema sp.* was found to be 79.6% whereas that of *Heterorhabditis sp.* was found to be 81.6%. Hence it was found out that the dose mortality response on the test insect with isolated *Heterorhabditis sp*. was observed to be more effective than that of *Steinernema sp.*

**Keywords:** EPN, *Heterorhabditis sp*., *Steinernema sp*., *Corcyra cephalonica*

## INTRODUCTION

The insect pests are one of the major limiting factors in agricultural production. To overcome the situation of development of resistance, secondary outbreak of minor or unknown pests, pesticide persistence and residue problems, contamination of food water resources other effective and environmental friendly method i.e. the used of bio pesticides have been developed and are becoming increasingly important as pest management tools in various cropping systems globally essentially to remedy problems associated with the indiscriminate use of hard inorganic pesticides and consumer-driven needs towards organic agriculture. During the recent past greater attention has been given to entomopathogenic nematodes to explore their bio control potential for the management of insect pests. Nematodes belonging to more than 30 families are known to parasitize insects but potential entomopathogenic nematodes belong to only two families, Steinernematidae and Heterorhabditidae. Entomopathogenic nematodes from these families possess wide host range and have been widely exploited for their bio control potential (Lacey *et al.*, 2001; Kaya and Gaugler, 1990). The impressive attributes of EPN have stimulated strong commercial interest in nematodes as biological insecticides. This includes their wide spectrum of insecticidal activity, ability to kill most hosts within short periods and efficient mass culturing techniques. (Kaya and Gaugler, 1993).EPNs have a great potential as biological control agents against insect pests of crops due to their wide host range, easy of handling, short life cycle, economically large scale production and environment safety (Cabanillas *et al.*, 1994; Poinar, 1990; Ali *et al.*, 2005) .They have the potential for long term establishment in soil through recycling on infected insects. All Steinernematids and Heterorhabditid infective juveniles carry symbiotic bacterial species belong to genus, *Xenorhabdus* and *Photorhabdus*, but are specific for each nematode species (Akhurst and Boemare, 1988; Poinar and Brooks, 1977; Thomas and Poinar, 1979). All the species of *Steinernema* are associated with bacteria of genus *Xenorhabdus* and all *Heterorhabditis* nematodes species are associated with genus *Photorhabdus*. These bacteria are released into the insect haemoceal causing septicaemia and death of the insect (Kaya and Gaugler, 1993). However, the insect mortality is determined by the nematode-insect complex and not simply by the virulence of the bacterium. They are unique because they are the only nematodes which are capable of carrying and introducing symbiotic bacteria into the body cavity of insects and thus killing them within 48 hours. The parasitic cycle of the nematodes is initiated by the 3$^{rd}$ stage infective juveniles (IJs). These non-feeding juveniles locate and invade suitable host insects through natural body openings (i.e. anus, mouth and spiracles) once they enter inside the host, the nematode invade the haemoceal and release the symbiotic bacteria that are held in the nematode intestine which cause septicaemia, killing the host within 24-48 hours. The bio control potential of these nematodes is well established in laboratory and also in field under favourable conditions. Performance of EPNs, however, varies greatly with the isolate, in addition to insect host and climate conditions. So, there is need to explore the native strains of EPNs which could perform well under given set of

environmental conditions and could provide a better and long lasting control. The present study deals with a preliminary work to isolate local population (Allahabad district, India) of a potential EPN and to examine its pathogenicity against laboratory host.

## MATERIAL AND METHOD

### Collection of soil samples

A survey was conducted from twenty different villages of Allahabad district, during October 2009 to February 2010 in which a total of sixty composite soil samples were collected. The information regarding the locality, soil type, habitat and raised/standing crop in the field was recorded. The soil samples were collected from the upper soil profile at a depth of 5-20 cm into polythene bags (500g).Each sample was than mixed thoroughly in each plastic container with some quantity of distilled water to maintain soil moisture of 15±1%.

### Soil baiting

Soil baiting was done by using the living instars larvae of cabbage semi-looper, *Thysanopulsia orichalcea* in the collected soil samples. To each soil samples, five insect larvae were released and the plastic containers were filled completely with moist and sterilized soil and were covered with muslin cloth. The moisture level in pots was maintained by adding sterilized water. Larval mortality was checked first after 24 hours. After five days of baiting all the larvae was found death. Dead insects were identified by lack of movement, lack of response to gentle prodding, colour change, growth of fungus on the dead insects etc. We have also observed that the dead insect does not pupate (Rishi *et al*., 2008; Hussaini *et al*., 2002).

### Nematode collection

The dead insect larvae were kept in harvesting container consisting of a small Petri dish (~6 cm) as a lid with a slightly larger diameter than the base and so it enable commercial filter disks to fit inside the larger Petri dish without raising or touching the lid of the larger petri dish. A filter paper was placed in the smaller petri dish. The dead insects were transferred to the paper and it's moistened just enough so that it was evenly wet, but not so much that there were no excess water for the nematodes to emerge .Into the base of the larger petri dish enough water was added so that the water touches all the sides of the dish .The lid of the smaller dish with the insect and paper was placed in the larger plate with the lid. After 24 hours, entomopathogenic nematodes were seen emerging out from the dead insects. They look like clumps of off white matter when seen through the naked eye. They are attracted towards the water and move to edge of small dish and finally to the larger dish (White, 1927).

### Preparation of Nematode suspension

Harvested infective juveniles were collected in a beaker and sterilized distilled water was added to make the nematode suspension. Ten ml of each of the samples were prepared by measuring in a measuring cylinder and 1ml from each samples were collected in the counting dish and the entomopathogenic nematodes were observed under a binocular and counting of nematode population was done .

### Rearing of rice grain moth (*Corcyra cephalonica*)

The conventional method of rearing was followed in the present investigation. In this method, rearing from egg to adult stage was undertaken using broken grain of sorghum in wooden rearing cage of 45x30x15 cm size; covered with wooden lid. Broken sorghum grains were first sterilized at 110°C for 2 hours in a hot air oven and used for mass rearing. The sterilized grains were mixed with dried yeast powder @2g/kg .0.001% streptomycin, pinch of sulphur dust and kept 2.5 kg of sorghum in each tray. At the beginning of rearing 1cc of *Corcyra cephalonica* eggs were sprinkled and kept for the development. The larvae feed on grains and pupated inside the tray itself. The moth started emerging from the $30^{th}$ day onwards, the emerged moth were collected and kept for egg lying in ovipositional cage provided with honey solution .The moth laid most of the eggs within 3days after emergence. The moth were collected daily and then transferred to the especially designed ovipositional cages. Moth emergence reduced after 100 days of initial infestation and boxes were reused after cleaning.

### Bioassay of EPNs against laboratory host

### Dose mortality response

The bioassay of isolated EPN was studied under laboratory condition on larvae of rice grain moth, *Corcyra cephalonica*. Different inoculums levels *viz* 50, 100, 150, 200 and 250 I Js/ml was prepared following serial dilution technique and were used in bioassay study. The prepared inoculum of EPNs was applied on filter paper kept in the petri dish and sufficient amount of water was applied to maintain the moisture of filter paper. Ten full grown larvae of *Corcyra cephalonica* were released into each petri plates. A control was taken for each inoculum where in the filter paper was treated only with distilled water and whole experiment was replicated four times. The mortality count of the larvae was recorded from 24 hours to 120 hours at 24 hours interval.

## RESULT AND DISCUSSION

### Survey for native isolates of EPNs

After the survey conducted we found out that out of sixty soil samples examined, entomopathogenic nematodes

were recovered from ten soil samples (16.67%) of total (Table 4).Four samples (6.7%)were found positive for the occurrence of Steinernematids at Baswar sample no. (BA 2) and (BA 3), Hadgan (HAD 1) and Ubhari (UB 2). Six samples (10%) were found positive with *Heterorhabditidis* at Balapur (BAL 1), Dandupur (DA 3), Jasra (JA 2), Maraoka (MA 2), Perpersa (PE 1) and (PE 2). No nematodes was recovered from twelve sampling sites *viz.*,Bhandra, Gahonia , Hadgani, Jhansi, Kheri , Karchana , Murlikot , Muhodipur , Mohabatganj , Shyamlalkapura , Umari and Udaidaskapura .

The nematodes were found at varying pH ranging from normal (6.50 to 7.60 pH) to slightly alkaline (8.00 pH) e.g., the *Heterorhabditis sp.* isolated from Dandupur. The *Heterorhabditis sp.* isolates were found in sandy loam and alluvial soils whereas, *Steinernema sp.* was found in sandy soil and alluvial soil, respectively. This may be due to various factors such as soil texture, moisture, temperature and host availability which is thought to be important in determining their distribution. Although these nematodes seem ubiquitous, Allahabad and its adjoining areas are virtually unexplored; therefore the objective of the present study was to survey the cultivated areas in Allahabad district and to isolate entomopathogenic nematodes from soil. From the experiment was found out that nematodes were found at varying pH ranging from normal (6.50 to 7.60). The *Heterorhabditis sp*. was found in sandy loam and alluvial soil whereas *Steinernema sp*. was found in sandy soil and alluvial soil.

**Table I:** Survey of entomopathogenic nematodes from different locations of Allahabad district

| Location | Sample code | No of positive samples | Vegetation | Soil pH | Soil type |
|---|---|---|---|---|---|
| Baswar | BA1 | Sample 1(-ve) | Orchard | 7.00 | Alluvial soil |
| | BA2 | Sample 2(+ve) | Orchard | 7.30 | Alluvial soil |
| | BA3 | Sample 3(+ve ) | Orchard | 7.40 | Alluvial soil |
| Bhandra | BH1 | Sample 1(-ve) | Cultivated land | 6.90 | Alluvial soil |
| | BH2 | Sample 2(-ve) | Cultivated land | 7.60 | Alluvial soil |
| | BH3 | Sample 3(-ve) | Cultivated land | 7.00 | Alluvial soil |
| Balapur | BAL1 | Sample 1(+ve) | Cultivated land | 7.20 | Sandy loam |
| | BAL2 | Sample 2(-ve) | Cultivated land | 6.50 | Sandy loam |
| | BAL3 | Sample 3(-ve) | Cultivated land | 7.42 | Sandy loam |
| Dandupur | DA1 | Sample 1(-ve) | Orchard | 7.30 | Alluvial soil |
| | DA2 | Sample 2(-ve) | Orchard | 7.50 | Alluvial soil |
| | DA3 | Sample 3(+ve) | Orchard | 8.00 | Alluvial soil |
| Gahonia | GA1 | Sample 1(-ve) | Cultivated land | 6.88 | Alluvial soil |
| | GA2 | Sample 2(-ve) | Cultivated land | 6.50 | Alluvial soil |
| | GA3 | Sample 3(-ve) | Cultivated land | 7.00 | Alluvial soil |
| Hadgani | HA1 | Sample 1(-ve) | Cultivated land | 7.50 | Sandy loam |
| | HA2 | Sample 2(-ve) | Cultivated land | 6.87 | Sandy loam |
| | HA3 | Sample 3(-ve) | Cultivated land | 6.55 | Sandy loam |
| Hadgan | HAD1 | Sample 1(+ve) | Orchard | 7.00 | Sandy soil |
| | HAD2 | Sample 2(-ve) | Orchard | 7.54 | Sandy soil |
| | HAD3 | Sample 3(-ve) | Orchard | 7.21 | Sandy soil |
| Jasra | JA1 | Sample 1(-ve) | Orchard | 6.75 | Alluvial soil |
| | JA2 | Sample 2(+ve) | Orchard | 6.50 | Alluvial soil |
| | JA3 | Sample 3(-ve) | Orchard | 6.00 | Alluvial soil |
| Jhansi | JH1 | Sample 1(-ve) | Orchard | 7.86 | Alluvial soil |
| | JH2 | Sample 2(-ve) | Orchard | 7.90 | Alluvial soil |
| | JH3 | Sample 3(-ve) | Orchard | 7.43 | Alluvial soil |
| Kheri | K1 | Sample 1(-ve) | Cultivated land | 6.30 | Alluvial soil |
| | K2 | Sample 2(-ve) | Cultivated land | 6.66 | Alluvial soil |
| | K3 | Sample 3(-ve) | Cultivated land | 6.80 | Alluvial soil |
| Karchana | KA1 | Sample 1(-ve) | Orchard | 7.83 | Alluvial soil |
| | KA2 | Sample 2(-ve) | Orchard | 7.00 | Alluvial soil |
| | KA3 | Sample 3(-ve) | Orchard | 7.00 | Alluvial soil |
| Murlikot | M1 | Sample 1(-ve) | Cultivated land | 6.56 | Alluvial soil |
| | M2 | Sample 2(-ve) | Cultivated land | 6.84 | Alluvial soil |
| | M3 | Sample 3(-ve) | Cultivated land | 6.70 | Alluvial soil |
| Muhodipur | MU1 | Sample 1(-ve) | Cultivated land | 7.00 | Alluvial soil |
| | MU2 | Sample 2(-ve) | Cultivated land | 6.50 | Alluvial soil |

| | | | | | |
|---|---|---|---|---|---|
| | MU3 | Sample 3(-ve) | Cultivated land | 6.00 | Alluvial soil |
| Maraoka | MA1 | Sample 1(-ve) | Cultivated land | 6.65 | Sandy loam |
| | MA2 | Sample 2(+ve) | Cultivated land | 6.50 | Sandy loam |
| | MA3 | Sample 3(-ve) | Cultivated land | 6.89 | Sandy loam |
| Mohabatganj | MO1 | Sample 1(-ve) | Cultivated land | 6.10 | Alluvial soil |
| | MO2 | Sample 2(-ve) | Cultivated land | 6.00 | Alluvial soil |
| | MO3 | Sample 3(-ve) | Cultivated land | 6.43 | Alluvial soil |
| Perpersa | PE1 | Sample 1(+ve) | Orchard | 7.00 | Sandy loam |
| | PE2 | Sample 2(+ve) | Orchard | 7.60 | Sandy loam |
| | PE3 | Sample 3(-ve) | Orchard | 7.40 | Sandy loam |
| Shyamlalkapura | SH1 | Sample 1(-ve) | Orchard | 8.10 | Alluvial soil |
| | SH2 | Sample 2(-ve) | Orchard | 8.00 | Alluvial soil |
| | SH3 | Sample 3(-ve) | Orchard | 7.71 | Alluvial soil |
| Ubhari | UB1 | Sample 1(-ve) | Orchard | 7.98 | Sandy soil |
| | UB2 | Sample 2(+ve) | Orchard | 7.50 | Sandy soil |
| | UB3 | Sample 3(-ve) | Orchard | 6.90 | Sandy soil |
| Umari | UM1 | Sample 1(-ve) | Cultivated land | 7.00 | Alluvial soil |
| | UM2 | Sample 2(-ve) | Cultivated land | 7.54 | Alluvial soil |
| | UM3 | Sample 3(-ve) | Cultivated land | 6.20 | Alluvial soil |
| Udaidaskapura | UD1 | Sample 1(-ve) | Cultivated land | 7.60 | Sandy loam |
| | UD2 | Sample 2(-ve) | Cultivated land | 7.00 | Sandy loam |
| | UD3 | Sample 3(-ve) | Cultivated land | 7.03 | Sandy loam |

**Identification of isolated EPNs**

The isolated entomopathogenic nematodes were studied for their morphological characters and camera Lucida diagrams were prepared however, species level confirmation is awaited. The measurements were taken by an ocular micrometer. The entomopathogenic nematodes isolated was found to be of the family Steinernematidae and Heterorhabditidis. The adult of *Steinernema sp*. were found with six lips partially fused, stroma short and wide, nerve ring encircling the isthmus, excretory pore anterior to nerve ring, oesophagus muscular with cylindrical procorpus, narrow isthmus, tail tip with a mucron. Females have amphidelphic, didelphic reflexed ovaries. Males have single testis reflexed at tip. Whereas the adults of *Heterorhabditis sp.* have six lips which are fused at the base, excretory pore posterior to nerve ring, nerve ring distinct and surrounding the isthmus in females and basal bulb in males, excretory pore always located anterior to nerve ring, long and acutely pointed tail. Females have didelphic, amphidelphic reflexed ovaries. Male have single testis and reflexed. The isolated nematode was identified as *Steinernema sp*. and *Heterorhabditis sp* .These morphological features were compared with the taxonomic reviews of entomopathogenic nematodes (Hominick *et al.*, 1997; Adams *et al.* 2002; Ganguly, 2006).

**Table II.** Comparative measurements of adult males and females of the Allahabad isolates and the original population of *Steinernema sp.* and *Heterorhabditis sp.*

| **Characters** | ***Steinernema sp*** | | ***Heterorhabditis sp.*** | |
|---|---|---|---|---|
| Body length (L) | 446 (398-495) | 558 ( 438-650) | 528(479- 573) | 588(512- 670) |
| Body width(W) | 11.3 (10.3-14.8) | 10.6 (9.1-11.2) | 20(19-22) | 23(18-34) |
| Oesophagus (ES) | 94.5 ( 80-107) | 120 (103-190) | 117(109-123) | 125(100-139) |
| Excretory pore (EP) | 35 (29-38) | 38( 30-60) | 101(93-109) | 98(83-112) |
| Tail (T) | 35.5 (31-41) | 53(46-61) | 98(88-107) | 103(87-110) |

(All the measurements are taken in mm)

**Bioassay of EPNs against laboratory host**

The filter paper method was used in the present investigation to record the mortality of EPNs on *Corcyra cephalonica* , the choice of the substrate has always been consider to relevant to the bioassay because of its effect on nematode seeking behaviour. A filter paper environment favour nematodes, which use an ambush strategy to find the host, whereas the species that are more active, should be more infective on sand and soil environment (Grewal *et al.*, 1994).

**Dose mortality response of Steinernema *sp*. against *Corcyra cephalonica***

It is clear from the data presented in Table no. III that the % mortality of *Corcyra cephalonica* with different inoculum levels of 50-250 IJs/ml of *Steinernema sp.* was found to be ranging from 2.5 to 97.5 % after 120 hours of inoculation. The data in Table no. III shows that net mortality of *Corcyra cephalonica* was found to be ranging from 1.0 to 79.6%. The lowest % net mortality of 1.0% was observed with 50 IJs/ml after 24 hours of inoculation whereas the highest net mortality of 79.6 % was observed with 250 IJs/ml after 120 hours of inoculation.

**Dose mortality response of *Heterorhabditis sp* against *Corcyra cephalonica***

It is clear from the data presented in Table no.IV shows that the % mortality of *Corcyra cephalonica* with different inoculum levels of 50-250 IJs/ml of *Heterorhabditis sp.* was found to be ranging from 2.5 to 100 % after 120 hours of inoculation. The data in Table no.IV shows that net mortality of *Corcyra cephalonica* was found to be ranging from 1.0 to 81.6%. The lowest % net mortality of 1.0 % was observed with 50 IJs/ml after 24 hours of inoculation whereas the highest net mortality of 81.6 % was observed with 250 IJs/ml after 120 hours of inoculation. Comparing the mortality response of both *Heterorhabditis sp*. and *Steinernema sp*. we found out that 100% mortality was observed with *Heterorhabditis sp.* In this study *Heterorhabditis sp.* was found to be more virulent against *Corcyra cephalonica*. Virulence of entomopathogenic nematode differs greatly among species and isolates. Ricci *et al.*, 1996 detected differences in virulence towards *C. cephalonica* when comparing *Steinernema sp*. and *Heterorhabditis sp*. in petri dish bioassays. The present study shows that there is increase in the nematodes inoculums level the insect mortality level also increases.

**Table III:** Efficacy of *Steinernema sp.* against *Corcyra cephalonica* with different inoculum levels after 24, 48, 72, 96 and 120 hours of inoculation.

| Exposure time | | Treatments ( No. of IJs / ml ) | | | | | |
|---|---|---|---|---|---|---|---|
| | | $T_0$ Control | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ |
| 24 hours | % mortality | 02.5 | 10.0 | 12.5 | 22.5 | 32.5 | 42.5 |
| | % net mortality | 01.0 | 04.1 | 05.1 | 09.1 | 13.1 | 17.2 |
| 48 hours | % mortality | 02.5 | 27.5 | 37.5 | 47.5 | 57.5 | 67.5 |
| | % net mortality | 01.0 | 11.1 | 15.2 | 19.2 | 23.3 | 27.3 |
| 72 hours | % mortality | 02.5 | 40.0 | 50.0 | 60.0 | 70.0 | 80.0 |
| | % net mortality | 01.0 | 16.2 | 20.2 | 24.3 | 28.3 | 32.3 |
| 96 hours | % mortality | 02.5 | 50.0 | 60.0 | 70.0 | 80.0 | 90.0 |
| | % net mortality | 01.0 | 40.8 | 49.0 | 57.2 | 65.3 | 73.5 |
| 120 hours | % mortality | 15.0 | 60.0 | 70.0 | 80.0 | 90.0 | 97.5 |
| | % net mortality | 04.1 | 49.0 | 57.2 | 65.3 | 73.5 | 79.6 |

**Treatments**

$T_0$ – Control
$T_1$ – 50 IJs/ml of *Steinernema sp.*
$T_2$ – 100 IJs/ml of *Steinernema sp.*
$T_3$ – 150 IJs/ml of *Steinernema sp.*
$T_4$ – 200 IJs/ml of *Steinernema sp.*
$T_5$ – 250 IJs/ml of *Steinernema sp.*

**Table IV:** Efficacy of *Heterorhabditis sp.* against *Corcyra cephalonica* with different inoculum levels after 24, 48, 72, 96 and 120 hours of inoculation.

| Exposure time | | Treatments ( No. of IJs / ml ) | | | | | |
|---|---|---|---|---|---|---|---|
| | | $T_0$ Control | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ |
| 24 hours | % mortality | 02.5 | 12.5 | 22.5 | 32.5 | 42.5 | 52.5 |
| | % net mortality | 01.0 | 05.1 | 09.1 | 13.1 | 17.2 | 21.2 |
| 48 hours | % mortality | 02.5 | 32.5 | 45.0 | 57.5 | 67.5 | 77.5 |
| | % net mortality | 01.0 | 13.1 | 18.2 | 23.2 | 27.3 | 31.3 |
| 72 hours | % mortality | 02.5 | 42.5 | 55.0 | 67.5 | 77.5 | 85.0 |
| | % net mortality | 01.0 | 17.2 | 22.2 | 27.3 | 31.3 | 34.4 |
| 96 hours | % mortality | 05.0 | 52.5 | 65.0 | 77.5 | 85.5 | 95.5 |
| | % net mortality | 04.1 | 42.9 | 53.1 | 63.3 | 69.4 | 77.6 |
| 120 hours | % mortality | 05.0 | 62.5 | 75.0 | 85.0 | 95.5 | 100.0 |
| | % net mortality | 04.1 | 46.9 | 61.2 | 69.4 | 77.6 | 81.6 |

**Treatments**
$T_0$ – Control
$T_1$ – 50 IJs/ml of *Heterorhabditis sp.*
$T_2$ – 100 IJs/ml of *Heterorhabditis sp.*
$T_3$ – 150 IJs/ml of *Heterorhabditis sp.*
$T_4$ – 200 IJs/ml of *Heterorhabditis sp.*
$T_5$ – 250 IJs/ml of *Heterorhabditis sp.*

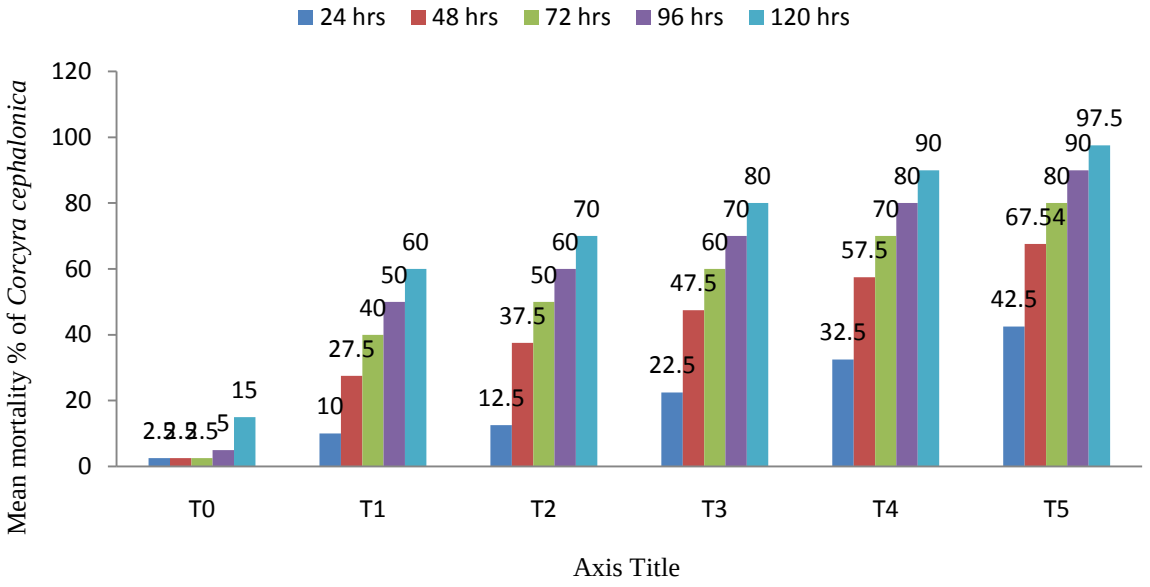


**Fig. I**: Mean mortality % of *Corcyra cephalonica* at different hours after inoculating with different inoculum levels of *Steinernema sp.*

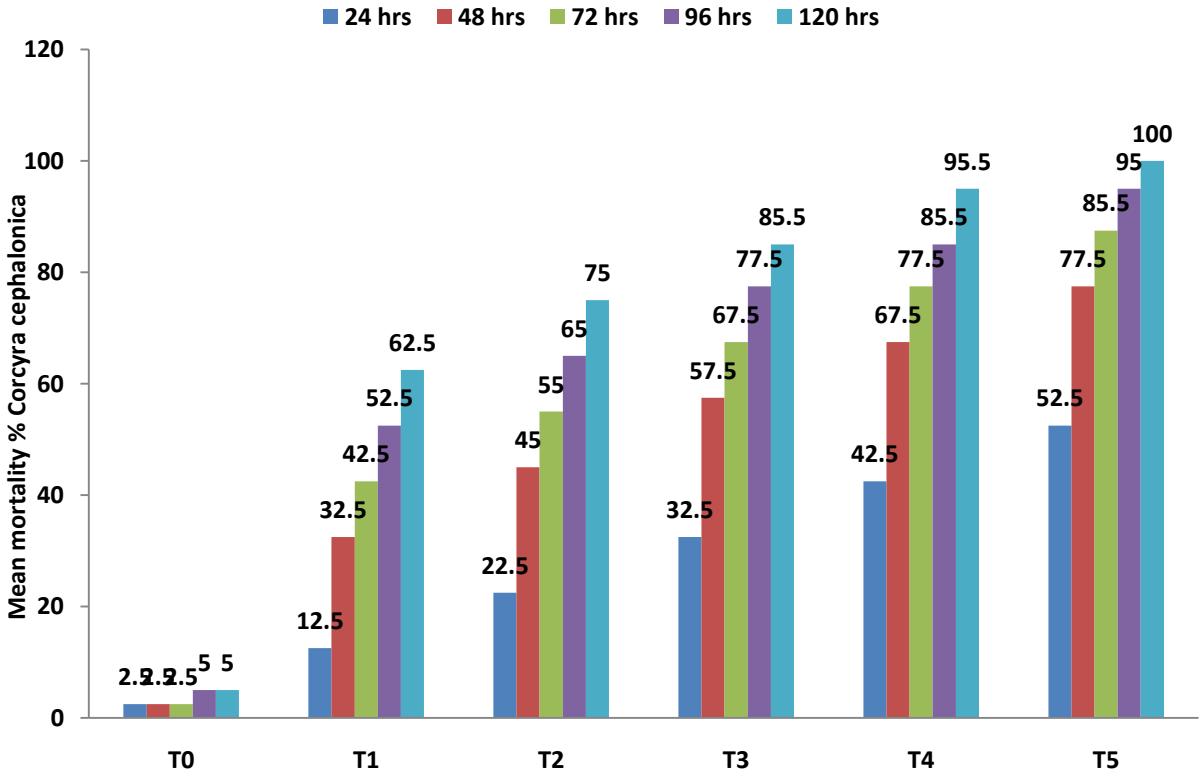


**Fig. II:** Mean mortality % of *Corcyra cephalonica* at different hours after inoculating with different inoculum levels of *Heterorhabditis sp.*

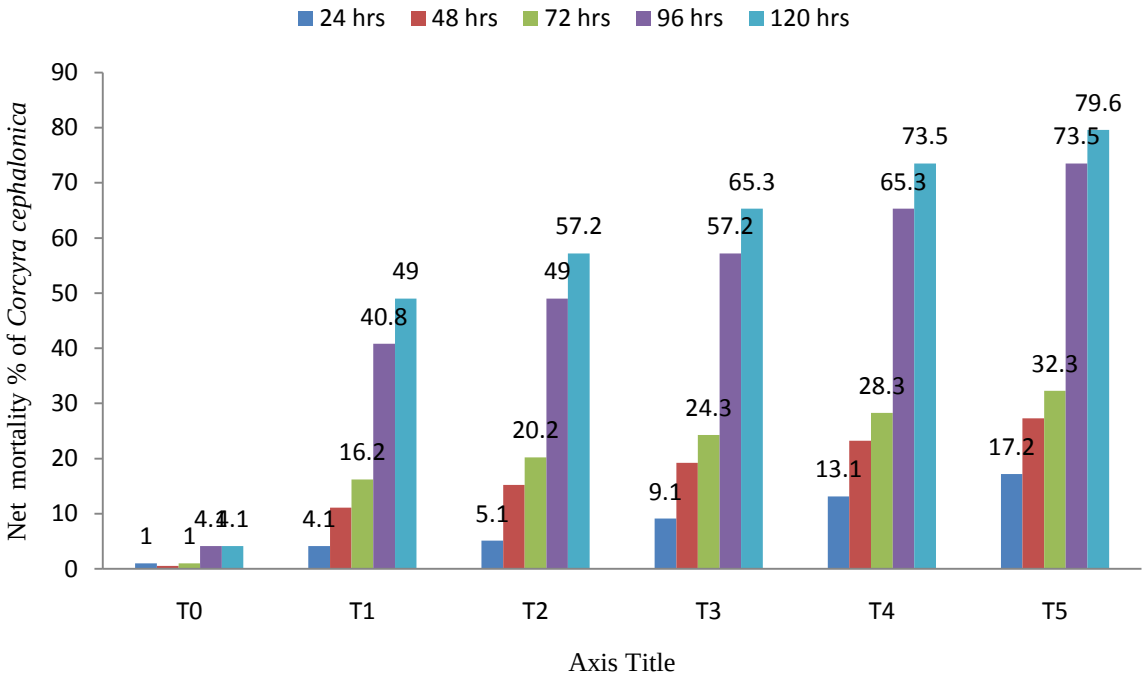


**Fig. III:** Net mortality % of *Corcyra cephalonica* at different hours after inoculating with different inoculum levels of *Steinernema sp.*

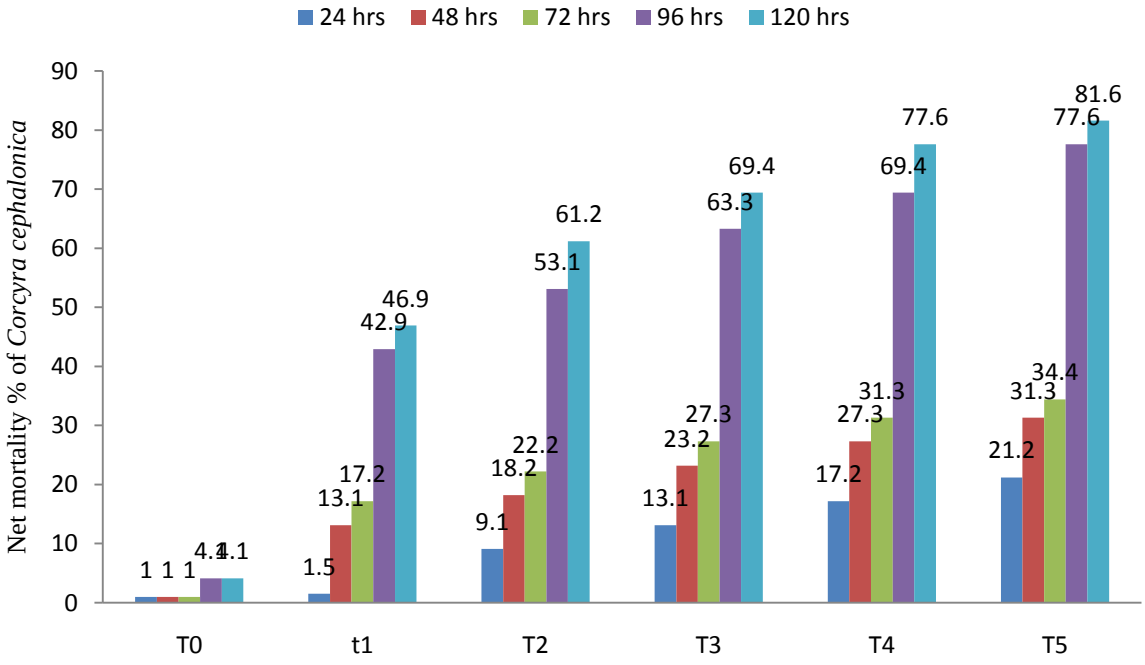


**Fig. IV:** Net mortality % of *Corcyra cephalonica* at different hours after inoculating with different inoculum levels of *Heterorhabditis sp.*

## CONCLUSION

The present study entitled "Isolation and Authentication of Entomopathogenic Nematodes from Allahabad region" was conducted in the laboratory of Department of Plant Protection, Sam Higginbottom Institute of Agriculture, Technology and Sciences, Allahabad, India.

It may be concluded that the use of naturally occurring nematodes as biological agents may also reduce the risk to non- target organisms.

Entomopathogenic nematodes characteristically have a wide host range in insect members of the order Lepidoptera and Coleoptera .In tropical country like India; Lepidopteran insect larvae are usually easily available for most of the year due to multiple and varied cropping sequences. The number of insect species susceptible to entomopathogenic nematodes seems unlimited. Hence, there is need to conduct extensive survey for isolation and identification of potential strains of entomopathogenic nematodes, which can be used for the management of crop pests.

## ACKNOWLWDGEMENT

The authors are grateful to Mr.Rajkaran and Mr.Mithailal, Department of Plant Protection, SHIATS, Allahabad for their help in collecting and processing soil samples and rearing of *Corcyra cephalonica.*

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