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Survey for the occurrence of Entomopathogenic Nematodes in few districts of Tamilnadu.

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Abstract

The soil samples from various districts approximately nine districts of Tamil Nadu were surveyed for the presence of EPNs which are used successfully as biological control agents or bio-pesticides. Four samples out of fifteen from Coimbatore district, two out of five from Karur, two out of five of Pollachi , one out of five from Tirupur, and two out of five from Udumalpet were found harbouring the EPN of the genus of S*teinernema*, while two samples of Coimbatore, two samples of Dharmapuri. One sample of Madurai and two of Salem were recorded to harbour *Heterorhabditis*. It was also noticed that samples from Ooty do not contain any EPN.

Keywords: *Entomopathogenic, Steinernema, Heterorhabditis, Xenorhabdus, Photorhabdus.*

Introduction

Biological control agents play a major role in Integrated Pest Management (IPM). Entomopathogenic nematodes, the carriers of insect pathogenic bacteria showed considerable potential as a biological agent for insect pest in modern agriculture.

The Entomopathogenic nematodes come under the families *Steinernematidae* and *Heterorhabditidae*. *Steinernematidae* is represented by the genera *Steinernema* and *Neosteinernema* and *Heterorhabditidae* is represented by the genus *Heterorhabditis*. They are associated with mutualistic bacteria in the genus *Xenorhabdus* for *Steinernema* and *Photorhabdus* for *Heterorhabditis.*

Thus, it is a nematode - bacterium complex that works together as a symbiont in bio control to kill an insect host. These two nematode families belong to the order *Rhabditidae* and are not closely related, but because they possess many biological similarities, they can be considered together **(Kaya., and Gaugler., 1993).**

The positive attributes of these nematodes as biological control agents are that they have a broad host range, are safe to vertebrates , plants and other non – target organisms , have no known negative effect on the environment , are easy to mass produce *in vivo* and *in vitro* , are easily applied by using standard spray equipment , can search for their host, can kill rapidly , have the potential to recycle in environment , are compatible with many chemical and other biological pesticides.

Steinernematids and Heterorhabditis are obligate pathogens in nature have the non feeding , free living , third stage infective juvenile that infect the insect host in the soil environment. The infective juveniles infect the host through natural openings (mouth, spiracles or anus) or thin areas of the host cuticle and penetrates into the host hemocoel . The infective juvenile then releases the bacteria either through the anus for *Steinernematids* or through the mouth for *Heterorhabditis.*

The mutualistic bacterium propagates and produces substances that rapidly kill the host and protect the cadaver from colonization by other micro organisms. Both bacteria (*Xenorhabdus* and *photorhabdus)* are motile , Gram negative , facultative , non - spore forming anaerobic rods in the family *Enterobacteriaceae* (**Anna et al., 2008**).

Surveys conducted around the world including other island habitats reveal a diverse fauna of entomopathogenic nematode sp. Native species of Entomopathogenic nematodes that are adapted to local environmental and climatic condition are especially good candidates for use as biological control agents **(Claudia et al.,2008).**

At present mainly non – native strains have been used in nematode applications. About 10 comericially available species are normally used worldwide and most of them have been isolated from different parts of the world. Since there are only relatively few commercial strains, it stands to reason that most are from places other than the locations where they are used. Therefore, these strains may not be well adapted to specific local climates and environmental conditions, hence, their efficacy might be reduced, whereas native species are adapted to local climatic conditions and are therefore more likely to survive in the target area after application. Such native nematodes can be developed as new biological controls agents and several previous surveys have searched for new EPN species with the intent to contrl important agricultural and horticultural pests under specific conditions. In India , alternatives to chemical pesticides are needed and EPNs are used as part of integrated pest management programs for several cropping systems including high value crops.

Rather than introducing a non native Entomopathogenic nematode for specific control, it will be very efficient to introduce the native Entomopathogenic nematode to the same suspective area and it might provide increased capabilities for persisting and recycling under the same soil and climatic conditions.

Therefore, it becomes necessary to perform a survey for the occurrence of native Entomopathogenic nematodes and also it is essential to collect the isolates of EPN. Hence this present study is taken to perform a survey for the presence of EPN within various districts of Tamil Nadu.

Materials and Methods

Rearing of *Galleria mellonella*

The larvae of greater wax moth *Galleria mellonella* (*Lepidoptera galleridic*) were used for baiting the nematodes . The larvae were reared in 1,500 ml containers at 32 C on an artificial diet. The lids of the containers had small holes for aeration Periodically the larvae were transformed to another container with fresh diet, on weekly basis. They reached the last instar stage between 5 – 6 weeks . They were collected and used for study. Some larvae were left in the container itself for pupation. When they reached the adult stage, they were placed in a separate container having wax coated butter paper in which the females lay eggs.

The eggs were washed in 0.01% formalin 3 times and washed once in distilled water and dried. The eggs were then placed on a butter paper over fresh diet in a container for rearing. The eggs hatch within 3-4 days and the procedure was repeated and the larvae were used for future studies.

Preparation of artificial diet

The greater wax moth larvae were bred on artificial diet contains 400g Commeal,200g Wheat flour,200g Wheat rava,200g Milk powder,100g Yeast,350gm Honey and 350ml Glycerine. The diet thus prepared was dispensed in each rearing sterile container and kept at room temperature.

Collection of soil sample

A total of fifteen soil samples were collected in and around the Coimbatore district, five samples were collected from each of the following districts , Salem, Dharmapuri and Madurai. From all the places 25gm of soil samples were collected at a depth of 15cm from the surface of soil and were stored in a clean polyethene bags and were brought to the laboratory and stored at 25 C for future study.

Isolation of nematode and proliferation

Entomopathogenic namatodes were recovered from soil samples using the insect baiting methods as described by Bedding and Akhurst., (1975). Insect baits (five last – instar larvae) of *Galleria mellonella* were placed in 100ml plastic containers which contained 25gm of collected soil. Larvae were checked for infection every day and the dead ones were

removed and the alive larvae were placed in the containers . The dead larvae were isolated and thoroughly rinsed in 0.01% formalin and placed in White's trap **(Kaya and Stock, 1997)** until the emergence of third stage infective juveniles of nematodes in another two to three days.

The emerging nematodes were pooled from each sample and stored in culture flask (T-flask) by changing the formalin once in a week. These nematodes were used to infect fresh larvae of *Galleria mellonella* for mass propagation of nematodes, for identification and establishment of culture .The culture flasks with nematodes were maintained at 25^0 C.

Culturing of symbiotic bacteria

Nematodes act as vector by carrying the bacterium and infects the larvae. They enter through the natural openings like mouth, anus and spiracles. It multiplies within the larvae by using body parts of the larvae as nutrient source. The symbiotic bacteria in the nematodes also multiply along with nematodes. They lead to the death of the larvae by septicemia. The dead larvae were sterilized with 70% ethanol by immersing the larvae in ethanol for 3 times and air-dried. The larva harboured the symbiotic bacteria, so as to isolate the bacteria a loopful of haemolymph was streaked on Nutrient agar plate containing 0.004 % triphenyl tetrazolium chloride and 0.025 % Bromothymol blue (NBTA). The plates were incubated at 28^0 C for 24 hrs. The two forms phase I and II were differentiated based on the colour of the culture. Only primary from bacteria were used in the study.

Results and Discussion

Four samples out of fifteen from Coimbatore district, two out of five from Karur, two out of five of Pollachi , one out of five from Tirupur , and two out of five from Udumalpet were found harbouring the EPN of the genus of S*teinernema*, while two samples of Coimbatore, two samples of Dharmapuri. One sample of Madurai and two of Salem were recorded to harbour *heterorhabditis*. It was also noticed that samples from Ooty do not contain any EPN.

S.No	Districts	No. of Samples	EPN Recorded	EPN Identified
1.	Coimbatore	15	4 2	*Steirnernema* *Heterorhabditis*
2.	Dharamapuri	15	2	*Heterorhabditis*
3.	Karur	5	2	*Steinernema*
4.	Madurai	5	1	*Heterorhabditis*
5.	Ooty	5	-	-
6.	Pollachi	5	2	*Steinernema*
7.	Salem	5	2	*Heterorhabditis*
8.	Tripur	5	1	*Steinernema*
9.	Udumalpet	5	2	*Steinernema*

The identification of the EPN can be done by the observation of the colour of the cuticle of dead *Galleria* larva infected by the EPN. That is if the colour of the cuticle of the dead *Galleria* larva is black that indicates the presence of EPN of *Steinernema* species. Whereas the colour of the cuticle of the EPN infected dead *Galleria* larva brick red that indicates the presence of EPN of *Heterorhabditis* species. **(Woodring and Kaya, 1980)**.

The genus of the EPN can be confirmed further by the colour of the colonies formed during the culture of bacteria in NBTA media. If the colony is blue green in colour that indicates the presence of symbiotic bacteria *Xenorhabdus* which is a symbiont of *Steinernema sp.* And if the colony is white in colour and bioluminescent, then it indicates the symbiotic bacteria of *Photorhabdus* which is a symbiont of *Heterorhabditis.* (**Akhurst R.J., 1982**).

The occurrence of Entomopathogenic nematodes in soil depends on various factors of both biotic and abiotic components. A variety of environmental factors including the effect temperature, soil depth, type , moisture, presence and type of vegetation and proximity of host insects affect the distance and rate of vertical and horizontal movements of infective juveniles **(Georgis and Poinar 1983)**. The temperature also influences the nematode's mobility , reproduction and development(**Mason and Homnik 1995**). Moreover the negative attributes of EPN include their broad host range (although no negative

effects on non-target host have been observed, this broad host range may include some beneficial insects), narrow tolerance to environmental conditions (e.g. moisture requirement) poor long-term storage, poor field persistence and relatively high host in comparison to chemical pesticides. **(Kaya and Gaugler., 1993)**

The occurrence of different EPNs (*Heterorhabitis and Steinernema species)* in different areas of (various districts studied here) is due to the differences in soil type, vegetation, moisture and temperature etc, .And also frequent disturbance to agricultural habitats like tilling disturbs the soil eco system and so EPN populations.

Abiotic factors such as extreme temperatures, soil moisture, osmotic stress, soil texture, UV radiations and biotic factors such as antibiosis, completion, natural enemies are the primary causes that affect the survival of EPNs.

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