- Irrigate the field prior and after EPN application and keeping the treated area wet for at least 5days post application
- The ideal time of application is at evening hours so EPN can become active at night without risk of sunlight damage
- Give more concentrated application in the border rows extending to five to seven meters inside the field (white grub infestation generally seen on the boundaries of the plots and extending to a few meters inside).
- Mix the EPN formulation with water (150g EPN formulation/ sprayer tank) and can be drenched with a sprayer (nozzle and inside filters of the spray equipments should be removed to prevent them from becoming clogged with nematodes) in each sugarcane clump / any crop by making a 15-20 cm pit using a crowbar (sugarcane / maize) or
- Thoroughly Mix the EPN powder formulation with 100kg soil or FYM and broadcast in the field and irrigate the field immediately.

FIELD EVALUATION OF EPN

Five different field trials were conducted to evaluate the efficacy of EPN against white grub *H. serrata*. In all the trials, reduction in number of grubs was observed in the EPN treated plots as compared to the control plot. The reduction of grub population due to EPN treatment is presented in Table 1.





Steinernema infection

Heterorhabditis infection

Table 1. Field evaluation EPN against white grub Holotrichia serrata on sugarcane

Location	% reduction of grubs
Field trial I. Ramapuram village, Thalavadi, Erode Dt. Tamilnadu	77.0
Field trial II. Bannaenahalli, Anumanthapuram, Dharmapuri Dt. Tamilnadu	78.0
Field trial III. Vadakahalli Village K. R. Pet, Karnataka	80.0
Field trial IV. B. Anaikarapatti, Bodinayakanur Theni Dt. Tamilnadu	87.5
Field trial V. Banagahalli village, Thalavadi, Erode Dt. Tamilnadu	79.0

To obtain the license for ICAR-SBI EPN BIOPESTICIDE

FORMULATION, the interested companies / firms may

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BIOPESTICIDE FORMULATION

Non chemical, Non toxic, Environmental friendly and Ecologically Safe control of insect pests



BACKGROUND

White grubs generally known as Chafers, May or June beetles all over the world have become a challenging pests for our farmers in various parts of the country particularly in parts of Maharashtra, Rajasthan, Gujarat, Uttar Pradesh, Karnataka, Bihar and Tamilnadu. The damage caused by this pest range between 40 and 80 per cent in endemic pockets. The white grub family Scarabaeidae has a high diversity of species, and many of them have become important pests. The common white grubs are Holotrichia spp. Leucopholis spp. Maladera insanablis, Brahmina spp. Anomala spp. Melolontha spp. & Phyllognathus spp.. No crop is completely free from or resistant to the attack of these grubs. The larvae of white grub feed on the main roots and subsequently damage the underground portion of the crops. Generally, the damage caused by white grubs is in patches, but during epidemics the entire crop in a field may be dried up.

The pest is becoming more and more severe every year as they are spreading to regions where they had not been recorded as a pest in the past years. The problem is of very serious nature and need urgent attention for control. Until and unless concerted efforts or integrated pest control measures are made in endemic grub pockets, it would not be possible to suppress this menace.

Insecticide issue and white grub management

Though chemical insecticides such as carbaryl, endosulfan, chlorpyriphos, organophosphates, methyl parathion dust are effective in the management of earlier stages of white grubs but there are several limitations while using indiscriminately, such as ineffective in later stages of grubs due to resistance, extremely hazardous nature,



Beetles

carcinogenicity, pollution to environment and ground water sources. Hence there is an urgent need for development of an effective, ecologically safe and sustainable technologies of protection against white grubs.

Entomopathogenic nematodes: Potential biopesticide against insects especially white grubs

Among the suitable alternative control measure against insect pests, biological control using entomopathogenic nematodes is gaining greater attention world over. EPN become important in IPM programme as biopesticides and are commercially used to control insect pests worldwide. EPN occur naturally in soil environments, locate the insect pests in response to carbon dioxide and chemical cues from them. EPN infect different types of soil insects belongs lepidopteran (butterfly families), coleopteran (beetle families) and dipteran (flies family).

Entomopathogenic nematodes (EPN) of the genera Heterorhabditis and Steinernema having symbiotically association with bacteria of the genera Photorhabdus and Xenorhabdus respectively and kill the insects in 24 – 48h. EPN are microscopic, non-segmented roundworms that are obligate parasites of insects. The Infective Juveniles (IJs) of EPN are free-living nematode stage is well adapted to long-term survival in the soil. The IJs carries its symbiotic bacterium in the anterior part of its intestine. It invades the insect host through natural openings (mouth, anus, tracheae) or directly through the cuticle. The IJs

release the symbiotic bacterial cells into the insect's haemocoel. The bacteria produce toxins and other metabolites which kill the insect within 24 to 48 hrs after nematode invasion.

Entomopathogenic nematodes are beneficial for several reasons:

- 1. They have wide host range that they can be used successfully on numerous insect pests;
- 2. EPN kill their insect hosts within 48 hours.
- Amenability to mass production by in vivo on any insect host and in vitro culturing in artificial media allows for commercial production;
- 4. The IJs are durable and can stay viable for months when stored at the proper temperature;
- They are compatible with various insecticides, herbicides and fertilizers:
- 6. Besides having high biocontrol potential, these nematodes have the capability to recycle in the environment, are safe to the human health, plants, environment and beneficial insects. This makes nematode use for insect pest control safe and environmental friendly and they have been exempted from registration in many countries including India.

Description of ICAR - SBI EPN biopesticide formulation technology

Research efforts have been made at ICAR-Sugarcane Breeding Institute, Coimbatore in utilization of EPN against white grubs. Extensive survey was conducted in white grub endemic areas of sugarcane for isolation of EPN; bioefficacy of EPN was evaluated against different instars of white grubs and potential EPN was identified under laboratory, pot culture, microplot and field conditions.

A novel ICAR-SBI entomopathogenic nematodes (EPN) biopesticide formulation was developed first of its kind in a unique combination of carrier materials to attain a longer shelf life with viable infective nematode juveniles (IJs) of EPN. The formulation containing Heterorhabditis indica strain SBITND78 has a shelf life of nine months with 92% survival of nematodes and the formulation containing Steinernema glaseri strain SBILN1 has a shelf life of 12 months with 90% survival of nematodes. Successful control of white grub under field conditions obtained with ICAR-SBI EPN biopesticide formulation.

Mass production of EPN on larvae of greater wax moth Galleria mellonella and formulation

EPN mass production on larvae of greater wax moth Galleria mellonella has been standardized with economically cheaper available substrates on commercial level.

The Steinernema and Heterorhabditis spp. can be mass produced on larvae of greater wax moth Galleria mellonella, which is an excellent host for EPN multiplication. The



Galleria larvae

basic EPN mass production consists; rearing of Galleria larvae, infection of Galleria larvae with EPN, incubation. harvest of Infective Juveniles (IJs) from dead Galleria larvae and formulation of harvested IJs. The artificial diet developed for rearing of Galleria is economically viable to enhance the bio-mass production of EPN Mass production on Galleria Galleria larvae which in turn will



vield high EPN production with virulent IJs.

EFFICACY AGAINST WHITE GRUBS:

EPN is an effective and potential biopesticide for the management of white grubs associated with several cultivated crops like sugarcane, arecanut, coconut, banana, paddy, upland rice millets, wheat, sorghum, cardamom, groundnut, potato, maize, lucerne alfalfa, ginger, cocoa, vegetables, chilli, okra, brinjal, onion, cucurbits, amaranthus, forest nurseries etc.

ADVANTAGES OF ICAR-SBI EPN FORMULATION TECHNOLOGY AND COMMERCIAL POTENTIAL

- ICAR-SBI EPN biopesticide formulation is non toxic, environmental friendly and ecologically safe to human beings and beneficial organisms. They kill only target insect.
- EPN mass production is standardized by in vivo method on Galleria larvae. The artificial diet developed to enhance the biomass production of Galleria larvae is cheaper and cost effective.
- EPN technology can be commercially scaled up to larger scale as per the requirements
- Longer shelf life of 9-12 months.
- Successful control of white grub under field conditions.
- No special costly equipments and materials are needed for EPN production and Formulation.
- No special containers needed to store the formulation as locally available aluminum lined high density polyethylene sachets are sufficient
- Formulation is easy to transport and safety during storage and application
- Can be easily applied in the field with conventional equipments by broadcast, spot application, spraying with normal sprayers and water based application methods.
- Exempted from CIB registration in India

TARGET CUSTOMER/COMPANIES

The target customers are biopesticide manufacturing companies / firms / cottage industries / farmers. The EPN formulation can be used in tropical and subtropical regions of India.

FIELD APPLICATION OF EPN

- Recommended dose of EPN: 1 x 10⁸ IJs/acre (2-3 kg powder formulation/acre)
- EPN should be applied at first beetle emergence during onset of summer shower (April – June)