# PATENT GRANTED FOR NEW INNOVATION: USE OF ENTOMOPATHOGENIC NEMATODES (EPNs) FOR THE BIOLOGICAL MANAGEMENT OF INSECT PESTS

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## INTRODUCTION

Biological pest management is need of the hour and identification of potential bio-control agents is major objective of research for many plant protection researchers. National Institute of Plant Health Management (NIPHM) has pioneered in developing bio-control agents for both insect pests and diseases management. It has established its strength of research results both regionally and nationally. Among the microbial bio-control agents used for insect pest management, fungi, bacteria and viruses are common. However, nematodes are never tried and tested for insect pest management commercially in India. Nematodes that parasitize insects, also known as entomopathogenic nematodes (EPNs), have the wide potential to use as candidate bio-control agents for direct application and also to include in integrated pest management practices.

Entomopathogenic nematodes of families Steinernematidae and Heterorhabditidae are unique in their action and potential. They are considered as one of the most suitable entomopathogens in managing wide variety of insect pests particularly soil inhabiting ones. The uniqueness stems out from their symbiotic association with entomopathogenic bacteria, ease of mass production, storage and application. Due to their safety to non-target organisms and the environment, they are even exempted from Environment Protection Act in USA and many countries. Indian research on EPNs dates back to 1960s with use of DD-136 (a commercial product of *Steinernema carpocapsae*) against several lepidoteran pests. *Heterorhabditis indica* was first to be isolated from Indian soils during late 1980s. The NIPHM has identified highly potential *Steinernema* and *Heterorhabditis* species of EPNs from Steinernematidae and Heterorhabditidae families. The EPNs are tested in vitro and in vivo against many economically important insect pests and found very effective. Their ability to kill the insects such as white grub, diamond back moth, pod borer (*Helicoverpa*), cut worm, (*Spodoptera*), leaf webber and semilooper is well documented and confirmed. Their field demonstration against sugarcane white grub in Maharashtra has proved their ability beyond doubt. They confirmed most significant bio-control agent against root grub compared to chemical practices and other bio-control agents. Many farmers, agricultural officers and extension officials from different state departments are regularly getting trained on production and use of EPNs against sugarcane pests at NIPHM. The impressive results have become talk of the towns in sugarcane region of Maharashtra through mass media.

National Institute of Plant Health Management (NIPHM) has been instrumental in developing cutting edge technologies in the field of plant health management through biological control agents. The technologies are protected by patent rights. The institute offers rights of these technologies to interested public and private firms on agreement and competitive royalty basis. In order to bring the Entomopathogenic Nematodes on main platform of biological pest management, NIPHM has developed latest cutting edge technology with respect to mass production and formulation of Entomopathogenic Nematodes. Technology was invented by NIPHM and a **patent application no. 3948/CHE/2014 Journal No: 34/14 was filed by the institute on August 11, 2014, which is the first of its kind in India for which the patent has been granted on December 30, 2021 with Patent No. 385637**

**This technology is available for commercial use, interested person may contact to Registrar NIPHM, Rajendranagar, Hyderabad.**

**Transfer of technology to the stake holders**

Patent certificate

# SALIENT FEATURES OF PATENTED TECHNOLOGY FOR MASS PRODUCTION OF EPNs

- Can harvest pure culture of EPN compared to White trap method
- Low cost and uses local resources and easy to adopt (cost reduction by 80%)
- Without any sophisticated equipment's at room temperature EPN can be multiplied on standard host using new technology
- Can harvest more number of IJs of EPN (Increased yield of EPN)
- Effective, cheap and practical method for EPN mass production
- Can be promoted as rural home based cottage industry among rural women
- Upgradable to cottage industry level without much investment
- The new method accommodates more number of larvae
- No mortality of EPNs
- EPNs can be stored under shade and not exposed to direct light



# LIMITATIONS OF EXISTING MASS PRODUCTION AND FORMULATIONS TECHNIQUES

The in vivo multiplication method viz., White trap method (1927) is utilized in laboratories, glass house studies etc. Though the technology is simple the short comings are

- It requires specialized training.
- Requirement of huge space and large quantity of glass wares for mass production.
- Requirement of close monitoring for ensuring infection of the host.
- Requirement of high cost of production.
- Requirement of good laboratory facilities for large scale mass production.

NIPHM has established with the state of the art laboratory facilities with a designated scientists specialized in nematology and other infrastructures required for training, research and extension in the nematology science.

Officers training on EPN

Well furnished lab

# MASS PRODUCTION OF EPNs:

Mass production of EPN

# METHODS OF MASS PRODUCTION OF EPNs

Entomopathogenic nematodes are currently mass-produced by either in vivo or in vitro (solid and liquid culture). In vivo production system is based on the White trap (White, 1929), which take advantage of the IJ's natural migration away from host cadaver upon emergence. The most common insect host used for in vivo production is the last instar of the greater wax moth (*Galleria melonella*), because of its high susceptibility to most nematodes, ease in rearing, wide availability and ability to produce high yields. Insect hosts are inoculated on a dish or tray lined with absorbent paper. After approximately 2-5 days, infected insects are transferred to the White traps.

In vitro culturing of EPNs is based on introducing nematodes to a pure culture of their symbiont in a nutritive medium (contains peptone, yeast extract, eggs, soy flour and lard). A liquid medium is mixed with foam, autoclaved, and then inoculated with bacteria followed by the nematodes. Nematodes are then harvested within 2-5 weeks by placing the foam onto sieves immersed in water.

# FORMULATION OF EPNs:

Nematodes can be stored and formulated in different ways including the use of polyurethane sponge, water-dispersible granules, vermiculite, alginate gels, micronized vermiculite, and an aqueous suspension of nematodes and baits. Bait formulations and insect host cadavers can enhance EPN persistence and reduce the quantity of nematodes required per unit area. Formulated EPNs can be stored for 2 to 7 months depending on the nematode species and storage media and conditions.

# METHODS OF APPLICATION OF EPNs:

EPNs can be applied with nearly all agronomic or horticultural ground equipment including pressurized sprayers, mist blowers, and electrostatic sprayers or as aerial sprays. The application equipment used depends on the cropping system, and in each case there are a variety of handling considerations including volume, agitation, nozzle type, pressure and recycling time, system environmental conditions, and spray distribution pattern. It is important to ensure adequate agitation during application. For small plot applications, hand-held equipment (e.g., water cans) or back-pack sprayers may be appropriate. When nematodes are applied to larger plots, a suitable spraying apparatus such as a boom sprayer should be considered. Conceivably, applicators could also be using other methods such as through micro jet irrigation systems, subsurface injection or baits. Various formulations for entomopathogenic nematodes may be used for applying EPNs in aqueous suspension including activated charcoal, alginate and polyacrylamide gels, clay, peat, polyurethane sponge, vermiculite, and water dispersible granules (WDG). Optimum moisture (30% field capacity) temperature (25-30°C) soil type (lighter soils) should be checked

**Dosages:** EPNs usually must be applied to soil at minimum rates of 2.5 x 109 IJs/ha (=25/cm2) or higher.

# POPULARIZING EPNS AS POTENTIAL BIO- AGENTS IN THE MANAGEMENT OF ROOT GRUBS

In spite of the significant benefits of EPNs in managing insect pests, particularly soil borne insects, utilization of EPNs in our country is very low. There is a need for building awareness on role of EPNs as biological control agents among the extension functionaries and farmers. NIPHM has started to popularize the use of EPNs with special focus on management of root grub in sugarcane. Root grub is major pest which feeds on sugarcane roots. It is damaging thousands of sugarcane fields in Maharashtra NIPHM steps to popularize EPNs as an alternative strategy for root grub management in sugarcane. It opened a new chapter in management of root grub this area. Initially EPNs culture was supplied to sugarcane belt of Sangli & Kolhapur districts. Best practices of EPNs application were demonstrated. Farmers were also trained in EPNs application methodology for root grub management. NIPHM is with the assistance of district agriculture officer successfully demonstrated in farmers' fields the way forward in mitigating the menace caused by root grub with EPNs. Based on the success in management of root grub with EPN, farmer's co-operatives in Sangli & Kolhapur districts have established a laboratory for in vivo mass production of EPN with technical support of NIPHM.

# CONCLUSION:

There is a need for popularizing the use of EPNs for the management of both soil borne and foliar insect pests. Utilization of EPNs by the farmers can be increased only when production of EPNs is feasible at farm level besides reduction in cost of production for commercial operators. The agricultural extension officers should be trained with this technology of insect pests management with the help of EPNs. Reliance on EPNs for management of soil borne insect pests will also result in significant reduction of chemical pesticides and ensure that soil health of agroecosystems is protected. NIPHM is committed to build the capacity of different stake holders to popularize mass production of EPN and their utilization as an alternative to excessive reliance on chemical pesticides, the usage of which is detrimental to soil health.