International Journal of Entomology and Nematology Research,.8 (1), 16-33, 2024
Print ISSN: 2399-1151(Print)
Online ISSN: 2399-116X (Online)
Website: https://www.eajournals.org/
Publication of the European Centre for Research Training and Development-UK

Diversity of Entomopathogenic Nematodes and their Potential as Bio-pesticide in North East India: A Review

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doi: https://doi.org/10.37745/ijenr.16 vol8n11633 Published November 13 2024

Citation: Dey A., Singha S., Debnath N., Pandey P. and Singha B. (2024) Diversity of Entomopathogenic Nematodes and their Potential as Bio-pesticide in North East India: A Review, *International Journal of Entomology and Nematology Research*,.8 (1), 16-33

Abstract: *The menace of pesticides and demand for organically produced agricultural products has forced the Indian Government to promote organic agriculture in the North-eastern states through special schemes and programs such as 'Technology Mission for North East and through setting up of a special Agricultural Export Zone. In the absence of insecticides, Entomopathogenic Nematodes (EPNs) are the best and most useful agents for the management of insect pests ranging from Lepidoptera, Diptera to Coleoptera. EPNs and their symbiotic bacteria together form efficient biocontrol agents that are widely used worldwide for insect-pest management in different agro-ecosystems. The EPNs of the families Steinernematidae and Heterorhabditidae are associated with bacteria of genus* Xenorhabdus *and* Photorhabdus *respectively. Here, we present a brief review of the various species of EPN and EPB isolated from soils of North East India and the current trend of research on the efficacy of EPN as bio-pesticide in northeastern agro-climate. Reports on EPN research and its uses as bio-pesticide are available only from 4 out of 8 states of North East India. A total of 24 EPN species comprising14 from Assam, 5 from Meghalaya, 4 from Mizoram, and 1 from Manipur were identified under three genera-* Heterorhabditis *(7 spp.),* Steinernema *(15 spp.), and* Oscheius *(2 spp.). Studies on efficacy testing reported that EPN species* Heterorhabditis indica *showed great efficacy against tea mosquito bug, tea termite, white grubs, tobacco cutworm, mustard sawfly, and greater wax moth in Northeast India. This encouraging current status of EPN research in NE India will provide a footing ground for further exploration, isolation, characterization, and biosystematics of EPNs and their symbiotic bacteria (EPBs) from the soils of varied agro-climatic conditions of this region. We conclude that isolation together with testing of the efficacy of EPN strains through massive field trials against pests of seasonal crops are essential to realizing the potential of EPNs formulations as potent biological pest management tools to promote organic agriculture in North East India.*

Keywords: entomopathogenic nematode, heterorhabditis, steinernema, oscheius, organic agriculture, biopesticide.

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INTRODUCTION

Entomopathogenic nematodes (EPN) are recognized as the lethal parasites of insect larvae which are widely used as a biocontrol agent against many important insect pests of vegetable crops. Therefore EPNs are grabbing the attention of entomologists as well as nematologists in the field of bio-control research worldwide (Kalita et al., 2019; Devi et al., 2017; Ganguly, 2006). EPNs represent a group of soil-inhabiting nematodes that parasitize and kill a wide range of host insects within 24-48 hours of infection and are harmless to plant and non-target organisms. These nematodes belong to two families viz., Steinernematidae and Heterorhabditidae which have a mutualistic partnership with strain-specific anaerobic bacteria, and together they kill a wide range of insect species. Xenorhabdus bacteria associated with Steinernema and Photorhabdus bacteria associated with Heterorhabditids. Nematodes after entering into the host insect body release their symbiotic bacteria into the hemolymph which kills the insect by massive septicemia. The bacteria degrades insect-larval tissues making it as the food source for the nematodes. It facilitates them to mature and multiply. Usually, 1 or 2 generations of adult nematodes are produced within the insect cadaver. The progeny of the last adult generation reassociates with few bacterial cells and nurture them in their intestine. Then nematodes move out of the insect cadaver into the soil and wait for another insect to parasitize (Ganguly, 2006).

Several studies have been conducted to isolate and identify EPN strains from across NE India, many of which are new to this part of India. The earliest report by Nematologists on EPNs was the isolation of Steinernema and Heterorhabditis from around the root zones of different plants across Assam (Deuri et al., 2000). Studies on EPNs from the Northeast can be traced back to 2010 when Promodini and Mohilal reported for the first time on the occurrence of Heterorhabditis strain from Manipur. In the same year, the author of the present paper reported three strains of EPN viz., *S. carpocapsae* (GenBank Acc. No. MH204152), *S. surkhetense* (MG 976890), and *Steinernema sp*. (MG976891) from Cachar district of Southern Assam through molecular and microscopic assays. Similarly, a report on the isolation of another new species *Oscheius indicus* also came from the Cachar district of Assam (Kumar, et al., 2019).

Even more encouraging was the report of successful isolation of a new species of EPN, *Steinernema sangi* and its symbiotic bacteria *Xenorhabdus vietnamensis* from Mizoram for the first time in North East India (Lalramnghaki et al., 2017). Following that success, *Heterorhabditis baujardi* was also reported for the first time from Mizoram, which was originally described in Vietnam (Vanlalhlimpuia et al., 2018). Nematologists from Mizoram contributed greatly to EPN research by identifying three EPN associated bacteria (EPBs), *viz*., *Xenorhabdus vietnamensis*, *Xenorhabdus stockiae*, and *Photorhabdus luminescens* which could produce a great potency against pathogenic fungi, *Fusarium oxysporum* (Lalramchuani et al., 2020).

Many studies, especially from four states of the North East confirmed the potency of the isolated EPNs against one or the other pests. Although *Heterorhabditis indica* is the first indigenous isolate

of Meghalaya which showed potency against Greater Wax Moth, *Galleria mellonella* (Lalramliana et al., 2005), *Steinernema meghalayensis* is the first new species of this genus isolated in Meghalaya whose efficacy was tested also against *Galleria mellonella*. (Ganguly et al., 2011). From Assam Borgohain (2015) isolated and identified as *Heterorhabditis sonorensis*. *Heterorhabditis bacteriophora* isolated from Assam proved to be potential against agricultural pest White grubs, *Lepidiota albistigma* (Devi et al., 2016 and 2020). *H bacteriophora* and *H. indica* from Assam also exhibited great potential in killing pests like tea mosquito bug and tea termite respectively (Amuri et al., 2020; Singha et al.,2014).

The aim of this paper is to review the studies on EPN undertaken in North East India in the last 20 years in order to assess the trend of study of EPNs to assess the diversity and potential in insect pest management in this part of India. Accordingly vigorous Journal and literature searches were made utilizing all available resources including Search Engines For Academic Research' like Research Gate, Google Scholar, Science Direct, Shodhganga, etc. Sincere attempts have been made to merge the investigations that were carried out to see the diversity, distribution, and efficacy of EPNs in Northeast India with a view to understanding the potential of EPNs as bio-pesticide in this agriculturally diverse land of India. Through this piece of work, the authors also tried to throw light on the prevailing knowledge gap in EPN research towards its successful implementation as a potential tool in biological pest management in North Eastern agro-ecosystem and suggesting some possible strategies to reach the goal.

Nematode

The word Nematode is derived from the Greek word 'nema' meaning thread. Nematodes are tiny roundworms that belong to the phylum Nematoda. They are one of the most abundant creatures found on Earth (Blaxter., 1998). Nematodes are triploblastic and possess an organ-system level of body organization. They are bilaterally symmetrical, elongated, cylindrical, unsegmented body basically worm-like. They are unisexual and males are smaller than females (Ganguly et al., 2012). They feed on bacteria, fungi, algae, yeasts, diatoms, and may also found to be the predators of several small invertebrate animals, including other nematodes also. Most of the nematodes in soil range in between 0.25-5.5 mm in length (Ingham, 1994). Nematodes are proved to play a significant role in the decomposition of organic matter, mineralization of plant nutrients, and nutrient cycling (Ingham et al., 1985). Nematodes are also proved useful in indicating pollution levels in aquatic and soil systems, and in industrial toxicology (Kessel et al., 1989). In recent years the use of EPNs as biopesticides against insect pests has grown rapidly. Many formulations have focused on increasing the storage time and preserving EPN infectivity in the field since the late 1970s (Heriberto et al, 2017). The most economically important groups of nematodes are reviewed to be the sedentary endoparasites, which include the genera Heterodera and Globodera (cyst nematodes) and Meloidogyne (root-knot nematodes) (Williamson et al., 2003).

Entomopathogenic Nematode (EPN)

The term entomopathogenic is derived from the Greek word 'entomon' meaning insect, and pathogenic means causing disease. EPNs are lethal insect parasites found in soil that belong to the Phylum Nematoda from the families Heterorhabditidae (genus *Heterorhabditis*) and Steinernematidae (genera

Steinernema and *Neosteinernema*). The genus *Oscheius* from the family Rhabditidae was also identified as EPN recently (Torres-Barragan, A., 2011). EPNs form symbiotic relationships with bacteria of the family Enterobacteriaceae. The third and only free-living stage is the "Infective Juvenile" (IJ) which carries the bacterium of genus *Xenorhabdus* and *Photorhabdus* in families Steinernematidae and Heterorhabditidae respectively. IJs forage in the soil for several months in search of a suitable host insect. IJ infects the host by entering into the host hemocoel through natural openings like spiracles, mouth, or anus. Once entered they release symbiotic bacteria. These bacteria multiply and cause massive septicemia and thus bring death within 24-48 hours of infection (Gozel et al., 2016). They are found to have an enormous potential for insect pest control both under and above ground. These nematodes are able to kill a large number of insect species, especially among the Lepidoptera, Diptera, and Coleoptera, in both laboratory and field assays (Simoes et al., 1996). Heterorhabditidae and Steinernematidae are two widely studied families because they both show great potential as inundative biological agents in addition to or in replacement of chemical pesticides against numerous insect pests and secondly, their short lifespan and easy culture and handling in the lab, they are increasingly used as model organisms in fundamental research into symbiosis and parasitism, interalia (Lacey., 2015).

EPN Systematics (Ref: Ganguly, 2006; Sharma et al 2016, Devi et al.; 2017)
Phylum: Nematoda
Class: Secernentea
Order: Rhabditida
Suborder: Rhabditina
Superfamily: Rhabditoidea
Family: i) Steinernematidae ii) Heterorhabditidae iii) Rhabditidae
Genus: i) *Steinernema, Neosteinernema;* ii) *Heterorhabditis;* iii) *Oscheius*

Global reports on EPN Strains

A total of 100 species were recorded to date under Genus *Steinernema* while 21 species were recorded under *Heterorhabditis* from different countries of the world (Bhat et al., 2020). Genus Oscheius has been divided into two groups, viz., Insectivora and Dolichura. Under the Insectivora group, 31 species were reported while 14 species have been identified under Dolichura. Thus, a total of 166 EPN species have been reported across the World to date.

Bionomics of EPN

Bionomics of family Steinernematidae

Steinernematids are free-living Infective juveniles (IJs), their foraging behavior includes either 'ambush' i.e., hunting behavior or 'cruising' strategy where they continuously roam around in the soil in search of larval stages of potential insect host and sometimes they become preyed (Ganguly, 2006). IJs, the free-living 3rd stage carries symbiotic bacterium of the genus Xenorhabdus in the vesicle of their anterior ventricular part of the intestine. EPNs in the soil can detect and sense their specific host in response to carbon dioxide, the physical structure of insect's integument, vibration, and other chemical stimuli (Kaya et al., 1993). IJs invade into host hemocoel through natural openings like spiracles, mouth, or anus and then release the bacteria into the hemocoel where they multiply and

provide nutrition to nematode and also prevent further contamination of host cadaver from the secondary intruder. It takes 24–48 hours for bacterial toxins to elicit sepsis to kill the host completely. The host cadavers turn brown or black when infected by Steinernema species (depending upon nematode and bacterium species involved). IJ's progenies feed on the bacteria as well as degraded contents of the hemolymph and reproduce and grow until the food supply becomes limiting, by that time progenies were grown into 3rd stage IJs. Progeny of nematodes goes through four juvenile stages to turn into adults. Within 2-4 days of infection, IJs undergo two molts to become 1st generation males and females. The 1st generation progeny mate and reproduce ovoviviparously. Steinernema species are amphimictic i.e involvement of fusion of male and female gamete for successful reproduction (Gozel et al., 2016). Progeny of the first generation again develop into amphimictic females and males. Reproduction is amphimictic in the second generation. After completing 2-3 generations in 5-7 days within insect hemocoel, the nematodes become fully ready for emergence in great number from the cadaver as the third and only free-living stage the Infective Juveniles (IJs), which are ready to infect another healthy insect (Gozel et al., 2016; Ganguly., 2006).

Bionomics of family Heterorhabditidae

Heterorhabditis is a 'cruiser' in their foraging behavior (Ganguly., 2006). The third infective juvenile stage carries symbiotic bacteria, Photorhabdus luminescens in their anterior ventricular part of the intestine. IJs enter into the host coelom via natural openings like spiracles, mouth, anus, and in some cases through intersegmental membranes of the cuticle. After invasion bacteria are released into the coelom and within 24-48 hours host dies due to massive septicemia. The cadaver turns brick red to purple color when parasitized by Heterorhabditis. Infected cadavers also throw luminesce effect in dark due to the presence of a characteristic feature in symbiotic bacteria. Bacteria convert the host body into nematode biomass by digesting host tissues into a nutritious soup for IJs and their progenies. Bacteria also protect the cadaver resources from different competitors, by producing antimicrobials, bacteriocins, and other antibiotics (Akhurst et al.,1982; Bode et al., 2009). Heterorhabditis are hermaphroditic and able to reproduce in the absence of conspecifics (Gozel et al., 2016). Reproduction is amphimictic in the second generation i.e the 1st generation hermaphrodite's progeny develops into second-generation amphimictic females and males. After completing 2-3 generations in cadaver third stage juveniles emerges and are ready to infect new healthy insect (Gozel et al., 2016; Ganguly., 2006).

Advantages of EPN

EPNs and their symbiotic bacteria have proven to be safe on non-target organisms (Ganguly, 2006), warm-blooded vertebrates, mammals including humans (Poinar et al., 1982; Boemare et al., 1996; Kumar et al., 2016). Problems associated with chemical pesticides are pest resistance, resurgence, and residue effect and also it requires days or weeks to kill the pest but whereas EPN causes quick mortality within 24–48 hours with no side effects. These nematodes have a wide host (pest) range including but not limited to Lepidoptera, Diptera to Coleoptera, and have the ability to search target insects in the soil. EPNs are inexpensive and are easy to culture under laboratory conditions. Therefore, they are extremely useful bio-control agents with enormous potential to accelerate organic agriculture.

Efficacy of EPN

EPNs with high biocontrol potential and harmless to a non-target organism have attracted researchers across the globe to isolate more and more indigenous strains for the effective management of several insect pests. A study found that local strains are better adapted to the local climatic and topographic conditions and more efficiently kill local pests than non-native strains (Devi et al., 2017). EPNs with their mutualistic partnership with anaerobic bacteria make them potential insecticidal agents and together they kill a wide range of insect species ranging from order Lepidoptera to Coleoptera (Simoes et al., 1996). Bacterium genus *Xenorhabdus* is associated with *Steinernema* and while *Photorhabdus* is associated with Heterorhabditids.

Although the testing of the efficacy of EPNs has not been conducted in any agricultural crop or vegetable fields, the stories of successful testing of efficacy against particular pest species are not rare from NE India (Table-2). In Jorhat, Assam efficacy of native isolates *Heterorhabditis bacteriophora* and *Oscheius chongmingensis* tested against tea mosquito bug, *Helopeltis theivora*, and bunch caterpillar, *Andraca bipunctata*. The result showed both the species were efficient and brought about 70-90% mortality and also *H. bacteriophora* proved to be more efficient than *O. chongmingensis* (Amuri et al., 2020). Out of three EPNs isolated from Meghalaya, *H. indica* and *S. thermophilum* showed great potency against mustard sawfly, *Athalia lugens* (Yadav, 2012). On the other hand, all the three isolates *Heterorhabditis indica*, *Heterorhabditis baujardi*, and *Steinernema sangi* were found to be highly effective against tobacco cutworm, *Spodoptera litura*. Among them, *S. sangi* showed the highest pathogenicity against the pest (Lalramnghaki et al., 2020).

Studies on EPN in Northeast India

There are many reports on the isolation and characterization of EPN from Northeastern states. EPN strains identified across North East India till date tabulated in table 1

EPN reports from Assam

The soil of Assam rich in entomopathogenic agents. Ample of reports on isolation and molecular characterization of EPN available from this state. In a survey conducted in Sorbhog, Barpeta, Assam, EPNs were isolated cadavers of white grubs, *Lepidiota albistigma*. 10 samples tested EPN positive out of 100 cadavers. Based on morphological and morphometrical studies, the isolated nematode was identified as *Heterorhabditis bacteriophora* (Devi et al., 2020). Similarly, there are reports of isolation of EPNs from 1 out of 200 soil samples from tea plantation areas of Jorhat through baiting technique using Galleria larvae. A new strain of EPN *Heterorhabditis bacteriophora* was identified through comparative Morphological and Morphometrical analysis (Amuri et al., 2020). In another study by the same author, *Oscheius chongmingensis* was isolated from the tea garden soil sample. Identification was based on morphological similarities like body length, body width, anterior end to excretory pore, anterior end to nerve ring, anterior end to esophagus base, tail length, anal body width, and distance from anterior end to the vulva as a percentage of length, etc. as described by Zhang et al., 2008 from Chongming Island in eastern China (Amuri et al., 2020). Report of new species *Oscheius indicus* under Insectivora group obtained also from Cachar district of southern Assam based on morphometrical, morphological observations and molecular phylogenetic analysis. (Kumar, et al., 2019). Using baiting technique with Galleria larvae, Devi et al., (2019) successfully isolated,

characterized, and identified another strain *S. kushidai* from 1 out of 100 soil samples from the Golaghat district. Kalita et al. (2019) identified two strains *viz*., *Heterorhabditis bacteriophora* and *Oscheius chongmingensis* based on the morphological and morphometric character from 8 positive soil samples out of 200 collected from Assam Agricultural University Campus.

The first report of EPN from Barak Valley of southern Assam was the isolation and identification of *Steinernema sp.* from rhizospheric soil of Bamboo and Crop field during rabi and monsoon season (Sharma et al., 2016, 2018). In another soil EPN survey conducted by Singha et al. (2019), 2 out of 100 garden soil samples were found EPN positive. Based on molecular characterization the EPN Genus was identified as *Heterorhabditis* (Singha et al., 2019). In 2010 the author reported the occurrence of a strain of Steinernema from the soil of Cachar, Barak Valley which was subsequently characterized through molecular and microscopic assays as *S. carpocapsae* (Fig.1) (Acc. No. MH204152). Singha et. al. also successfully isolated two more isolates of Steinernema from their subsequent investigations which are identified and submitted to the GenBank as *S. surkhetense* (MG 976890) and as *Steinernema sp.* (MG976891). These are the known records of occurrences of EPN of the genus *Steinernema* from the soils of Cachar district, Barak Valley, Southern Assam (Sharma R. 2018). Very recently, another local strain *H.indica* has been characterized from the Cachar district of Assam through microscopic (Fig.2) and molecular approaches, and subsequently its symbiotic bacterium *Photorhabdus sp*. has also been confirmed through culture (Accession no. MF417383, Nath et al., 2021).

Devi et al., (2017) conducted a survey across Assam and collected 100 soil samples. Out of these only 5 samples were found to be EPN positive, which comprises of 2 from Barak Valley, 2 from hill areas, and 1 from Upper Brahmaputra Valley. Based on morphological and morphometrical analysis three strains were identified as *Steinernema abbasi, S. ceratophorum*, and *S. tami*. The author also successfully identified EPNs Heterorhabditis and Steinernema in Soil and dead white grub *Lepidiota mansueta* from the white grub endemic field of Majuli, the world's largest river island. Moreover, morphometric and cross-breeding studies *Heterorhabditis* isolates were identified as *H. bacteriophora* (Devi et al., 2016). With the objective to identify EPNs across Assam Borgohain (2015) collected 305 soil samples from various regions of Assam. He identified four EPN species *viz*., *Steinernema abbasi*, *S. karii*, *Heterorhabditis bacteriophora*, and *H. sonorensis* from 45 EPN positive soil samples. Interestingly, this was the first report of *H. sonorensis* from India. According to the author, the sample positivity rate was found to be highest in Karbi Anglong while lowest in the Dhubri district of Assam. With the aim to isolate indigenous EPN Deuri et al. (2000) reported a large number of *Steinernema* and *Heterorhabditis* species around the root zones of different plants across Assam.
Studies on testing of the efficacy of EPN conducted on live wood-eating termites found abundantly across North-eastern tea estates, which cause nuisance by eating young and mature tea leaves. Field trials conducted in Assam tea gardens to check the potency of bio-pesticide against the termites in tea plantations indicated that EPN strain *Heterorhabditis indica* is effective both alone and IPM method in controlling the pest in comparison to the commonly used chemical thiamethoxam (Roy et al., 2020). Singha et al., (2014) conducted a study of laboratory efficacy and potency of two EPN spp. *Heterorhabditis indica* and *Steinernema thermophilum* (obtained from Nematology Division, IARI,

New Delhi) against tea termites and found that although both were efficient against the pest, *Heterorhabditis indica* exhibited higher potency.

EPN Reports from Mizoram

The first report of new species of EPN *Heterorhabditis baujardi* which was identified through morphological and multigene (ITS rRNA, 28S rRNA, and COI) sequence characterization also came from Mizoram. This species was originally described from Vietnam (Vanlalhlimpuia et al., 2018). Phylogenetic analysis revealed that *H. baujardi* belongs to *H. indica* clad. Following this report, Lalramnghaki et al., (2020) isolated indigenous EPNs from fertile lands of Mizoram which include *Heterorhabditis indica*, *Heterorhabditis baujardi*, and *Steinernema sangi*. Although all species are highly effective against 3rd, 4th, and 5th instar larvae of tobacco cutworm, *Spodoptera litura*, *S. sangi* showed the highest pathogenicity against the pest in terms of LC50 at 48 hr post-inoculation.

The pioneer and sole report of EPN associated bacteria (EPB) also came from Mizoram. Three identified EPB strain are *Xenorhabdus vietnamensis* from *Steinernema sangi*, *Xenorhabdus stockiae* from *S. surkhetense* and *Photorhabdus luminescens akhurstii* from both *Heterorhabditis indica* and *H. baujardi*. Out of three *X. stockiae* and *P. luminescens akhurstii* were reported for the first time from Mizoram. All three also showed great potency against pathogenic fungi, *Fusarium oxysporum* (Lalramchuani et al., 2020). This study reveals the possibility of future use of EPNs as potential biocontrol agents against many fungal diseases of agricultural crops. Morphological and molecular analysis (ITS rDNA for *Steinernema* and 16S rRNA for *Xenorhabdus*) revealed that *Steinernema sangi* is associated with the bacteria *Xenorhabdus vietnamensis* and also it is the first report from India (Lalramnghaki., 2017).

EPN Reports from Meghalaya

Steinernema meghalayensis is the first new species of this genus isolated from the Eastern Himalayan region of India. Findings revealed that the new species can infect the larvae of *Galleria mellonella* and can induce mortality, multiply (reproduce), and emerged from the cadavers within 6–8 days at a temperature ranging from 20–30°C (Ganguly et al., 2011). Three more indigenous EPNs *Heterorhabditis indica*, *Steinernema thermophilum* and *Steinernema glaseri* were also isolated from Meghalaya. However, out of three EPNs, H. indica and S. thermophilum showed great potency against mustard sawfly, *Athalia lugens* (Yadav, 2012). In another study of the efficacy of EPN, Yadav et al. (2012) found that *S. glaseri* produced 100 % mortality in 48 hr against the last instar larvae of taro leaf beetle, *Aplosonyx chalybaeus* under laboratory condition. Devi et al. (2011) also reported indigenous species *Steinernema carpocapsae* and Heterorhabditis indica from Jawai and Mawsynram towns of Meghalaya. Further, these species provide inconsistent suppression to Root-Knot Nematode Meloidogyne incognita on Tomato (Devi, 2011)

Apart from efficacy studies against pests, a few studies were also conducted to assess the effect of temperature and moisture on the viability and virulence of local EPN strains. In one such study co-relation between storage temperature on viability and virulence was tested. Result showed 25 °C is the optimum temperature for storage. However, *S. glaseri* survived well in all temperature (5 ± 2°C and 25 ± 2 °C) at different storage durations (Yadav, 2016). Similarly, optimum soil moisture for different nematode species was reported as *H. indica* (8–18%), *S. thermophilum* (6–20%), and *S.*

glaseri (8–25%). Further, this study also revealed that a minimum of 6% soil moisture is essential for all the three nematode species for achieving 100% host mortality (Yadav et al., 2012). The viability and effectiveness of two indigenous EPNs *Steinernema carpocapsae* and *Heterorhabditis indica* were tested using various spray adjuvants like Sunflower oil, Glycerol, Tween 20, Triton X-100, and Paraffin liquid at 1% concentration. The result showed the toxicity of Triton X-100 and Tween 20 were less compared to Paraffin liquid and Sunflower oil (Devi, 2011). Effect of agrochemicals (Monocrotophos, Topcin M, Glyphosate, Dicofol 18.5 E and Oxamyl (0.1%)) and botanicals (pine seed, pine needle, plant parts of *Tithonia spp*., plant parts of marigold (1%)) on these indigenous species indicated that both the genera can withstand agrochemicals and botanicals for 24 h. Mortality rate directly proportional to exposure duration (Devi, 2011). The potency of EPN *Heterorhabditis indica* isolated from the forest soil of Meghalaya was tested at various temperatures and relative humidities (RH). The result indicated that approximately 100 IJs of *H. indica* used to infect *Galleria mellonella* larvae successfully at 25 °C and 100% RH. IJs could emerge only at 15, 20, 25, and 30 °C. The earliest emergence (8 days) of IJs occurred at 25 °C and at 85, 96 (Lalramliana et al., 2005).

EPN Report from Manipur There is only one report on EPN from this state. From a preliminary survey conducted in Manipur one EPN spp. *Heterorhabditis sp*. isolated from soil by using grasshopper following insect trap method (Pramodini et al., 2010).

Table 1: List of identified EPN strains across Northeast India state wise till date

State	**No. of species under Genus *Heterorhabditis***	**No. of species under *Steinernema***	**No. of species under Genus *Oscheius***	**Total EPN sp. state wise**
Assam	*i) Heterorhabditis bacteriophora* ii) *H. sonorensis* iii) *H. indica* **(MF417383)**	i) *Steinernema kushidai* ii) *S. aciari* iii) *S. abbasi* iv) *S. ceratophorum* v) *S. tami* vi) *S. karii* vii) *S.carpocapsae* **(MH204152)** viii) *S. surkhetense* **(MG 976890)** ix) *Steinernema* sp. **(MG976891)**	i) *Oscheius chongmingensis* *ii) O. indicus*	**14**
Mizoram	i) *Heterorhabditis indica* ii) *H. baujardi*	i) *Steinernema sangi* ii) *S. surkhetense*	0	**4**

Meghalaya	i) *Heterorhabditis indica*	i) *Steinernema thermophilum* ii) *S. glaseri* iii) *S. meghalayensis* iv) *S. carpocapsae*	0	**5**
Manipur	i) *Heterorhabditis sp.*	0	0	1
Total	**7**	**15**	**2**	**24**

Table 2: List of indigenous EPN strains and their efficacy against local insect pests in NE India

Indigenous EPN species	**Efficacy study against**	**Method of Isolation and Identification**	**Reported from**	**Reference**
Heterorhabditis bacteriophora	White grubs, *Lepidiota albistigma.(Sugar cane)*	Isolated from dead White Grub; Morphological and Morphometrical	Sorbhog, Barpeta, Assam	Devi et al., 2020
Heterorhabditis bacteriophora and *Oscheius chongmingensis*	Tea mosquito bug, *Helopeltis theivora* and bunch caterpillar, *Andraca bipunctata*	Baiting with Galleria larvae; Morphometric characterization	Jorhat, Assam	Amuri and Devi, 2020; Kalita et al., 2019
Heterorhabditis bacteriophora and *Steinernema*	White grubs, *Lepidiota mansueta*	From Soil and White Grub; Morphometric and Cross-breeding studies	Majuli, Assam	Devi et al., 2016
Heterorhabditis indica	Tea termite	Baiting technique with Galleria larvae; Morphological characterization	Cachar, Assam	Singha et al., 2014
S. khushidai		Baiting technique with Galleria larvae; Morphometric characterization	Golaghat, Assam	Devi et al., 2019

Heterorhabditis indica, H. baujardi and *Steinernema sangi*	LC_{50} at 48 hr against Tobacco cutworm, *Spodoptera litura*.		Mizoram	Lalramnghaki et al., 2020
Steinernema sangi, S. surkhetense ,	Pathogenic fungi, *Fusarium oxysporum*		Mizoram	Lalramchuani et al., 2020
Heterorhabditis indica and *H. baujardi*	-	Morphological and multigene (ITS rRNA, 28S rRNA, and COI) sequence characterization; Phylogenetic analysis	Mizoram Assam	Vanlalhlimpuia et al., 2018 Nath et al., 2021
Heterorhabditis indica and *S. thermophilum*	Mustard sawfly, *Athalia lugens* Taro leaf beetle, *Aplosonyx chalybaeus*		Meghalaya	Yadav, 2012
Steinernema meghalayensis	Greater Wax Moth, *Galleria mellonella.*		Meghalaya	Ganguly et al., 2011
Steinernema carpocapsae and *Heterorhabditis indica*	Viability and effectiveness tested using various spray adjuvants like Sunflower oil, Glycerol, Tween 20, Triton X-100 and Paraffin liquid at 1% concentration		Jawai and Mawsynram, Meghalaya	Devi, 2011
Heterorhabditis indica	*Galleria mellonella;* varying temperature and Relative Humidity			Lalramliana et al., 2005
Heterorhabditis sp.	Grasshopper	From Soil; Insect Trap Method	Manipur	Pramodini et al., 2010

CONCLUSION

Organic agriculture is on the rise in the Northeastern states of India. In the absence of insecticides for the management of insect pests in bio-farming, entomopathogenic nematodes hold a promise to be a useful and most effective tool for pest control. With more and more documentation and realization of

their efficacy, EPN research is certainly going to gain momentum with the attention of entomologists, nematologists, agricultural scientists, and environmentalists alike. The number of studies on EPNs in north-eastern states is very few. Earlier work was confined to the isolation of EPN, therefore, a large number of strains isolated from the NE states are yet to be identified. To date, only 4 out of 8 states of North East India viz., Assam, Mizoram, Meghalaya, and Manipur could produce some reports on EPN research (Fig.4). The highest number of EPN species isolated from Assam (14 sp) followed by Meghalaya (5 sp.), Mizoram (4 sp.), and Manipur (1 sp.) Reports are also rare from Arunachal Pradesh, Tripura, Nagaland, and Sikkim. In India, previously 35 strains of EPNs were isolated from different geographical regions of India representing different agro-climate zones (Kumar et. al., 2015). However, a total of 24 EPN species already identified across the Northeast under three genera viz., 15 species under genus *Steinernema*, 7 under genus *Heterorhabditis*, and 2 species under genus *Oscheius* (Table-1; Fig.3). Significantly, no other state of NE India reported EPN of Genus *Oscheius*, and both the species identified and reported from Assam indicate that the genus may be endemic to this state.

In addition to the isolation and identification of 4 indigenous species of EPNs, the Mizoram is the only state to report regarding isolation and identification of EPN associated bacteria (EPB). Not less than three EPB strain were described viz., *Xenorhabdus vietnamensis*, *Xenorhabdus stockiae* from *Steinernema sangi*, *S. surkhetense* while *Photorhabdus luminescens akhurstii* from *Heterorhabditis indica* and *H. baujardi*. Adding to the credit is the finding that these EPBs can effectively be applied against pathogenic fungi, *Fusarium oxysporum*. This report will undoubtedly open a new research avenue towards utilizing the EPNs against many fungal diseases of crops and vegetables.

Significantly, the trend of EPN research in NE India in the last 20 years indicates that in 2019 alone a total of 8 species have been reported from Assam (Fig.5). However, despite this encouraging trend it is surprising to observe that apart from isolation and identification of EPNs and laboratory efficacy testing, there was no such trial made in the agricultural field to test the efficacy of particular EPN or their combinations against pests of seasonal vegetables and crops. Therefore the present paper identifies the most important gap in the field of EPN research in NE India. It can now be realized that EPN research should go hand in hand with current Integrated Pest Management strategies in order to obtain greater success in Organic farming in NE India. In spite of the knowledge that local EPN strains are better adapted to local agro-climatic conditions than exotic EPN strains and will have better biofarming potential against seasonal vegetable pests, exploitation of such soil treasure towards biological pest management is still in its infancy.

Therefore, we suggest that acceleration of collaborative research efforts among Agricultural Universities, other state and central Universities, and the Research Institutes of NE India is the only alternative to pave the way for establishing and promoting EPNs as the most useful bio-control agent and fill the prevailing knowledge gap. Industry-Academia exchange Programmes together with mass awareness of farmers is the key to substantially encourage both mass culture and production of local EPNs and their associated bacteria as well as their applications in the seasonal and vegetable crop fields in order to realize the potential of EPNs formulations as potent biological pest management tools to promote organic agriculture in North East India.

Acknowledgment:
This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical approval: This review article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of Interest: The Authors of the present paper have no competing interest.

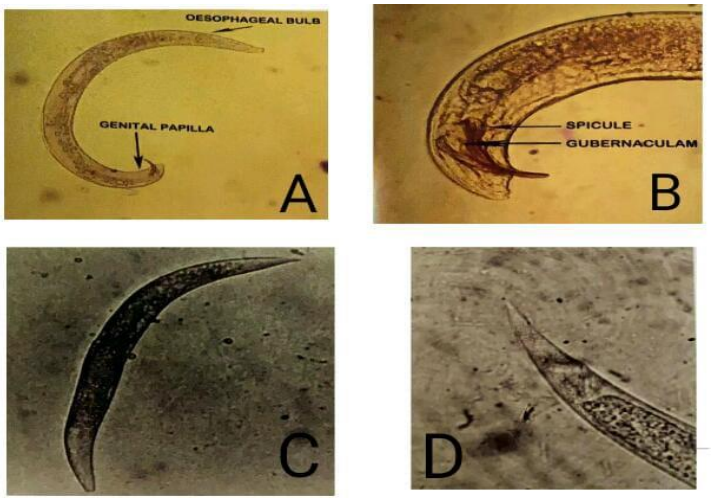
References:

1. Amuri, Bharath, and Gitanjali Devi 2020. Isolation, Morphometrics and Morphological Characterization of *Oscheius chongmingensis* from Assam, India. Int. J. Curr. Microbiol. App. Sci 9.4: 2474-2487.
2. Amuri, Bharath, and Gitanjali Devi 2020. Bio efficacy of *Heterorhabditis bacteriophora* and *Oscheius chongmingensis* against *Helopeltis theivora* and *Andraca bipunctata*. Int. J. Curr. Microbiol. App. Sci 9.4: 2460-2473.
3. Bhat, Aashaq Hussain, Ashok Kumar Chaubey, and Tarique Hassan Askary 2020. Global distribution of entomopathogenic nematodes, *Steinernema* and *Heterorhabditis.* Egyptian Journal of Biological Pest Control 30: 1-15.
4. Blaxter ML, De Ley P, Garey JR, Liu LX, Scheldeman P, Vierstraete A, Vanfleteren JR, Mackey LY, Dorris M, Frisse LM et al. 1998 A molecular evolutionary framework for the phylum Nematoda. Nature 392: 71-75.
5. Bode, H. B. 2009. Entomopathogenic bacteria as a source of secondary metabolites. Current opinion in chemical biology, 13(2): 224-230.
6. Boemare N.E., Laumond C., Mauleon H. 1996. The entomopathogenic nematode-bacterium complex: biology, life cycle and vertebrate safety. Biocontrol Science Technology, 6: 333–346.
7. Bongers, T., and Bongers, M. 1998. Functional diversity of nematodes. Applied soil ecology, 10(3): 239-251.
8. Borgohain, Nivedita 2015. Diversity Of Entomopathogenic Nematodes (EPN) In Assam Diss. AAU, Jorhat
9. Chatterjee, S., Saikia, A., Dutta, P., Ghosh, D., Pangging, G. and Goswami, A. K. 2006. Background Paper on Biodiversity Significance of North East India. Forests Conservation Programme. WWF-India, New Delhi.
10. Deuri, Rimi, et al. 2000. Occurrence of entomopathogenic nematodes in Assam." Journal of Nematology 10: 183-191.
11. Devi, G., et al. 2016. Occurrence of entomopathogenic nematode (*Rhabditida: Heterorhabditidae, Steinernematidae*) in white grub infested areas of 8, Assam, India." Journal of Biopesticides 9.2: 148.
12. Devi, Gitanjali, et al. 2017. Isolation and Identification of Entomopathogenic Nematodes from Assam, India." Indian Journal of Nematology 47.1: 65-74.
13. Devi, Gitanjali, Jisna George, and Mitali Saikia. 2019. Occurrence of entomopathogenic nematodes in Golaghat District of Assam."International Journal of Environment, Agriculture and Biotechnology (IJEAB) Vol-4, Issue-1, Jan-Feb- 2019 http://dx.doi.org/10.22161/ijeab/4.1.31 ISSN: 2456-1878
14. Devi, Gitanjali, et al. 2020. Report of entomopathogenic nematode, *Heterorhabditis* bacteriophora from parasitised white grubs in lower Brahmaputra valley.
15. Devi, Gitanjali, and Dhrubajyoti Nath 2017. Entomopathogenic nematodes: A tool in biocontrol of insect pests of vegetables-A review. Agricultural Reviews 38.2

16. Devi, Gitanjali., 2011. Compatibility of entomopathogenic nematodes (Meghalaya isolates) with spray adjuvants. Indian Journal of Nematology 41.1: 52-55.
17. Devi, Gitanjali., 2011. Influence of Agrochemicals and Botanicals on the Mortality of Entomopathogenic Nematodes (Meghalaya isolates)." Indian Journal of Nematology 41.2: 127-130.
18. Devi, Gitanjali., 2011. Efficacy of entomopathogenic nematode (Meghalaya isolates) against root-knot nematode on tomato." Indian Journal of Nematology 41.1: 115.
19. Lalramliana, Shylesha AN, A. K. Yadav, and A. N. Shylesha., 2005. Effects of temperature and relative humidity on the emergence of infective juveniles of *Heterorhabditis indica* from Meghalaya, India. Indian J Nematol 35: 157-159.
20. Ganguly et al., 2012. Biology of Animals Vol. 1 pg. 452-471 ISBN: 978 81 7381 110 4
21. Ganguly, Sudershan, et al. 2011. *Steinernema meghalayensis* sp. n.(*Rhabditida: Steinernematidae*) from northeastern hilly region of India." Indian Journal of Nematology 41.1: 83-97.
22. Ganguly, Sudershan., 2006. Recent taxonomic status of entomopathogenic nematodes: a review. Int J Nematol 36: 158-176.
23. Gozel, Ugur, and Cigdem Gozel., 2016. Entomopathogenic nematodes in pest management. Integrated Pest Management (IPM): Environmentally Sound Pest Management
24. Kaya H.K., Gaugler R. 1993. Entomopathogenic nematodes. Annual Review of Entomology, 38: 181–206.
25. Heriberto, C. M., Jaime, R. V., Carlos, C. M., and Jesusita, R. D. 2017. Formulation of entomopathogenic nematodes for crop pest control–a review. Plant Protection Science, 53(1): 15-24.
26. Ingham, R. E., Trofymow, J. A., Ingham, E. R., and Coleman, D. C., 1985. Interactions of bacteria, fungi, and their nematode grazers: effects on nutrient cycling and plant growth. Ecological monographs, 55(1): 119-140.
27. Ingham, R. E., 1994. Nematodes. Methods of Soil Analysis: Part 2 Microbiological and Biochemical Properties, 5: 459-490.
28. Kalita, Rinki Mani, et al. (2019) "Isolation of entomopathogenic nematodes from Assam Agricultural University, Jorhat, Assam." Journal of Entomology and Zoology Studies 7.5: 208-214.
29. Kalita, Rinki Mani, et al. 2019. Occurrence of Entomopathogenic nematodes (EPNs) from fields of Assam Agricultural University, Jorhat, Assam."
30. Kumar, Puneet, Sudershan Ganguly, and Vishal Singh Somvanshi (2015) "Identification of virulent entomopathogenic nematode isolates from a countrywide survey in India." International Journal of Pest Management 61.2: 135-143.
31. Kumar, Puneet, et al. 2016. Assessment of virulence of various indian *Steinernema spp.* isolates. Indian Journal of Nematology 46.2: 197-201.
32. Kumar, Puneet, et al. 2019. Description of *Oscheius indicus n. sp.(Rhabditidae: Nematoda)* from India. Journal of nematology 51
33. Lacey, L. A., Grzywacz, D., Shapiro-Ilan, D. I., Frutos, R., Brownbridge, M., and Goettel, M. S., 2015. Insect pathogens as biological control agents: back to the future. Journal of invertebrate pathology, 132: 1-41.
34. Lalramnghaki, H. C., 2017. Characterization of a new isolate of entomopathogenic nematode, *Steinernema sangi (Rhabditida, Steinernematidae),* and its symbiotic bacteria *Xenorhabdus vietnamensis* (γ-Proteobacteria) from Mizoram, northeastern India." Journal of parasitic diseases 41.4: 1123-1131.

35. Lalramchuani, M., et al., 2020. Characterization and screening of antifungal activity of bacteria associated with entomopathogenic nematodes from Mizoram, North-Eastern India. Journal of Environmental Biology 41.4: 942-950.
36. Lalramnghaki, H. C., H. T. Lalremsanga, and S. Lalliansanga (2020) "Evaluation of pathogenicity of indigenous entomopathogenic nematodes (*Steinernematidae* and *Heterorhabditidae*) from Mizoram, India, against tobacco cutworm, *Spodoptera litura* (Fabricius, 1775). Journal of Environmental Biology 41.4: 851-860.
37. Laznik, Ž., and Trdan, S. (2008). Entomopathogenic and entomoparasitic nematodes as biological control agents of thrips. Acta Phytopathologica et Entomologica Hungarica, 43(2): 317-322.
38. Maibam, Pramodini, and Mohilal Naorem. A preliminary survey on entomophagous nematodes of Manipur. Advances in Parasitology: A Novel Approach Towards a Disease Free World. Proceedings of the 22nd National Congress on Parasitology, University of Kalyani, West Bengal, India, October 30-November 1, 2010. University of Kalyani, 2011
39. Nath, Gayatri, et al. 2021. First occurence of *Heterorhabditis indica (Rhabditida: Heterorhabditidae*), an entomopathogenic nematode strain from the soil of Barak Valley, Southern Assam, India. Communicated to Zootaxa journal.
40. Poinar G.O. Jr., Thomas G.M., Presser S.B., Hardy J.L., 1982. Inoculation of entomogenous nematodes, *Neoaplectana* and *Heterorhabditis* and their associated bacteria, *Xenorhabdus spp.*, into chicks and mice. Environmental Entomology, 11: 137–138.
41. Roy, Somnath, et al. 2020. Nonchemical based integrated management package for live-wood eating termites in tea plantations of north-east India." International Journal of Tropical Insect Science: 1-6.
42. Sharma R., 2018. Morphological and molecular characterization of Entomopathogenic Nematodes occurring in Barak Valley, Assam (Ph. D. Thesis, awarded in 2018)
43. Sharma, Rupak., Singha, Baby., Subhadeep Roy Choudhury and Gouriduttsharma., 2018. Isolation and Microscopic Investigation of Entomopathogenic Nematodes (EPNs) Occurring in Barak Valley, Assam.Int.J.Curr.Microbiol.App.Sci. 7(4): 1835-1839
44. Singha, D., B. Singha, and B. K. Dutta., 2014. Natural occurrence and virulence of entomopathogenic nematodes on tea termites in barak valley of Assam, India. IJTS: 64.
45. Singha, Baby et al., 2019. The Occurrence of Two Isolates of Entomopathogenic Nematodes of the Genus *Hetereorhabditis* (Nematode: *Heterorhabditidae*) from Garden Soils of Cachar, Southern, Assam, India. Nematodes: A Threat to Food Security and Farmer's Livelihood pg 113
46. Torres-Barragan, A., Suazo, A., Buhler, W. G., and Cardoza, Y. J. 2011. Studies on the entomopathogenicity and bacterial associates of the nematode Oscheius carolinensis. Biological control, 59(2): 123-129.
47. Van Kessel, W. H. M., Zaalberg, R. B., and Seinen, W., 1989. Testing environmental pollutants on soil organisms: A simple assay to investigate the toxicity of environmental pollutants on soil organisms, using CdCl2 and nematodes. Ecotoxicology and environmental safety, 18(2): 181-190.
48. Vanlalhlimpuia, Lalramliana, and Vanramliana Hrang Chal Lalramnghaki., 2018. Morphological and molecular characterization of entomopathogenic nematode, *Heterorhabditis baujardi (Rhabditida, Heterorhabditidae)* from Mizoram, northeastern India. Journal of Parasitic Diseases: Official Organ of the Indian Society for Parasitology 42.3: 341.
49. Williamson, V. M., and Gleason, C. A., 2003. Plant–nematode interactions. Current opinion in plant biology, 6(4): 327-333.
50. Yadav, Arun K., 2012. Evaluation of the efficacy of three indigenous strains of entomopathogenic nematodes from Meghalaya, India against mustard sawfly, *Athalia lugens proxima Klug* (Hymenoptera: *Tenthredinidae*). Journal of parasitic diseases 36.2: 175-180.

51. Yadav, Arun K., 2012. Efficacy of indigenous entomopathogenic nematodes from Meghalaya, India against the larvae of taro leaf beetle, *Aplosonyx chalybaeus* (Hope). Journal of parasitic diseases 36.2: 149-154.
52. Yadav, Arun K., 2012. Evaluation of the efficacy of three indigenous strains of entomopathogenic nematodes from Meghalaya, India against mustard sawfly, *Athalia lugens proxima Klug* (Hymenoptera*: Tenthredinidae*). Journal of parasitic diseases 36.2: 175-180.
53. Yadav, Arun K., 2016. Effects of storage temperature on survival and infectivity of three indigenous entomopathogenic nematodes strains (*Steinernematidae* and *Heterorhabditidae*) from Meghalaya, India. Journal of Parasitic Diseases 40.4: 1150-1154.
54. Yadav, Arun K., 2012. Soil moisture effects on the activity of three entomopathogenic nematodes (*Steinernematidae* and *Heterorhabditidae*) isolated from Meghalaya, India. Journal of Parasitic Diseases 36.1: 94-98.
55. Zhang, Chongxing, et al., 2008. *Heterorhabditidoides chongmingensis gen. nov., sp. nov. (Rhabditida: Rhabditidae*), a novel member of the entomopathogenic nematodes. Journal of invertebrate pathology 98.2: 153-168.



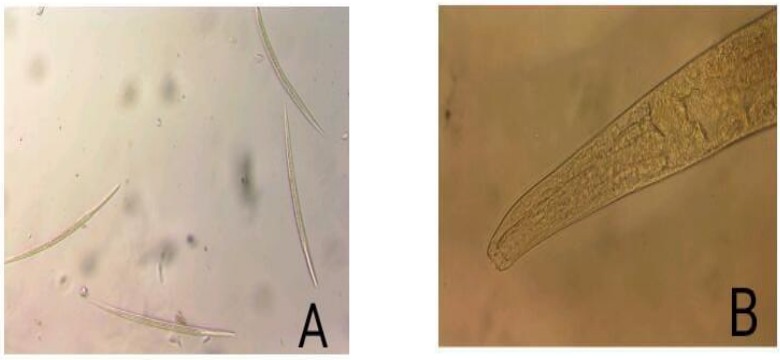


Fig.1: Light microscopic images of - A) Adult Male 1st generation (B) Spicule and Gubernacula of male of 1st generation (C) Infective juvenile (D) Tail region of infective juvenile of *Steinernema carpocapsae* (Adopted from Sharma et al., 2018)

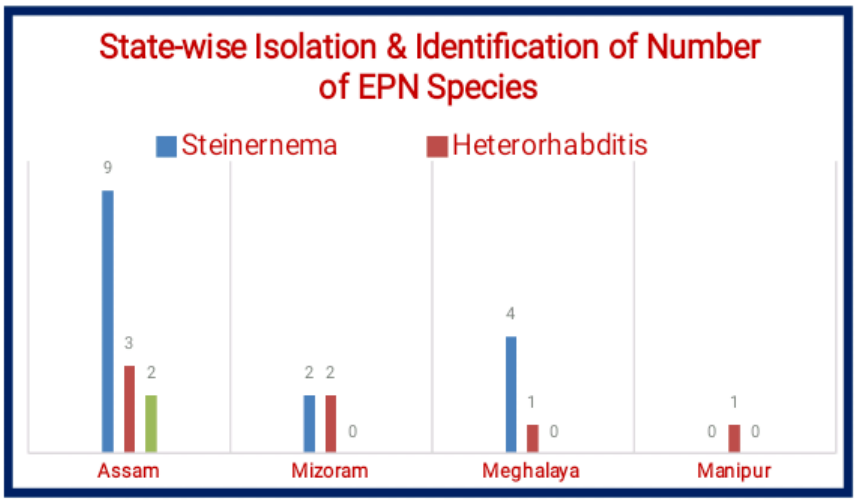


Fig.2: Light microscopic images of- (A) Infective juveniles (10X) (B) 1st generation hermaphrodite (40X) of *Heterorhabditis indica* (Adopted from Nath et al., 2021)

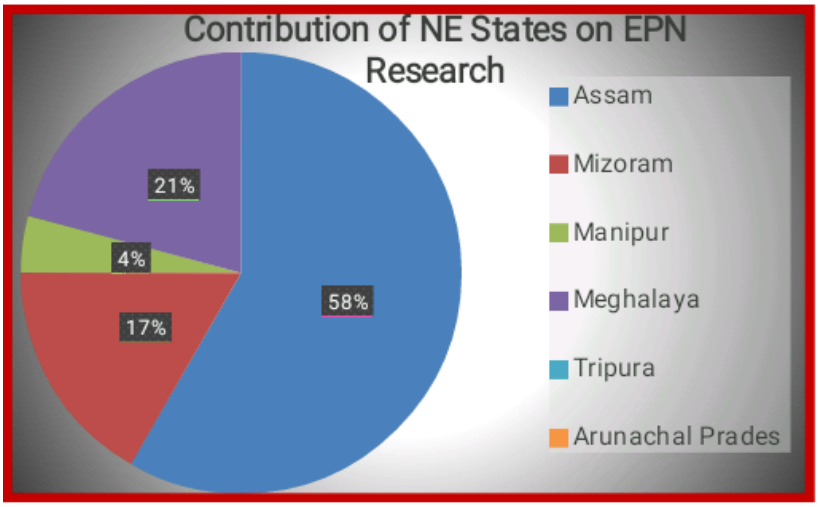


Fig.3: State-wise presentation of number of EPN species identified under three predominant genera - *Heterorhabditis, Steinernema* and *Oscheius* across Northeast India.

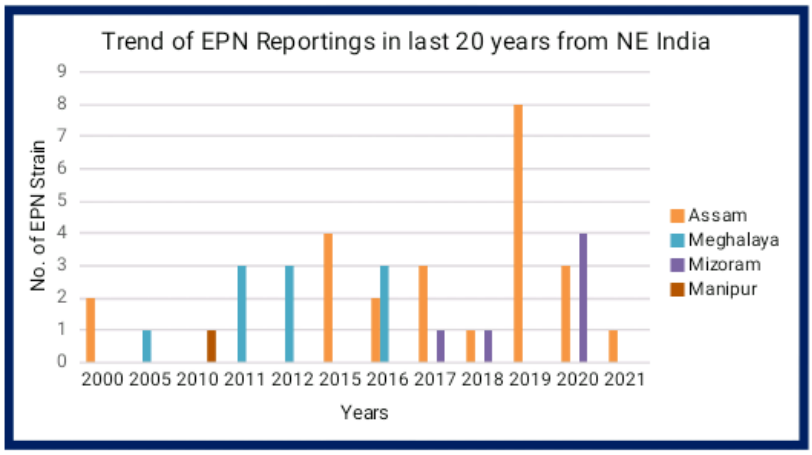


Fig.4: Represents the contribution of various states of NE India in EPN research