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Isolation and Microscopic Investigation of Entomopathogenic Nematodes (EPNs) Occurring in Barak Valley, Assam, India

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A B S T R A C T

Entotomopathogenic nematodes (*Steinernema* sp.) isolated from the soil samples in the Barak Valley of Assam, India. The nematodes were isolated by baiting technique using greater wax moth (*Galleria mellonella)*. Soil samples were collected during rabbi and monsoon season from the different locality of Barak Valley especially from crop fields, rubber plantation, pineapple garden, tea gardens and also from rhizospheric soil of bamboo bushes. Nematodes are isolated from rhizospheric soil of Bamboo and from crop field. Only on the basis of morphological characters isolates resembles with the *Steinernema* sp. Female having prominent vulva, anus, females juvenile bearing before emergence; males having prominent spicule and gubernaculum; juvenile with excretory pore anterior to nerve ring, tail is slightly curved and killed the insects larvae within 48 hours of infection. This is the first report for the occurrence of entomopathogenic nematodes from this region of Assam, India.

Introduction

Entomopathogenic nematodes are obligate lethal parasites of insect pests and thus use as effective bio-control agent in Integrated Pests Management. Due to successful use of these nematodes and non – pathogenic nature to other fauna, several surveys were conducted from decades to isolate the EPNs from different regions all over the world. Entomopathogenic nematodes (EPNs) in the family Steinernematidae (Chitwood and Chitwood) are insect parasites and capable of infecting a broad range of insect species. They have been used as biological control agents of insect pests in a variety of crops (Gaugler and Kaya, 1990; Kaya and Gaugler, 1993). The third stage juvenile is only the infective stage and they are free – living form present in soil. The IJ carry symbiotic bacteria of genus *Xenorhabdus* Poinar and Thomas. The IJ carries cells of the bacterial symbionts in its intestine. When the IJ finds a susceptible host, it invades and penetrates into the host's haemocoel through natural openings (i.e., anus, mouth, or spiracles). The IJ then releases the symbiotic bacterium that kills the host within 48 hr by septicemia. The bacterium produces antibiotics that prevent other microorganisms from colonizing the cadaver.

In addition to serving as a food source for the nematode, the bacterium digests the host tissues, thereby providing suitable nutrients for nematode growth and development.

The family Steinernematidae comprises of two genera, *Steinernema* Travassos 1927 with more than 59 described species and *Neosteinernema* Nguyen and Smart 1994, with only one species. The most updated bio-geographic account indicates that these nematodes have been isolated from all continents (except Antarctica). However, soil surveys conducted in different areas of the world have demonstrated variability in abundance across seasons, habitats, and geographic regions.

Materials and Methods

Propagation of isolates

The soil has been collected from different locality of Barak Valley, Assam. Soil samples are collected from different habitat includes vegetable fields, Tea garden, Pineapple garden, and Bamboo bush etc. Relatively shady and moist sites were chosen for sampling. Soil samples were collected at a depth of 10-15 cm of 27-37°C. The nematodes were propagated in last in-star larvae of greater wax moth, *Galleria mellonella* at 28-30◦C by baiting technique (Bedding and Akhurst, 1975). Emerging IJ was harvested in modified White traps following procedures described by Kaya and Stock (1997).The harvested nematodes are washed with distilled water two to three times. To maximize the recovery of nematodes, fresh *G. mellonella* larvae were used for second baiting round.

Light microscopy

For light microscopy, both infective juveniles (IJs) as well as adult nematodes are collected from insect cadaver. Adult males and females were collected by dissecting the 5^{th} instar larvae of *G. mellonella* after 2 DAI (Day After infection) as suggested by Nguyen and Smart (1995). On the other hand infective juveniles were obtained from insect cadaver by using white trap method (White, 1927) from insect cadaver 5 – 6 DAI. For light microscope observations, 20 IJS, 20 adults are examined alive. In order to study more morphological features nematodes were heat killed first at 50^0C in warm water. The heat killed nematodes were placed in triethanolamine formalin (TAF) fixative (Kaya and Stock, 1997) and processed to glycerine for mounting (Seinhorst, 1959). Type specimens are mounted in using glycerine. Cover glass supports were used to prevent the flattening of nematode specimens. Observations were made from live and mounted specimens using an Olympus CX 31microscope equipped with differential interference contrast optics.

Results and Discussion

The soil samples were collected from different habitat of Cachar district, Assam. The habitat includes A total of 170 soil samples were collected from different sites of Cachar district of which from only 4 sites the entomopathogenic nematodes were recovered. The percent recovery was 2.6 %.the present recovery was very low in comparison with a survey conducted by Hazir *et al.,* (2003) in Turkey. Other recovery includes 3.9% recovered by Rosa *et al.,* (2002) Northern Ireland and by Rajkumar *et al.,* (2002) in Udaipur with 3.3% etc. The soil type of positive sites was sandy and loamy with pH 5.5-5.9 and the temperature was 19^0C to 23^0C. All the recovery was done during rabbi season from rhizospheric soil of bamboo and from vegetable field. All the isolates from the family Steinernematidae of the genus *Steinernema* were recovered. Over the past few years the proper diagnosis is incomplete without morphometrics along with molecular

identification. So without this aspect the work is incomplete and has to overcome in this field of diagnosis in near future.

Family Steinernematidae

Diagnosis

Order Rhabditida, superfamily Alloionematoidea, family Steinernematidae, Typegenus: Steinernema Travassos, 1927. Obligate insect parasites of insects. Infective stage carry symbiotic bacteria in the bacterial pouch of the intestine. Both males and females are necessary for reproduction.

Adult female

Adult females are larger in size in comparison to adult male. Body cuticle smooth. Esophagus with procorpus cylindrical, muscular; meta-corpus swollen; isthmus distinct. Excretory pore is anterior to nerve ring with opposed reflexed ovaries. Vulva with lips at the mid region of the body (Fig. 1).

Fig.1 Microscopic pictures of adult female: [a] anterior body part showing excretory pore and oesophageal bulb, [b] vulva, [c] posterior body part with anus, [d] adult female containing IJs inside the body

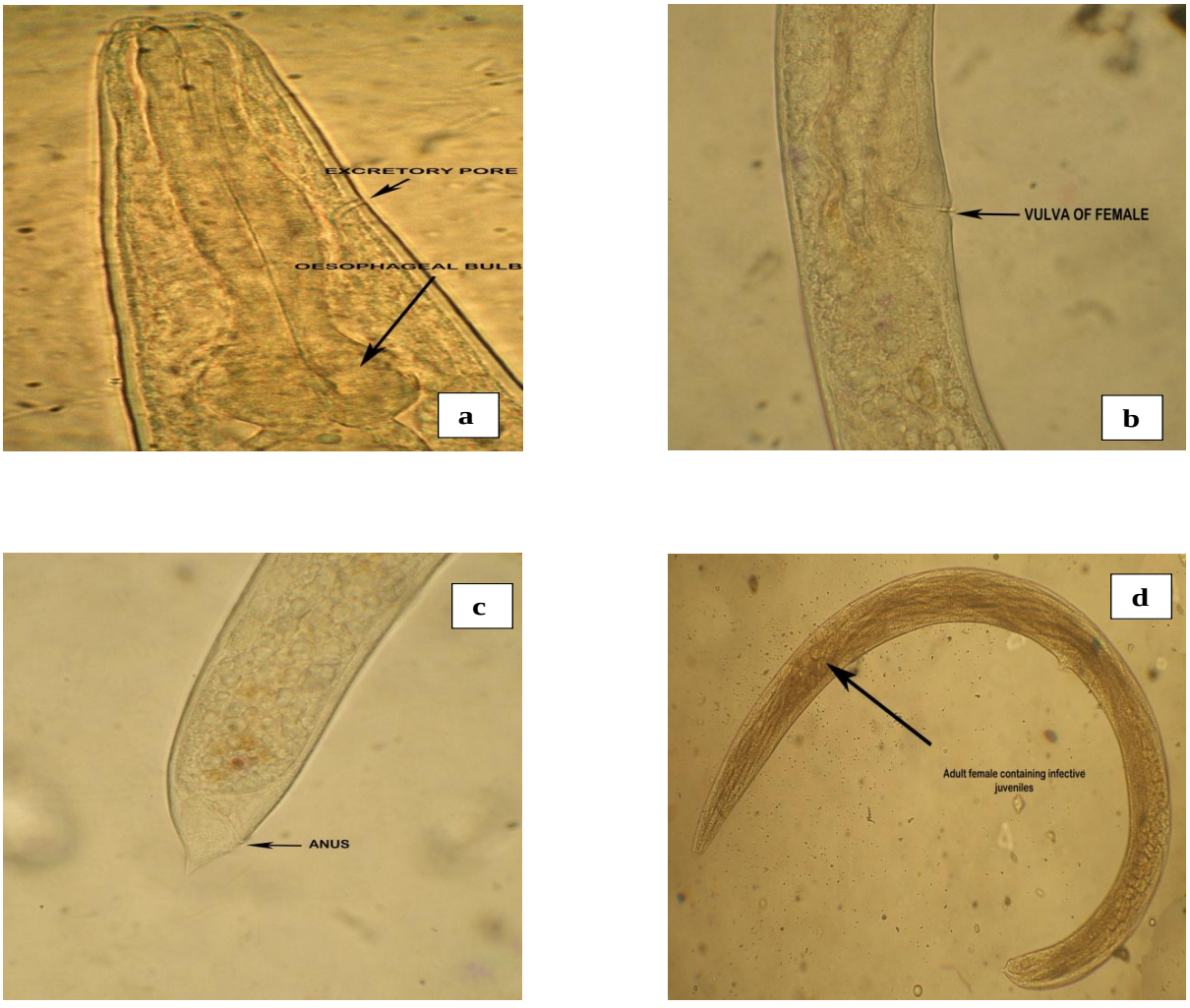
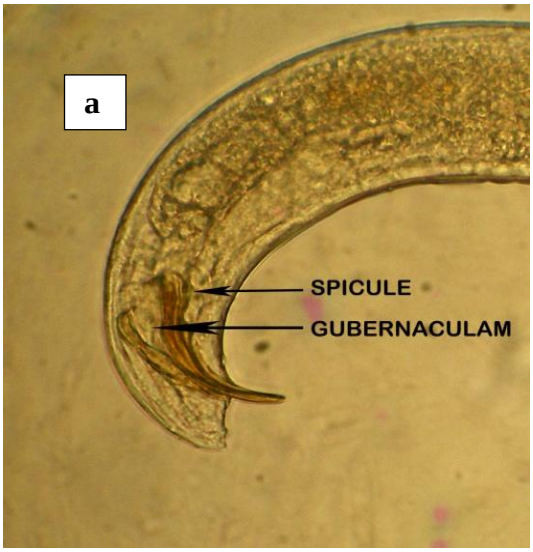


Fig.2 Microscopic pictures of adult male: [a] Posterior part showing spicule and gubernaculum [b] whole body of adult male



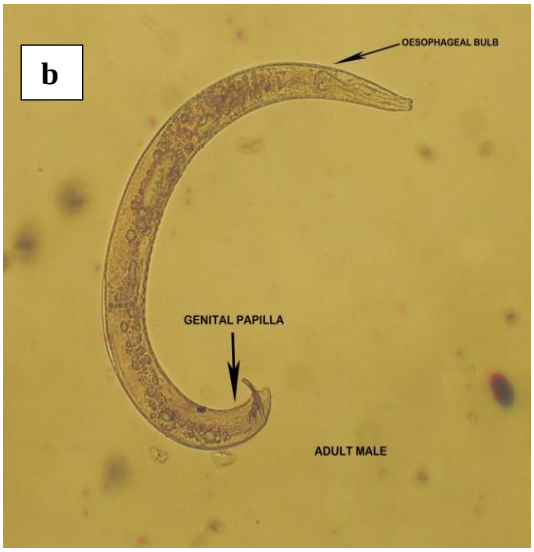


Fig.3 Infective juvenile

Tail somewhat long with tail mucron. Tail shorter than anal body width. The older adult female were consumed by young infective juveniles and eventually killing the female. Nervering surrounding isthmus, just anterior to basal bulb.

Adult male

Male is smaller than the female. Posterior region of the body curved ventrally. Spicules paired and separate brown in color and gubernaculum long. Gubernaculam is curved ventrally at the anterior end. Second-generation male similar to that of the first generation except body, spicule and gubernaculum shorter and thinner. Tail mucron is present (Fig. 2).

Excretory pore located mostly anterior to basal bulb. Esophagus with cylindrical procorpus. Head (manubrium) of spicules with rounded anterior end.

Infective juvenile

Body is slender with closed mouth and anus. Sheath (second-stage cuticle) present immediately after harvesting, but many IJ will lose their sheath in storage. Labial region smooth, rounded anteriorly, continuous with body. Excretory pore is anterior to the nerve ring. Tail is long and tail attenuate, tapering gradually with constriction on dorsal side (Fig. 3).

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