White Grub (Coleoptera: Scarabaeidae) Management through Biocontrol Soldier *Heterorhabditis indica* (Entomopathogenic Nematode) on Sugarcane Crop in Western Uttar Pradesh, India (A Success Story)

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Abstract

Heterorhabditis indica from genera Heterorhabditis is an efficient biological control agent, due to their ease of culture, their high fatality against key soil insect pests, and their safety in use. The utilization of entomopathogenic nematodes (EPNs) from the Heterorhabditidae and Steinernematidae families has witnessed rapid growth in recent years, with approximately 100 valid EPN species identified worldwide. These nematodes have gained widespread application as biopesticides for managing insect pests. Numerous EPN species have been successfully commercialized for biological control purposes. In this study, we focused on native EPN strains, which were isolated, identified, and assessed for their effectiveness against coleopteran pests in their larval stage. These locally adapted bioagents exhibit suitability for pest control in sugarcane crops, particularly due to their acclimatization to the local climate. They offer several advantages over chemical pesticides, including enhanced safety for both operators and end-users, absence of withholding periods, precise treatment targeting based on insect population monitoring, a broad host range, effectiveness in controlling larval pest stages within subsoil conditions, active and passive host-seeking capabilities, rapid host-killing ability, soil recycling potential, no harmful effects on non-target soil organisms, beneficial soil insects, humans, and animals, environmental friendliness, and the feasibility of EPN production through both in vivo and in vitro methods. By harnessing EPNs as biological control agents, we can promote responsible and selective pesticide use, reducing the reliance on chemical pesticides for managing soil arthropod pests.

Keywords: White Grub, Bio-control, Entomopathogenic Nematode, *Heterorhabditis indica,* Sugarcane

Introduction

Sugarcane and sugar industry is a vital industry of Uttar Pradesh. A total of 118 sugar mills operated in the state during crushing season 2022-23. The total sugarcane area of the state is 28.53 lakh hectares out of total sugarcane cultivation area 50.00 lakh hectares in India and sugarcane productivity is 839 quintals per hectare of the state. Western UP is the main sugarcane growing belt of the India where most of farmers are cultivating sugarcane cane crop. Farmers of Western UP are facing the problem of white grub infestation in sugarcane and other kharif crops due to resistance development of chemical control. Farmers heavily depend on synthetic pesticides, specifically organophosphates and carbamates, as their primary means of addressing the white grub problem. This practice persists despite reports indicating that numerous species have developed resistance to these pesticides (Qu. et al 2011).Considering the seriousness of the problem, we have conducted field demonstration of application of EPN for the management of white grub on sugarcane and other kharif crops in western UP.

White Grub

White grubs, which are the soil-dwelling larvae of scarab beetles (Coleoptera: Scarabaeidae), pose a significant threat to various agricultural and horticultural plants across the globe (Liu et al. 2009). These pests are particularly destructive to crops such as sugarcane, groundnut, chillies, potato, maize, wheat, barley, jowar, bajra, sesame, sunflower, cotton, tobacco, soybean, brinjal, cucurbits, bhindi, as well as turf, meadows, lawns, and forest trees, among others. India witnessed its first major white grub epidemic in sugarcane in Bihar during 1956. In the Indian subcontinent, there are more than 2000 known species of white grubs, with over 40 of them causing significant damage to a wide range of crop plants. The impact of their infestation is particularly severe in sugarcane, sometimes leading to staggering losses of up to 80-100% (Thamarai Chelvi et al., 2011). We have recorded 32 species of white grubs, prevailing in western UP by conducting pilot survey through light traps and pheromone traps. There are five predominant species of white grub; *Anomala dimidiata* Hope, *Holotrichia consanguinea* Blanchard, *Holotrichia nagpurensis* Khan and Ghai, *Holotrichia serrata* Fabricius and *Maladera insanabilis* Brenske prevailing in western UP. White grubs, once categorized as regional pests, are now recognized as national pests, with reports of their presence and crop damage spanning every state in the country. Presently, white grubs pose a substantial challenge to farmers and scientists across various regions of the nation. Virtually no crop remains entirely immune to or resistant against their infestations. Regions characterized by loose soils and moderate to low

rainfall create favorable environments for the survival and proliferation of these insects, leading to their increasing destructiveness in several states. The larval stage, known for its extended duration and significant destructiveness, results in initial symptoms such as yellowing, stunted growth, and subsequently, the drying and wilting of plants (Joshi et al., 1969).

White grubs are part of the Scarab beetles (Scarabaeidae), a very large family of beetles with more than 30,000 known species around the world (Mittal, 2000). The most common grubs associated with sugarcane fields are in the genera *Holotrichia, Anomala, Cyclocephala,* and *Phyllophaga* and the larvae of White grub beetles are causing damage to a number of crops and sometimes cause heavy economic losses (GC et al 2009). The severity of this pest continues to escalate each year as it expands its presence into regions where it was previously undocumented as a pest. This issue is of utmost gravity and necessitates immediate attention for effective control. Notably, across the nation, several million hectares of farmland are grappling with invasions by white grubs and other subsoil arthropods like root borers, termites, and cutworms. Currently, in India, the management of white grubs and other subsoil arthropod pests relies on chemical pesticides. However, growing concerns about safety, environmental contamination, and the limited efficacy of recommended insecticides underscore the increasing need to develop integrated pest management (IPM) strategies for these pests.

Entomopathogenic Nematodes (EPNs)

Entomopathogenic Nematodes (EPNs) are microscopic non-segmented, elongated, soil inhabiting roundworm without appendages and parasitic exclusively to insect pests. Taxonomical classification of EPNs in animal kingdom is as under:-

Kingdom	: Animals
Phylum	: Nematoda (roundworms)
Class	: Chromadorea
Order	: Rhabditida
Family	: Heterorhabditidae, Steinernematidae
Genus	: Heterorhabditis, Steinernema

Entomopathogenic nematodes (EPNs) are nematodes known for their ability to infect and control insects, making them valuable candidates for biological pest control. They offer distinct advantages over chemical control agents. EPNs have gained recognition as effective biocontrol agents against the larval stages of white grubs (Koppenhöfer et al., 2002). Importantly, EPNs are

harmless to plants and vertebrates, and despite their extensive application in fields, gardens, and pastures worldwide, there have been no significant findings of acute or chronic toxicity to humans or other vertebrate populations (Poinar G.O. Jr. et al., 1982 & Boemare N.E. et al., 1996). Consequently, regulatory bodies like the Environmental Protection Agency (EPA) in the USA, as well as counterparts in India, Australia, and numerous European countries, have exempted EPNs from registration requirements and associated regulations. Entomopathogenic nematodes have a global distribution (Hominick, 2002).

Biological formulations based on entomopathogenic nematodes have been established as one of the most efficient and effective means for the management of white grub and other soil arthropods such as root borer, termites, and cutworm etc. The production of EPN in India is being majorly done through *in vivo* mass multiplication using wax moth (*Galleria mellonella*) caterpillars as the host insect. We have established excellent facility for *in vivo* production of the EPN in our laboratory.

EPNs for the biological control of White Grub

EPNs are capable of managing most soil arthropod pests and completing at least one stage of life cycle inside the host and are considered beneficial to farmers. EPNs' species belongs to family of Heterorhabditidae and Steinernematidae are most effective as they possess certain special characters which suits to restrain insect pest population. The genera Heterorhabditis (family: Heterorhabditidae Poinar 1976) and Steinernema (family: Steinernematidae Chitwood and Chitwood, 1937) have generated much interest as potential biocontrol agents as they carry the lethal symbiotic bacteria Photorhabdus and Xenorhabdus respectively, in their guts. EPNs are quicker in killing soil pests with the help of a symbiotic bacterium (*Photorhabdus* Spp. or *Xenorhabdus* Spp.) live inside their gut. Optimum temperatures for infection and reproduction vary among nematode species and strains (Hazir et al., 2001). Entomopathogenic nematodes (EPNs) are obligate parasite in nature, which gives them the possibility of being used as bio-control agents and therefore represent a good alternative to chemical insecticide (Kaya and Gaugler, 1993). EPNs are versatile and found in every part of the world except Polar Regions. Dauer stage (IJ_3) of EPNs is strong and can survive outside of insect body in soil without food for several months under harsh weather conditions. EPNs are eco-friendly and attack target insect pests only and not beneficial insects like earthworms.

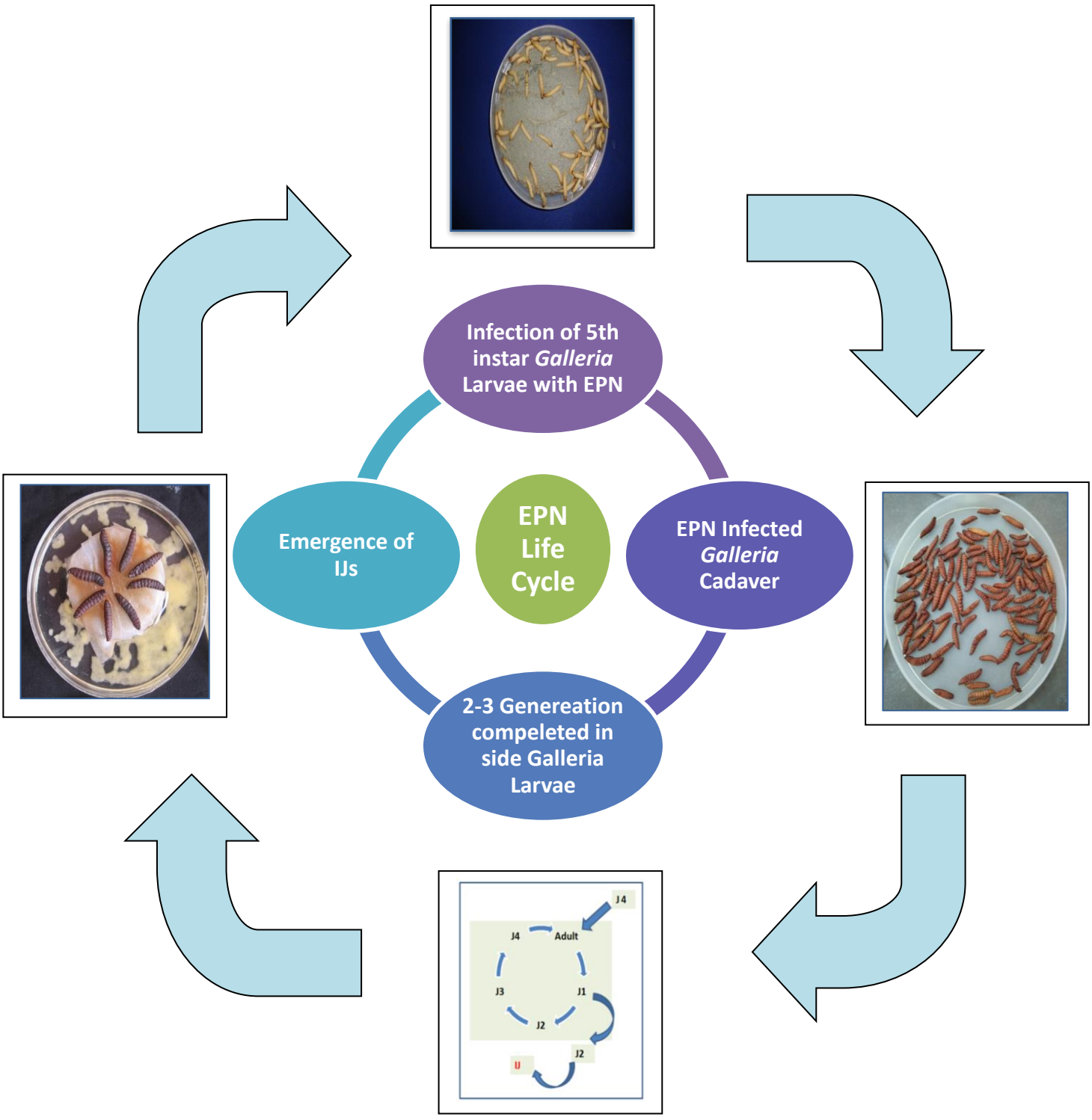
EPNs can be cultured and multiplied in laboratory on host insects. Wax moth (*Galleria mellonella*) is the most suitable host due to its short life cycle, large size and higher biomass. EPNs can be stored in different forms; Wettable Powder (W.P.), granules and sponge for months at ambient temperature. Therefore, EPNs are being considered a noble biological control agent suitable for almost all Indian agro-climate zones and are becoming one of the best components in Integrated Pest Management (IPM) for control of a wide range of soil insect pests of different crops.

Life cycle of EPN

The third juvenile stage of EPNs is known as the "infective juvenile (I.J.)" or "dauer" stage, and it is the sole nematode stage present in soil. Heterorhabditis and Steinernema nematodes exhibit differences in their reproductive methods. In Heterorhabditis, the initial generation individuals are produced by self-fertile hermaphrodites, while subsequent generations result from cross-fertilization involving both male and female (amphimictic). In Steinernema nematodes, except for one species, all generations are generated through cross-fertilization involving both males and females (amphimictic). The emergence of infective juveniles from the host typically takes around 5-7 days for Steinernema and 8-10 days for Heterorhabditis. Depending on the availability of food resources, they typically complete 2-3 generations within the insect cadaver before emerging as I.J.s to seek new hosts.

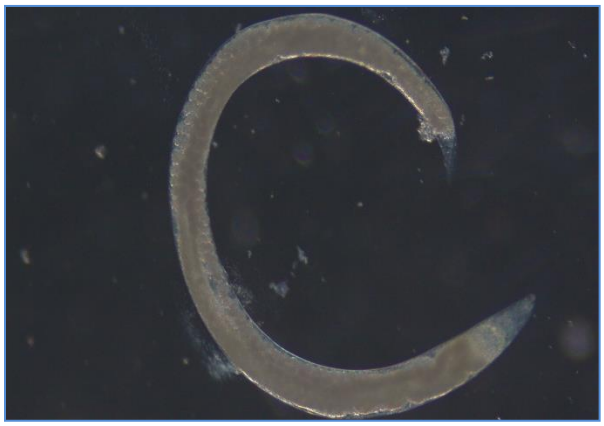
EPNs IJs are free living and always seen in the soil water film hunting insects by adopting either of the two strategies; ambushing and cruising to locate the enemies. Ambushers belong to *Steinernema* genus, remains stationary at or near the soil surface by standing on their tail, so that most of their body is in the air. Ambushers most effectively control insect pests that are highly mobile at the soil surface, such as cutworms, armyworms, and mole crickets. Other group of EPNs is cruisers who usually belong to *Heterorhabditis* genus, are highly mobile and can move through the soil profile. Cruisers locate their host by sensing carbon dioxide or other volatiles released by the host and are most effective against sedentary and slow-moving insect pests at various soil depths, such as root (white) grubs and root weevil grubs.

Once the target pests are located, numerous EPN soldiers infiltrate a single host's body through its natural openings, which include the mouth, anus, and spiracles. In the case of Heterorhabditis, they can also enter through the inter-segmental membrane of the host's cuticles. The infective juveniles (I.J.s) then actively penetrate the insect's mid-gut wall, entering the insect's hemocoel filled with hemolymph. Inside the insect host's body, they release their bacterial symbionts and transform into fourth-stage juveniles and subsequently into adults. The insects typically succumb due to septicemia, with occasional instances of bacterial toxaemia preceding the septicemia (Forst S. et al., 1997). Once released into the host's body, the bacteria rapidly multiply and lead to the host's demise within 24 to 48 hours, sometimes longer depending on the insect pest's biomass and developmental stage. The EPNs feed on both bacteria and the decomposing host tissue, maturing into the adult stage. These adults can give rise to hundreds or even thousands of new juveniles, and multiple life cycles may occur within a single insect host. When the insect host's internal resources are fully consumed, the infective juveniles, equipped with a fresh supply of bacteria in their gut, exit the empty host shell, enter the soil, and initiate their quest for a new host. An outer protective cuticle shields the infective juvenile, safeguarding it from harsh environmental conditions and potential predators.



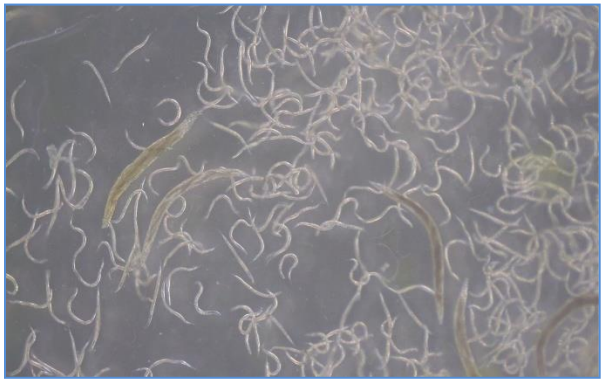
Life Cycle of EPN inside the Host Body (*Galleria mellonella)*

Photographs showing the different stages of EPN (*H. indica*) multiplication inside the *Galleria mellonella*

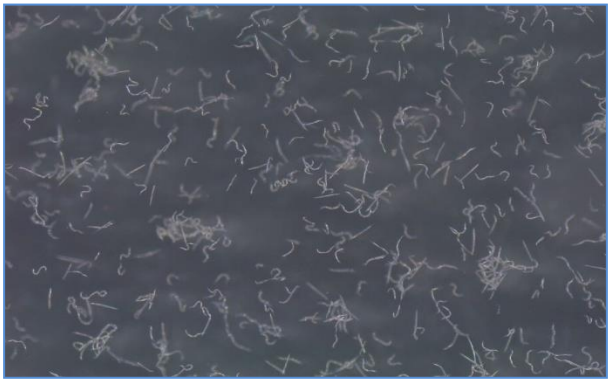


Hermaphodictic female with eggs 1st Generation

Male and Female 2nd Generation



Endotokiamatricida Releasing IJ_3



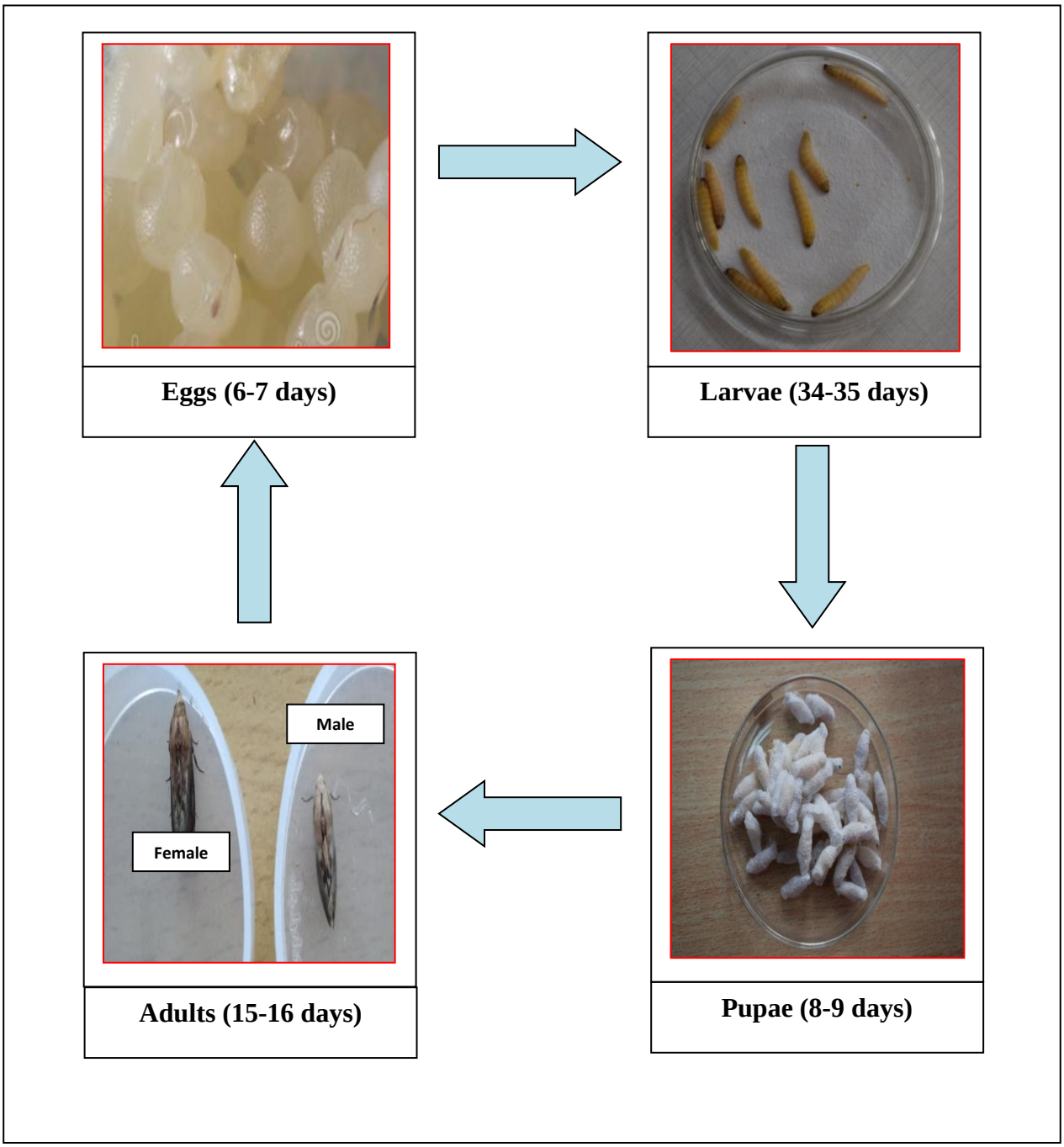
Infective Juveniles 3rd Generation (IJ_3)

EPN (*H.indica*) emergence from *Galleria* cadaver

Mass Rearing of Host Insect *Galleria mellonella* for *in vivo* multiplication of EPN

For mass production of EPNs *in vivo*; the fifth instar larvae of wax moth is the ideal for maximized multiplication of EPNs. We have established an excellent Insectory of Mass Rearing of Host Insect *Galleria mellonella* at Ghaziabad in western Uttar Pradesh. Mass rearing in the laboratory using semi-synthetic diet offers the most dependable and preferred method of obtaining large-scale, uniform, and constant supplies of fifth instars wax moth larvae. Mass multiplication of EPNs can be developed as cottage industry by mass rearing of wax moth. A semi-synthetic diet with modified composition can be used for mass rearing of wax moth, at 28±2° C, relative humidity 65±5% and 12:12 scoto-photo-phase regime. This diet successfully supports the growth and development of wax moth. All the components of the diet are easily available and economical too, as the cost of 1 kg diet was up to Rs. 200-250 only, for rearing 1000 larvae successfully. The semi-synthetic diet developed is used for large scale production of larvae by mass rearing of wax moth throughout the year in laboratory and for commercial production of Entomopathogenic Nematodes (Jagpal Singh, et al 2019).

Life Cycle of *Galleria mellonella* (Wax Moth)



Mass Rearing of *Galleria mellonella* (Wax Moth) in Controlled Conditions

Mass rearing view in Insectory

Eggs

Larvae

Pupae

Adults

Isolation and Identification of Entomopathogenic Nematodes

A total number of 947 soil samples were collected from different locations in western Uttar Pradesh. A total of 3 random samples at a depth of 15 cm, within the area of 2-meter square for each sampling site, were taken out and placed each sample in a plastic bag and labeled with a waterproof marker (including locality/area/site name, date, crop, temperature). After conducted baiting method for isolation of EPN we have isolated 9 strains of EPN.

The nine strains of EPN isolated from soil samples collected from different locations were sent for identification to the Division of Nematology, Indian Agricultural Research Institute, New Delhi. Molecular Characterization and DNA sequencing were conducted in the identification process revealed that out of 9 strains of isolated EPN, five strains were identified as *Heterorhabditis indica* and four strains were identified as (2 *Steinernema siamkayai* & 2 *Steinernema abbasi*). The native strains of EPN are more suitable for release against local insect pests because of adaptation to local climate and other population regulators (Bedding, 1990).

The molecular identification of samples using ITS marker and partial sequence obtained by sequencing PCR amplicon are given below: -

Heterorhabditis indica
>S1_JPS_ITS
TGAGCTGTTTCGAGAAGAGTGGGGACTGCTATATCGGGGCTTTCGGGCTCTGGTATGATGGAAACCATTTTAATCG
CAATGGCTTGAACCGGGCAAAAGTCGTAACAAGGTATCTGTAGGTGAACCTGCAGATGGATCATCGTCGATACCTT
ATAGGTACATGCTGATCACGAGATGCCGATAATCATGGAATCAGGCTTGTTCTTGGTTCCAGTCGGTGTCTCACCC
CATCTAAGCTCTCCGTGAGGTGTCTATTCTTGATTGGAGCCGCTTTGAGTGACGGCAATGATAGTTGGGTATGTTCC
CCGTGAGGGTAGAGCATAGACTTTATGAACAGAGCTGGGCTGTCGCCTCACCAAAAACCATCGATAACTGGTGGC
TGAGTGAGAAATCACTGGATCTGCTATGCAGGGAGCCTTAATGAGTTGGTCTTCACC

Heterorhabditis indica
>S2_JPS_ITS *Heterorhabditis indica*
TGAGCTGTTTCGAGAAGAGTGGGGACTGCTATATCGGGGCTTTCGGGCTCTGGTATGATGGAAACCATTTTAATCG
CAATGGCTTGAACCGGGCAAAAGTCGTAACAAGGTATCTGTAGGTGAACCTGCAGATGGATCATCGTCGATACCTT
ATAGGTACATGCTGATCACGAGATGCCGATAATCATGGAATCAGGCTTGTTCTTGGTTCCAGTCGGTGTCTCACCC
CATCTAAGCTCTCCGTGAGGTGTCTATTCTTGATTGGAGCCGCTTTGAGTGACGGCAATGATAGTTGGGTATGTTCC
CCGTGAGGGTAGAGCATAGACTTTATGAACAGAGCTGGGCTGTCGCCTCACCAAAAACCATCGATAACTGGTGGC
TGAGTGAGAAATCACTGGATCTGCTATGCAGGGAGCCTTAATGAGTTGGTCTTCACCTACTCAACCGCCACTATCG
GTAATCTATTCCCAATTAACTTGTTTCTAGTAAAAGGCTAAATTAGTCAGTGGAAAATAGCCTTAGCGATGGATCG
GTTGATTCGCGTATCGATGAAAAACGCAGCTAGCTGCGTTATT

Heterorhabditis indica
>S3_JPS_ITS
TGAGCTGTTTCGAGAAGAGTGGGGACTGCTATATCGGGGCTTTCGGGCTCTGGTATGATGGAAACCATTTTAATCG
CAATGGCTCGAACCGGGCAAAAGTCGTAACAAGGTATCTGTAGGTGAACCTGCAGATGGATCATCGTCGATACCT
TATAGGTACATGCTGATCACGAGATGCCGATAATCATGGAATCAGGCTTGTTCTTGGTTCCAGTCGGTGTCTCACC
CCATCTAAGCTCTCCGTGAGGTGTCTATTCTTGATTGGAGCCGCTTTGAGTGACGGCAATGATAGTTGGGTATGTTC
CCCGTGAGGGTAGAGCATAGACTTTATGAACAGAGCTGGGCTGTCGCCTCACCAAAACCCATCGATAACTGGTGG
CTGAGTGAGAAATCACTGGATCTGCTATGCAGGGAGCCTTAATGAGTTGGTCTTCACCGACTCAACCGCCACTATC
GGTAATCTATTCCCAATTAACTTGTTTCTAGTAAAAGGCTAAATTAGTCAGTGGAAAATAGCCTTAGCGATGGATC
GGTTGATTCGCGTATCGATGAAAAACGCAGCTAGCTGCGTTATTTACCA

Sample 4 - Steinernema abbasi (= S. thermophilum)
>S4_JPS

TTAAAGTAGCTGGCTTGGCTCGCCACTATTTATCCATATTGTTTAACGTTTGTTATGTGTTGTTACACCACTTGCAG
GTGTATTGATTAATATAATCAAGTCTTACCGGTGGATCACTTGGTTCGTAGATCGATGAAAAACGGGGCTAGAACC
GTTATGTAGCGTGAATTGCAGACATATTGAGCGCTAAAATTTTGAACGCAAATGGCACTAACAGGTTTATATCTGT
TAGTACACTTAATTGAGGGTTGATTAACTTGTTACTTGCAGTCAGCTGTGACTGTTTTTTCGAATAGCTAAGTGCTT
TTTGCATTTACCTATTTGGCATGATTGCTACGATAGTACAATGAACTTTTCTGTTCTTAAGTTTCTTGACGAATTGTT
CGCTATCTTATCGACTCTTTGCAAAGTATTAGTTTTTGTTTGGCGTGTTTCTTGCCGACTGACTTATACACTTTCCGT
GTATGTAGACTGTTTTGTCATGTCAGTTAAAAACTTTTACTAATTCAACGCGTTTGTTGATTAGTGTTGCTTTTGCTA
AGATGTTTTGTTATCGA

Sample 5 - Steinernema siamkayai
>S5_JPS
AGACCGGCGGCCCGGGGTCTGAGTTGTTTCGAGAAAGCGGAGATTGCGATGTTGAATGTTTTCGGATGTTCTTTAT
TGCGAGAACCGCGTTAATCCAATCGGCTTGAACCGGGCAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGG
AAGGATCATTATTGAGCTAATTTCTTCCATTTAATCAGGTCTTTGCTGTTTGTCTCTAAGCATTGACTTGATCTTGCT
TTGAATGGTTTCTAGAGACGTTTGGAGCAGTCATTTAAGCGTGACTGTGATGATGAGCGTTTTACTTTGCTTGCATT
TCGCTGTTTCTTGAATGCTTAGCAATGAGAATTAAAGAGGTCTGCTGACTCTCCATTTTTATTGATAACAAAAACTT
TTTGTTTTGATTTTTGTGTCACTTGTTGATGCATTATTCAATTATCAAGTCTTATCGGTGGATCACTCGGTTCGTAGG
TCAATGAAAAACGGGGCAAAAACCGTTATTTGGCGTGAATTGCATACATATTGAGCGCTAAAATTTTGAACGCAA
ATGGCACTAACAGGGTTATCTGTTAGTATGTTCAATTGAGGGTCTTTTGACTAGAATCTGGCAATCGGCTGTGATTG
CTTTTTCGGAAAGTTATTTTGCTTTTTCTAAAGTGAAGTACC

Sample 6 - Steinernema Siamkayai
>S6_JPS
TGTTTTGATTTTTGTGTCACTTGTTGATGCATTATTCAATTATCAAGTCTTATCGGTGGATCACTCGGTTCGTAGGTC
GATGAAAAACGGGGCAAAAACCGTTATTTGGCGTGAATTGCAGACATATTGAGCGCTAAAATTTTGAACGCAAAT
GGCACTAACAGGGTTATCTGTTAGTATGTTCAATTGTGGGTCTTTTGACTAGAATCTGGCAATCGGCTGTGATTGCC
TTTTCGGAAAGTTATTTTGCTTTTTCTAAAGTGAAGTACCTTTTTGGTATGGCTATTTGATTGTCTAATGGATGTCTG
GTTAGCTGTTTCTTTGCTAGACGTCTGCAATCATTTGGCTTTGCGTAGTGTTTGAATAATAGGTTAGCGCGTTTCTTG
CTAAATGACTTTTGCACAAGCAAGTGTAATACGTTTCTTAAAGTCAGCTTTTATTCAATTTGGTTTTCTGACTTGATT
TGaCGGTTTACTGTGCTATGCTTTGTCAATCTTTTCGAACTAGACCTCAA

Sample 7 - Steinernema abbasi (= S. thermophilum)
>S7_JPS
AAAGTAGCTGGCTTGGCTCGCCACTATTTATCCATATTGTTTAACGTTTGTTATGTGTTGTTACACCAGTTGCAGGT
GTATTGATTAATATAAACAAGTCTTACCGGTGGATCACTTGGTTCGTAGATCGATGAAAAACGGGGCTAGAACCGT
TATGTAGCGTGAATTGCAGACATATTGAGCGCTAAAATTTTGAACGCAAATGGCACTAACAGGTTTATATCTCTTA
GTACACTTAATTGAGGGTTGATTAACTTGTTACTTGCAGTCAGCTGTGACTGTTTTATCGAATAGCTAAGTGCTTTT
TGCATTTACCTATTTGGCATGATTGCTACGATAGTACAATGAACTTTTCTGTTCTTAAGTTTCTTGACGAATTGTTCG
CTATCTTATCGACTCTTTGCAAAGTATTAGTTTTTGTTCGGCGTGTTTCTTGCCGACTGACTTATACACTTTCCGTGT
ATGTAGACTGTTTTGTCATGTCAGTTAAAAACTTTTACTAATTCAACGCGTTTGTTGATTAGTGTTGCTTTTGCTAAG
ATGTTTTGTTATCGATTTTGCTAT

H. indica Muzaffarnagar strain D2/D3 (GebBank Accession Number OM149711)
TTCCACCAGAGTTTCCTCTGGCTTCGTCCTGCTCAAGCATAGTTCACCATCTTTCGGGTCGCAACCTACACGCTCTA
CCGCTGCCCATCTGCAAGCAGACAAGACAGGGCCATGGTGCTCCGTTTCGAAGAAGTTCAGTCGGATCCCATGTCA
ACCGGTTAACCGGTCTTTACTTTCATTATGCCATAAGGTTTCCTCAGCCCTTTGACTCGCGTGTAAATTACACTCCT
CGGTCCGTGTTTCAAGACGGGTCGGAAAGGTGGTTAACTTTCACACTGACTCCCTAGAAACTAAGGCTTGACGTTA
ACCATGACAAACCTCCCAATAAGCAAGACACCACAATGTGGGCAACACTACATTGTTAGGAAAGCATGATCAACG
CAGTCAGCGCAACAACAGGTAGCGTCCACCCCCCAAAGCCACAGCTAAGCGACTATAGAGAATATAGCTACCAAG
TTATGTTAACTCTCTCCGGTTCCACTTCAGCGATTTCACGTTCTCTTGAACTCTCTCTTCAAAGTTCTTTGCAACTTT
CCCTCCGGGTACTTTGTAGAAAATAAAATCTCCAGGGACTTCGAAGTCGCGGGAGATCTATGACAAGTACCGTGA
GGGAAAGTTG

H. indica Saharanpur strain D2/D3 (GenBank Accession number OM149712)
TTTCCTCTGGCTTCCTCCTGCTCAAGCATATTTCACCATCTTTCGGGTCGGAACCTACACGCTCTACCGCTGCCCAT
CTGCAACCAGACAAGACAGGGCCATGGTGCTCCGTTTCCAAGAAGTTCAGTCGGATCCCATGTCAACCGGTTAACC
GGTCTTTACTTTCATATATGCCATAAGGTTTCCTCAGCCCTTTGACTCGCGTGTAAATTACACTCCTCGGTCCGTGTT
TCAAGACGGGTCGGAAAGGTGGTTAACTTTCACACTGACTCCCTAGAAACTAAGGCTTGACGTTAACCATGAGCA
AACCTCCCAATAAGCAAGACACCACAATGTGGGCAACACTACATTGTTAGGAAAGCATGATCAACGCAGTCAGCG

CAACAACAGGTAGCGTCCACCCCCCAAAGCCACAGCTAAGCGACTATAGAGAATATAGCTACCAAGTTATGTTAA
CTCTCTCCGGTTCCACTTCAGCGATTTCACGTTCTCTTGAACTCTCTCTTCAAA

All identified seven strains of EPN had been submitted to NCBI GenBank with allocated accession numbers. (Table 1)

Table -1 List of Isolated Native EPN

Sr. No.	Molecular identification by ITS marker	GenBank Accession Numbers
1.	*Heterorhabditis indica*	MK078600
2.	*Heterorhabditis indica*	MK078601
3.	*Heterorhabditis indica*	MK078602
4.	*Heterorhabditis indica*	OM149711
5.	*Heterorhabditis indica*	OM149712
6.	*Steinernema thermophilum/abbasi*	MK078603
7.	*Steinernema siamkayai*	MK078604
8.	*Steinernema siamkayai*	MK078605
9.	*Steinernema thermophilum/abbasi*	MK078606

Galleria mellonella larvae inoculation with EPN in Polylab Petri Plates

For obtaining satisfactory level of infection, the Pipette Method (Dutky et al., 1964) is followed for inoculation of *Galleria* larvae by using EPN, *H. indica*. Fully grown 5th instar larvae of host insects reared on artificial diet are infected / inoculated with EPNs. Inoculation process is done by inoculation of EPNs on absorbent paper placed in bottom of plastic Petri dish (8" Diameter) of Polylab by adding EPN,@ 50 I.J.s/larvae by pipette. After that 50 - 60 numbers of 5th instar larvae of host insects put into the each Petri and covered with lid and kept at 28±2^{0} C temperature for 48 hrs in the dark. In 48 hrs 95 % *Galleria* larvae will get infected with EPN. All EPN infected *Galleria* Cadaver (G.C.) are collected from all Petri dish and put in to the perforated tray for conditioning.

Petri Plates with Filter Paper

Filter Paper moist with EPN culture

Transferred 5th instar *Galleria* Larvae

Cover the plates and Keep in the dark at 28^0c

Galleria cadaver after infected by EPN

Efficacy of Entomopathogenic Nematodes against *Galleria mellonella* L.

As we have isolated local strain of EPN, we investigated the use of locally isolated three strains of EPNs; *H. indica* (MK078602), *S. abbasi* (MK078603), and *S. siamkayai* (MK078604) against *Galleria mellonella* larvae by conducting bio-assay in our laboratory. The detailed investigation is given as under: -

Materials and Methods

The wax moth *Galleria mellonella* was reared at 27 ± 2°C with a light/ darkness (LD) photoperiod of 12:12 hr and RH of 70 ± 10%, on artificial diet (Singh et al. 2019). The three native nematodes, *H. indica* (MK078602), *S. abbasi* (MK078603), and *S. siamkayai* (MK078604), isolated from districts of Uttar Pradesh were used. The nematodes were multiplied with fifth instar larvae. The infective juveniles (IJs) that emerged from the Galleria cadavers were gathered in a solution of 0.01% formalin in water, as described by Shapiro et al. (2006), utilizing White's trap. Subsequently, these IJs were stored in darkness within a temperature range of 10-15°C for a period of 2 weeks, pending further utilization. Prior to their use in bioassays, the IJs were permitted to acclimatize at room temperature for 1 hour, and their viability was confirmed by observing their movements through a Leica S9i stereozoom microscope. Bioassays to assess larval mortality were conducted in Petri dishes lined with a single layer of Whatman No. 1 filter paper. For each EPN species strain 10, 20, 40, 60, 80 and 100 IJs/ larva were applied. The lethal dose (LD_{50} and LD_{90}) were calculated at 24, 36 and 48 hr after inoculation. The experiment was repeated thrice with ten replicates including one untreated (control). The Petri dishes were placed in a BOD incubator at a controlled temperature of 27±2°C for a duration of 48 hours. The assessment of larval mortality was conducted at specific intervals, namely, 24, 36, and 48 hours following the inoculation. To determine the lethal dose, both LD50 and LD90 values were calculated using probit analysis as described by Finney (1971).

Results and Discussion

The virulence of three species of EPNs, viz., *H. indica, S. abbasi* and *S. siamkayi* was evaluated against the 5th instar larvae of *G. mellonella* under laboratory conditions. The number of IJs/ larva was observed to be positively correlated with the time of larval mortality and *H. indica* was found to be the most virulent (Table 2). After 48 hr of inoculation, *H. indica* was found to be the most effective (LD_{50} 4.603 IJs/larva) as compared to *S. abbasi* (LD_{50} 7.118 IJs/larva) and *S. siamkayai* (LD_{50} 10.663 IJs/larva); *S. siamkayai* consumed more time and required higher dose for causing maximum mortality. At initial inoculation of 10 and 20 IJs/ larva, zero % mortality in *G. mellonella* larvae was observed; however, at higher dose of 40, 60, 80 and 100 IJs/ larva caused 26.67, 40.00, 46.67 and 53.33% mortality after 24 hr of inoculation. Maximum mortality at lower dose (40 IJs/larva) was observed in *H. indica* (26.67, 83.33, 100 %) followed by *S. abbasi* (20, 73.33 and 93.33%) and *S. siamkayai* (13.33, 66.67 and 86.67%) at 24, 36 and 48 hr of inoculation. These observations are in agreement with those of Kalia et al. (2018), who studied the virulence of three native EPN strains of *Heterorhabditis* sp. against *Helicoverpa armigera, Spodoptera litura* and *G. mellonella*, and stated that EPN strains varied considerably in LC_{50} and LT_{50}.

Table - 2 LD50 and LD90 and dosage response of EPNs against *G. mellonella* larvae (Riazuddin et al. 2020)

Species/strains of EPNs	LD_{50}	Confidence limit 95%		LD_{90}	Confidence limit 95%	
		Lower	Upper		Lower	Upper
24 hr after inoculation						
H. indica	83.018	65.307	127.997	232.328	143.568	887.091
S. abbasi	115.021	85.157	248.927	375.512	195.543	2920.592
S. siamkayai	148.958	119.608	215.263	518.754	321.768	1213.863
36 hr after inoculation						
H. indica	12.304	5.721	18.016	44.538	30.927	88.060
S. abbasi	19.746	14.227	25.082	67.038	50.882	103.081
S. siamkayai	25.986	22.471	29.521	104.655	86.932	133.157
48 hr after inoculation						
H. indica	4.603	2.131	6.576	13.811	11.146	17.026
S. abbasi	7.118	4.992	9.029	24.607	20.906	29.912
S. siamkayai	10.663	6.064	14.730	39.354	29.643	60.695

Umamaheswari et al. (2004) and Saravanapriya and Subramanium (2007) reported *H. indica* as highly virulent against *S. litura* (LC_{50} 3.5 and 7 IJs/ larva with 50% mortality in a minimum time of 34.53hr/ larva. These observations align with the previously reported findings of Divya et al. (2010) who reported that early instar larvae of *H. armigera, S.litura* and *G. mellonella* were more susceptible to *H. indica*; *H. indica* registered lowest LC_{50} of 6.81 IJ/larva and LT_{50} of 23.42 hr/ larva for *S. litura* (Radhakrishnan and Shanmugam, 2017). Shapiro-Ilan et al. (2006) reported

that 25 IJs (10.2 IJs/ cm2) and 55IJs (20.5 IJs/cm2) are required to achieve 50% mortality of *G. mellonella* with *H. indica*; *H. indica* is a highly effective entomopathogenic nematode with different degrees of virulence depending on the dose and time. Present data shows that *H. indica* is more virulent as only 4.603 IJs were required to kill 50% and 13.811 IJs were required to kill 90% of the *G. mellonella* larvae at 24 hr. Rosalba et al. (2019) observed a LD_{50} of 1.4IJs/cm2 of *H. indica* MOR03 against *G. mellonella* larvae and Noosidum et al. (2010) reported LD_{50} as 1.99-6.9 IJs (0.9-3.4 IJs cm2). The objective of IPM is to significantly reduce the population of the target pests to bring it below the economic threshold level. If the application of EPNs proves effective against the target pest, longer-term management may be attainable. The current study has demonstrated that EPNs exhibit satisfactory efficacy against lepidopteran pests. It can be concluded that H. indica stands out as the most promising EPN for controlling G. mellonella. Furthermore, these EPNs have shown good compatibility with pesticides (Lalramliana and Yadav, 2009), indicating their potential inclusion in IPM strategies.

***In vivo* evaluation of indigenous strain of *Heterorhabditis indica* against *Holotrichia serrata* F.**

After investigated the use of locally isolated three strains of EPNs; *H. indica* (MK078602), *S. abbasi* (MK078603), and *S. siamkayai* (MK078604) against *Galleria mellonella* larvae, the investigation revealed that the locally isolated strain of *H. indica* (MK078602) was found very effective as compared to *S. abbasi* (MK078603), and *S. siamkayai* (MK078604). After that we have gone for *in vivo* evaluation of locally isolated strain of *Heterorhabditis indica* (MK078602) against the white grub species *Holotrichia serrata* F. The detailed study is given as under: -

Materials and Methods

The native strain of *H. indica* (MK078602) isolated from village Sabitgarh, Bulandshahr district in Uttar Pradesh was used. This EPN strain was propagated within fifth instar larvae of the wax moth Galleria mellonella. The infective juveniles (IJs) that emerged from the Galleria cadaver were harvested using White's trap and placed in a solution of 0.01% formalin water (Shapiro et al., 2006). They were subsequently stored in darkness at temperatures ranging from 10 to 15°C until they were ready for use, up to 2 weeks later. Prior to their use in bioassays, the IJs were allowed to acclimate at room temperature for one hour, and their viability was assessed by observing their movements under a Leica S9i stereozoom microscope. *H. serrata* adult beetles were collected from host trees, viz., Neem (*Azadiracta indica*), Sheesham (*Dalbergia sissoo*), Guvava (*Psidium guajava*) from Dabana village, Ghaziabad using light trap. The collected mated female beetles were placed in desiccators containing moist soil for oviposition and monitored for eggs on alternate days. The collected eggs were transferred to Petri dishes containing moist soil. The newly emerged neonates were transferred individually on live maize roots and reared individually up to pupation in controlled conditions (30±20 C, 70±5% RH, 12:12 hr Scotophase-photoperiod).

Grub mortality bioassay was carried out in individual plastic pot (6 cm deep and 3 cm diameter) by using soil incorporation method. The sterilized sandy soil (autoclaved at 121°C under 15 PSI before use) was adjusted by 25% moisture by adding distilled water. 100, 200, 300, and 400 IJs/100 gm soil were applied for both first and second instar grubs separately. The experiment was repeated thrice with ten replicates including one untreated control treatment. The experimental pots were kept at environmentally controlled chamber at 30±20 C, 70±5% RH, 12:12 hr scotophase- photoperiod) and grub mortality was checked after 24, 48, 72, 96, 120, 144 and 168 hrs after inoculation. The dead brick red coloured grubs were collected and kept on white trap for release the IJs to check the emergence reason of EPN. The number of dead grubs due to EPN infection was recorded, and mortality data were subjected to probit analysis (Finney, 1971).

Results and Discussion

The entomopathogenic nematode *H. indica* (strain MK078602) was found effective against first and second instar grubs of *H. serrata* under laboratory conditions. LD_{50} and LD_{90} values at different exposure times were calculated against 1st and 2nd instar grubs. No mortality was observed up to at 24 and 48 hr of inoculation. It was also observed that increasing dosage level resulted in a reduction in the values of estimate lethal time (Table 3). The number of IJs/grub was observed to be positively correlated with the time of inoculation. Among the lowest lethal dose IJs per grub; LD_{50} and LD_{90} (LD_{50} 89.601, LD_{90} 226.200 for 1st instar and LD_{50} 115.050 and LD_{90} 722.164 for 2nd instar) was obtained at the maximum of 168 hr after inoculation followed by (LD_{50} 102.256, LD_{90} 356.024 for 1st instar and LD_{50}189.854 LD_{90} 822.532 for 2nd instar) at 144 hr, (LD_{50} 130.798, LD_{90} 464.958 for 1st instar LD_{50} 263.104 and LD_{90}1229.798 for 2nd instar) at 120 hr, (LD_{50} 150.723, LD_{90} 705.839 for 1st instar and LD_{50} 263.104, LD_{90} 1229.798 for 2nd instar) at 96 hr and (LD_{50} 228.529, LD_{90} 957.248 for 1st instar and LD_{50} 304.621, LD_{90} 623.590 for 2nd instar) at 72 hr.

Table -3 Efficacy of *H indica* against *H. serrata* (Swati et al. 2020)

Mortality in early instar grub Confidence limit 95%				
Hr. after Treatment	1st instar		2nd instar	
	LD50	LD90	LD50	LD90
72	228.529	957.248	304.621	623.590
	(-)	(-)	(210.731±673.695)	()402.792±410.734
96	150.723	705.839	263.104	1229.798
	(119.181±177.808)	(518.259±1219.000)	(225.705±315.531)	(811.528±2634.811)
120	130.798	464.958	263.104	1229.798
	(105.217±152.565)	(378.113±642.162)	(225.707±315.531)	(811.528±2634.811)
144	102.256	356.024	189.854	822.532

	(77.275±122.921)	(-)	(-)	(-)
168	89.601	226.200	115.050	722.164
	(-)	(-)	(-)	(-)

These observations are in agreement with those of (Kalia et al. 2018) who studied the virulence of three native EPN strains of *Heterorhabditis* sp. against *Helicoverpa armigera*, *Spodoptera litura*, and *Galleria mellonella*; and stated that EPN strains varied considerably in terms of both LC_{50} and LT_{50}. *H. indica* is a highly effective entomopathogenic nematode against white grub *H. serrata* with different degrees of virulence depending on the dose and time. Present data shows that *H. indica* is virulent and only 89.601, 115.050 IJs are required to kill 50%; and 226.200, 722.164 IJs required to kill 90% of *H. serrata* 1st and 2nd grubs, respectively at 168 hr after treatment. These observations agree with those of (Chandel et al. 2018) who reported that LC_{50} for 1st, 2nd and 3rd grub of *H. serrata* was 85.25, 141.83 and 300.17, respectively after 10 day of inoculation. The superiority of *H. indica* was reported by (Maneesakorn P. et al. 2010) who reported that *H. indica* strains were more virulent at 5 days after treatment under laboratory conditions against Japanese beetle, *Papillia japonica* with LC_{50} value of 136 IJs/ grub. Likewise, (Singh et al. 2001) observed that *H. bacteriophora* was more virulent at 4 days after treatment against *H. consanguinea*.

The local strain of entomopathogenic nematode *H. indica* MK078602 was thus observed promising against white grub *H. serrata* within 7 days after treatment. The goal of IPM is to kill a large number of the target pests to bring it below the economic threshold level. If *H. indica* local strain application can occur successfully against the target pest, the longer term management might be achievable. The EPNs showed good compatibility with the pesticides (Lalramliana and Yadav, 2009). Hence, these can be incorporated as a potential biocontrol agent in IPM strategies of root grubs and other soil arthropods that are pests of sugarcane.

Application Techniques of Entomopathogenic Nematodes (EPN)

Most importantly, it is crucial to select the appropriate nematode species that matches the target pest. Factors to consider when choosing the right nematode include its virulence, host-finding ability, environmental tolerance, and, in some cases, persistence. Typically, an effective strain of EPNs is applied to the soil at a rate ranging from 100,000 to 250,000 IJs per square meter. Depending on the specific target pests, a higher application rate may be necessary. Recycling potential should also be taken into account. Generally, when environmental conditions are favorable, nematode populations will remain at a level that ensures effective pest control within 2 to 3 weeks after application. To enhance the efficacy of EPN applications, improved formulations can be employed. Considerable progress has been made in recent years in the development of EPN formulations, particularly for aboveground applications. These formulations may involve mixing EPNs with surfactants and polymers. Furthermore, increased

efficacy can be achieved by applying EPNs to leaves with the addition of surfactants to enhance leaf coverage.

The methods of application of EPNs in crop fields are as:

Application in the form of EPN infected *Galleria* Cadavers (GC)

A novel approach that has garnered interest involves delivering EPNs within their infected host cadavers. This cadaver-based application approach offers advantages over the conventional method of applying nematodes in aqueous suspension. Reported benefits include enhanced nematode dispersal, infectivity, survival, and efficacy, primarily due to the emergence of EPNs in their natural habitat. The period of 10 days after infection and application of GC in soil has thus been recommended, resulting in higher emergence of IJs when using the cadaver application approach. The EPN infected GC shall be applied in sub soil as 8-10 cm depth by making holes in soil (Dibbling method) followed by light irrigation after application and at interval of 10-15 days as per the requirement to maintain good moisture condition in soil. 2000 GC per acre having 2.5 lakh numbers of IJs in each GC may be applied in the field.

EPN Infected *Galleria Cadaver* (GC)


Photographs showing Application of EPN infected GC in the field by Dibbling Method

Application in the form of W.P. Formulation

The 8 kg of W.P. formulation containing 50,000 IJs of EPN per gram mix with 100 kg of dry sand/soil may be applied at root zone followed by light irrigation at an interval of 10-15 days as per requirement and maintain adequate moisture in the soil.




Photographs showing Application of W.P. formulation of EPN in the Field

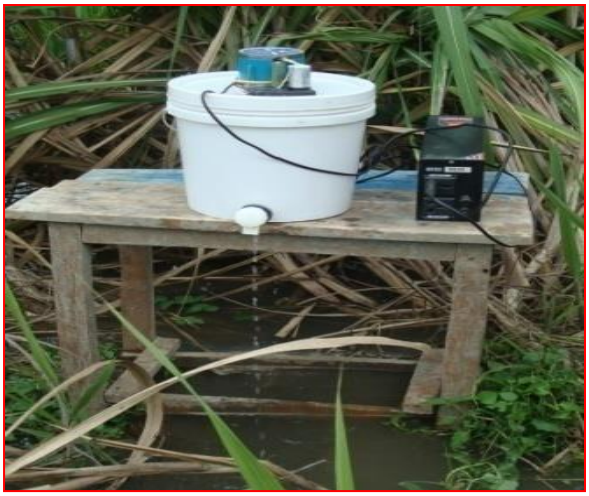
Application in the form of the liquid suspension

EPNs can be effectively applied using a wide range of agronomic or horticultural ground application equipment, which includes pressurized sprayers, mist blowers, electrostatic sprayers, or even aerial sprays. The choice of application equipment depends on the specific cropping system being employed. In each scenario, there are various factors to consider, such as the required volume, agitation, nozzle type, pressure, recycling time, environmental conditions, and the desired spray distribution pattern. It is crucial to ensure adequate agitation during the application process. For smaller plot applications, hand-held equipment or backpack sprayers may be suitable. However, for larger plots, it is advisable to consider the use of more extensive spraying apparatus like a boom sprayer.

We have developed an apparatus for the application of EPN in liquid form by which the EPN suspension is released along with irrigation water by regulating the outflow of liquid EPN suspension.

Design of Applicator for Application of EPN Liquid Suspension in the Field

Components of Applicator; (1) Electric Motor fitted with Stirrer, (2) UPS, (3) Vessel and (4) Outlet



Photographs showing the Application of Liquid Suspension of EPN

Multi locations field release of EPN for the management of white grub

After successful investigation of efficacy of EPN against their host *Galleria mellonella* and white grub species of *Holotrichia serrata* belonging to order coleoptera, family Scarabaeidae. We have found that *H. indica* species of EPN given immense results. We have conducted multi location field trial of *H. indica* for the management of white grub on sugarcane and other kharif crops.

Details of Field Demonstration

Our main operational area was Upper Gangetic Agro Climatic Zone of Western UP. We have laid 77 FLDs of EPN covering total area of 70.4 Acre of 18 villages in the 6 different districts; Amroha, Bulandshahar, Ghaziabad, Meerut, Muzaffarnagar and Saharanpur. The dissemination of technology of W.P. formulation of EPN and EPN infected *Galleria* Cadaver for the management of white grub and other soil arthropods on sugarcane and other Kharif crops has been found very effective as has been observed during pre-treatment and post-treatment pest population recorded. Use of Entomopathogenic Nematode (EPN) for the control of white grub infestation in sugarcane, cucumber and bottle guard crops could increase the crop yield by 26.92 – 55.00 %, 16.98 – 29.62 and 12.00 – 17.18 % respectively. The white grub management through application of EPN has been recognized as noble bio-agents to replace the chemical pesticides. Demand of W.P. formulation of EPN and EPN infected *Galleria* Cadaver for the management of white grub and other soil arthropods on sugarcane and other Kharif crops has been increasing day by day. The farmers belonging to different states of India; Maharashtra, Madhya Pradesh, Rajasthan, Karnataka, Gujarat, Himachal Pradesh, Uttarakhand who are facing white grub infestation in their crops, are approaching us for supply of W.P. formulation of EPN

and EPN infected *Galleria* Cadaver for the management of white grub and other soil arthropods on sugarcane and other Kharif crops. The details of all FLDs are given in the below (Table 4): -

Table - 4 District wise details of FLDs

Sr. No.	District	Village (In Nos.)	FLDs (In Nos.)	Covered Area (In Acre)	Crop	Percentage of Pest Population Reduction over Control	Crop Yield (Quintals / Acre)	Percentage increased over control	Soil Texture
1	Amroha	5	30	28.5	Sugarcane	68.51 – 85.00	250 – 330	30.43 – 55.00	Sandy, Sandy Loam
2	Bulandshahr	2	8	6.2	Sugarcane	65.51 – 84.61	280 – 305	40.00 – 55.00	Loam, Sandy, Sandy Loam
3	Ghaziabad	6	15	15	Sugarcane	70.83 – 83.33	310 – 340	26.92 – 50.00	Sandy Loam
			2	0.8	Cucumber	66.66 – 81.48	62 – 70	16.98 – 29.62	Sandy
			2	0.8	Bottle Guard	57.14 – 78.78	140 – 150	12.00 – 17.18	Sandy
4	Meerut	1	1	0.6	Sugarcane	82.92	300	50.00	Sandy Loam
5	Muzaffarnagar	3	17	16.5	Sugarcane	70.21 – 81.81	250 - 330	28.00 – 52.63	Sandy, Sandy Loam
6	Saharanpur	1	2	2	Sugarcane	79.16 – 79.54	280 – 290	36.58 – 45.00	Sandy
7	Total	18	77	70.4	-	-	-	-	-

Monitoring of sugarcane fields infested by White Grub:




Success Stories of Farmers

Management of white grub by applying the EPN at the time of sowing as well as in white grub infested field has given immense result for the control of white grub infestation. There are many successful controls of white grub in sugarcane crop however, here we are mentioning some examples of success story of farmers, who are benefitted “Management of White Grub through Entomopathogenic Nematode (EPN)” belonging to the Amroha district.

1. Shri Raju

Introduction:

Name	Shri Raju
Address	Village – Jallopur District – Amroha
Education	8th Class
Geo Tagging	Latitude:28.5846118 Longitude:78.2278556
Crop	Sugarcane
Area	2.5 Acre
Percentage increased over control	52.38 %

Achievement:

After successful application of EPN in the farmers field infested by white grub the pest population reduction was recorded day by day and at the last observation the field was free from infestation as 85.00 % reduction of pest population was recorded. The farmer obtained 52.38 % increase in crop yield over control.

2. Shri Jaipal Singh

Introduction:

Name	Shri Jaipal Singh
Address	Village – Sahbaajpur Gujjar District – Amroha
Education	12th Class
Geo Tagging	Latitude:28.609567 Longitude:78.2296358
Crop	Sugarcane
Area	1.5 Acre
Percentage increased over control	51.51 %

Achievement:

After successful application of the WP formulation of EPN in the sugarcane field of Shri Jaipal Singh' unhealthy and infested sugarcane crop has started to become healthy and at the last observation taken by us observed that pest population reduction was 79.31%. Finally, farmer obtained 51.51 % crop yield percentage increased over control.

At the time of feedback taken by us Shri Jaipal Singh Son Shri Shashi Kumar said that they are facing white grub infestation in their sugarcane field crop from last several years. Shri Shashi Kumar said that they had applied many pesticides for the control of white grub but they didn't have any perfect solution for white grub control. But after application of WP formulation of EPN they were happy, and they also appreciated and advised to other farmers to use EPN technology for the control of white grub.

3. Shri Aslam

Introduction:

Name	Shri Aslam
Address	Village – Nanai District – Amroha
Education	10th Class
Geo Tagging	Latitude:28.5540197 Longitude:78.2438766
Crop	Sugarcane
Area	1 Acre
Percentage increased over control	50 %

Achievement:

After successful dissemination of EPN technology in the white grub infested sugarcane field of Shri Aslam belong to village Nanai of Amroha district, Shri Aslam observed that reduction in pest population of white grub have started, and sugarcane crop started to become healthy. When we were taking last post treatment observation of the sugarcane field of Shri Aslam we have recorded 78.87 % of pest population reduction.

At their feedback about EPN Technology Shri Aslam was very happy to timely adoption of EPN technology for the control of white grub infestation in their sugarcane crop. He was also happy to get 50.00% more yield increase over control field. Shri Aslam told about EPN technology that he was very happy to get such type of biocontrol to overcome the white grub problem and he was very much thanks full to our efforts for timely aware, provide and application of EPN Technology.

4. Shri Kuldeep

Introduction:

Name	Shri Kuldeep
Address	Village- Jallopur District - Amroha
Education	8th Class
Geo Tagging	Latitude:28.5434441 Longitude:78.2257192
Crop	Sugarcane
Area	1 Acre
Percentage increased over control	43.18 %

Achievement:

After successful application of the EPN technology in the sugarcane field infested by white grub of farmer Shri Kuldeep. The infested field successfully recovered by 76.59 % reduction in pest population and total yield of 43.18 % increased over control farmer was obtained.

Shri Kuldeep was highly appreciated the EPN technology, and he was very much happy by recovery their infested field.

Dissemination of Entomopathogenic Nematode (EPN) Technology

Conducting Training Programme

For empowerment of rural women, youths, and farmers, two five days training programme were conducted on Mass Multiplication of Entomopathogenic Nematodes (EPN) and Bio-agents. About 54 numbers of farmers including 18 women farmers participated in the above training programmes for capacity building and to motivate them to undertake multiplication of EPN and Bio-agents at village level. During the training period, method of production and application of EPN and bio-agents in crops were demonstrated. By application of technologies, farmers were able to reduce cost of production substantially and to minimize losses due to pests and diseases resulting in increased cost-benefit ratio.

Four one day training and awareness programme were also organized on Mass Multiplication of Entomopathogenic Nematodes (EPNs) and Entomopathogenic Fungi (EPFs) and their use for

soil arthropod pest and other pest management. About 188 numbers of farmers including 11 women farmers have participated in the training programme.

17 more one day training programmes were also organized on “Integrated Pest Management of White Grub in sugarcane Crop – especially through Entomopathogenic Nematodes and Bio-pesticides” and also showcasing the technologies; EPN Infected Galleria Cadaver Technology for the control of white grub and other soil arthropods pests in sugarcane and other Kharif crops, Low cost technology for the multiplication of Bio-agents (Bio-fertilizers and Bio-pesticides) for the application of soil and seed treatment, *In vivo* mass multiplication of EPN on *Galleria mellonella* (Wax Moth) and Automation process for conditioning, harvesting and deflection/separation of EPN and WP formulation of EPN.

Organizing Field Day programme

Organized 8 field day programmes on the subject “Application of EPN & other Bio-agents for the management of white grub in sugarcane and other crops” at eight different locations of Amroha, Bulandshahr, Ghaziabad, Muzaffarnagar and Saharanpur districts. About 134 rural youth, women, farmers and sugar mills functionaries were participated in all Field Day Programme.




Organizing / Participating in KISAN Mela and Webinar

KISAN MELA

Participated in several Kisan Mela organized by ICAR-Indian Agricultural Research Institute (IARI), New Delhi, Sardar Vallabhbhai PatelUniversity of Agriculture & Technology, Modipuram Meerut, Department of Agriculture, Meerut Division, Meerut and Krishi Vigyan Kendra. Our Stall was visited by approximately 9708 Scientists, Research Scholar, Students, Rural Youths, Farmer and Women Farmers.

In all Kisan Melas we have demonstrated the technologies by installing stall as under;

- EPN Infected *Galleria* Cadaver Technology for the control of white grub and other soil arthropod pests in sugarcane and other Kharif crops.
- *In vivo* mass multiplication of EPN on *Galleria mellonella* (Wax Moth).
- Automation process for conditioning, harvesting and deflection/separation of EPN.
- WP formulation of EPN technology**.**

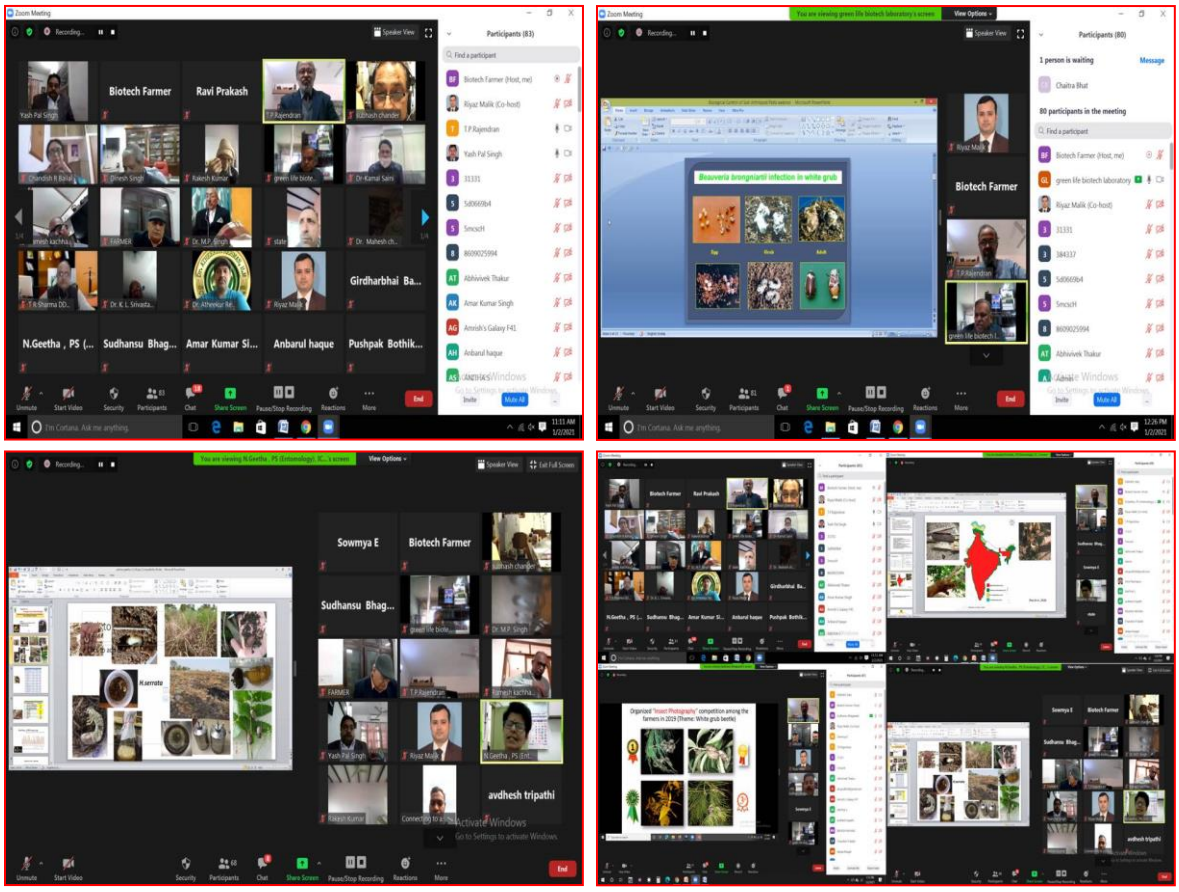



WEBINAR:

A webinar on **"Bio-control of Soil Arthropod Pests"** was also conducted by us. The webinar was inaugurated by **DDG (Crop Science), ICAR** and participated by Scientists from Research Institutions, SAUs, Sugar Industry, Formulation Industry, Research Scholars and Farmers. The total numbers of beneficiary participants were **179.**

The Executive Summary of Webinar Published in Indian Journal of Entomology, Volume 83, Part 1, March 2021 ISSN 0367-8288 (Print) & ISSN 0974-8172 (Online) Page No. 150.

Screen shot of conducted Farmer Webinar on the subject "Biological Control of Soil Arthropod Pests"



Key points for success in using Entomopathogenic Nematode (EPN)

Several critical factors influence the efficacy of EPNs in the biological control of crop pests (Amy, 2013).

- When utilizing agrochemicals in the same area where entomopathogenic nematodes will be applied, it is essential to ensure an adequate time gap between the applications of harmful substances and the entomopathogenic nematodes. Certain chemicals have been observed to impact the effectiveness of nematodes when they come into contact with them. Therefore, caution should be exercised when using these chemicals alongside nematodes.
- Entomopathogenic nematodes necessitate a soil environment that is adequately moist but not saturated. This moisture level enables them to maneuver and search for their host effectively.

- The soil temperature in the area where nematodes are to be deployed should range from 12°C to 32°C. Nematodes can also be influenced by factors like inappropriate soil type, the depth of thatch, and the frequency of irrigation.
- Safeguard nematodes from prolonged exposure to ultraviolet (U.V.) rays, as excessive exposure can deactivate and lead to their demise.
- Ensure that the application of entomopathogenic nematodes coincides with the vulnerable stage of the pest.
- Select the appropriate EPN species to match the most susceptible pest species and its life stage.
- Storage of formulated nematode species varies: *Steinernematids* at 4-8°C; *Heterohabditids* at 10-15°C. Do not leave in a hot vehicle.
- Select the suitable application rate and technique for the specific entomopathogenic nematode strain to optimize the interaction between nematodes and the targeted pest. Always consult the manufacturer's label for guidance in all instances.

Use of Entomopathogenic Nematodes (EPN) on crops

Globally the EPNs are utilized as biological control agents as detailed below. The effectiveness of EPNs as a biological control agent for soil pests has been well recognized by farmers in India for application in crops such as; sugarcane, maize, bajra, sorghum, groundnut, soybean, potato, ginger, turmeric, cucurbits, banana, areca nut etc. where root grubs are serious pests in various states. However, EPNs are globally being used for the control of various pests of different crops as detailed below (Table 5).

Table -5 List of EPNs with their host

Crop	**Pest**	**EPN species**	**Reference**
Rice	*Tryporyza incertulus*	*S. carpocapsae*	Rao and Manjunath (1966)
Rice	*T. incertulus,* *C. supressalis* *Cirphis compacta* *Pseudolatia separata*	*S. carpocapsae*	Isreal *et al.* (1969)
Maize	*Chilozonellus*	*Steinernema sp.*	Mathur *et al.* (1976)
Potato	*Anomola sp.* *and S. litura*	*S. carpocapsae*	Rajeshwari *et al.* (1984)
Maize	*Helicoverpa armigera*	*S. riobrave*	Cabanillas and Raulston (1996)
Cotton	*Spodoptera litturalis*	*S. carpocapsae*	Glazer *et al.*(1992)
Cotton	*Earius insulana*	*S. carpocapsae*	Glazer *et al.*(1992)
Grass	*Popillia japonica*	*H. bacteriophora*	Downing *et al.* (1994)

Sugarcane	*White grub*	*H. indica*	Shrad Mohan et al, (2017)
Chickpea	*H. armigera* Chickpea	*H. indica*	Prabhuraj and Shivaleela (2006) Prabhuraj and Shivaleela (2006)
Grape	*Sceledonta strigicollis*	*H. indica*	Prabhuraj *et al*. (2004)
Tomato	*A. ipsilon*	*S. bicornutum*	Hussaini *et al.* (2001)

Reasons for considering EPN as an effective bio – agent

- EPNs have a broad host range
- EPNs have capability to control larval stages of economically import insect pests in sub soil condition (White Grubs, Cutworms, Root Borers, Termites etc.).
- EPNs have the ability to search the hosts by active and passive modes.
- EPNs have the ability to kill hosts faster (24-48 hours).
- EPNs have recycling potential in soil.
- EPNs have no deleterious effects on non-target soil organism, beneficial soil insects, humans and animals.
- EPNs are eco-friendly.
- Mass production of EPN by *in vivo* and *in vitro* methodology is possible.
- EPNs can be easily applied by traditional spraying machines.
- EPNs have compatibility with many chemical insecticides and bio-pesticides.
- EPNs have been exempted from regulation requirement by US Environmental Protection Agency (EPA) and similar agencies in many other countries including Insecticides Act, 1968.

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