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RESEARCH ARTICLE

DEVELOPMENT OF NOVEL INSECTICIDAL WETTABLE POWDER FORMULATIONS OF *HETERORHABDITIS INDICA* STRAIN NBAII HI1 FOR THE BIOLOGICAL CONTROL OF ROOT GRUBS IN SMALL CARDAMOM

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ABSTRACT

The complex including entomopathogenic nematodes (EPNs) of the genera *Steinernema sp* and *Heterorhabditis* sp and their mutualistic partner, i.e., *Xenorhabdus* and *Photorhabdus* bacteria, respectively possesses many attributes of ideal biological control agents against numerous insect pests as a third partner. Despite authenic opportunities for their practical use as bio-control agents globally, they are challenged by major impediments especially their cost and reliability. This review article presents major attributes of EPNs to familiarize growers and stakeholders with their careful application. Novel Entomopathogenic nematode (EPN) formulation for the biological control of insect pests the wettable powder formulation of the EPN, *Heterorhabditis indica* (strain NBAII Hi1) has been developed and found effective for the management of white grubs in arecanut, banana, sugarcane, potato and corn. The novel formulation has improved shelf-life wherein at least 90% of the juveniles are viable even after eight to twelve months of storage at a temperature of between 25 and 37°C and exempted from CIB registration. Field demonstrations of the formulation were carried out in Kerala and Around 13000 Ha small cardamom fields were applied with WP formulations of *H. indica* @ 4-5 kg/acre respectively. The technology could reduce the small cardamom root grub incidence by 62-78%. ICAR-KVK, IDUKKI has distributed, more than 11000 Kg of WP EPN formulation to the farmers for the management of root grubs in small cardamom. The conditions and practices that affected the use of EPNs for integrated pest management (IPM) are identified. Besides, efforts have been made to address such practices in various ways that grasp their effective approaches, identify research priority areas, and allow refined techniques. Additionally, sampling factors responsible for obtaining more EPN isolates with differential pathogenicity and better adaptation to control specific pest(s) are discussed. Specific improvements of EPN production, formulation, and application technology are reviewed which help in their broader may use. Other diverse factors that optimize EPNs to constitute a cost-effective, value added approach to IPM are also demonstrated.

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INTRODUCTION

Entomopathogenic nematodes (EPNs) are microorganisms which parasitize, cause disease, and kill the insects. Although the group of EPNs was recently expanded to comprise other nematodes such as certain species in the genus Oscheius (Dillman *et al*. 2012), only the genera Heterorhabditis and Steinernema are addressed herein since they have been exclusively developed as EPNs for commercial pest control. This is mainly due to their wide host range, short life cycle, easy in vitro and in vivo mass production, and ability to resist for survival especially under subterranean conditions (Kaya and Stock 1997; Askary and Abd-Elgawad 2017).

The third stage juvenile also called infective juvenile (IJ) of EPN is mutually symbiotic with the bacteria Xenorhabdus (in Steinernema) and Photorhabdus (in Heterorhabditis), which produce toxins after entering into the insect host body. The bacterial toxins are mainly responsible for the death of host which generally occurs within 48 h (Kaya and Gaugler 1993). Besides, EPNs are usually non-hazardous, improve the soil quality, compatible with many agrochemicals, biocontrol agents, specific to target pests, and generally non-toxic to beneficial organisms (Askary and Ahmad 2017). Therefore, nematode–bacteria complexes are considered one of the best alternative non-chemical insect pest control. In this review, the nematode-bacteria-insect interactions were briefly presented to give evidence for the usefulness of optimizing trends for integrated pest management and discussing the different factors affecting their maximum exploitation. Opportunities, conditions, and practices that can enable stakeholders to face challenges for wider exploitation of EPNs as a biological component in integrated pest management (IPM) programs are discussed.

Natural damage and symptoms of attack of root grub: Soil insect pests including whitegrubs, cutworms, termites and root grubs cause 24–40% yield losses in sugarcane, corn, arecanut, cardamom, groundnut, potato, banana, guava, turmeric, pulses, vegetables, grasses, etc. with direct plant loss to the tune of 20–60%. The larvae of root-feeding insects remain underground near the root zone of the plant, feeding upon the roots resulting in yellowing, wilting of leaves and drying of the entire plant. Due to continuous depletion of forest cover, organic carbon, soil microbial activities, antagonistic potential and ecological services of natural soil summarily attributed to anthropogenic and geological events, the soil-borne insect pests are increasingly invading the crops and causing a serious threat. Root grub is a serious pest damaging the roots of cardamom. Nutrient uptake is reduced due to root damage leading to yellowing of leaves; the pest problem is severe in less shaded area. Adult of the pest is a small beetle four and six mm length shiny metallic blue, green or greenish brown colour. Females are bigger than males. They are seen on cardamom leaves during morning and evening hours but do not feed on cardamom. The beetles feed on leaves of jack (*Artocarpusheterophylla*, mango (*Mangiferaindica*), guava (*Psidiumguajava*), dadeps (*Erthrypinalithosperma*), etc. Beetles occur in March,April and August-September. Females lay about 124-393 eggs in batches of 12-63 on dry cardamom leaves or mulches. The minute creamy white grubs hatch out from eggs, fall on the ground, reach root zone and start feeding the roots. Grubs have two periods of occurrence, the first during April-July and the second during September to January. Grubs (larvae) feed on roots, become mature in 45-60 days; they are short and stout, C shaped. Pupation takes place in an earthen cocoon. The pest completes life cycle in 65-102 days during first generation (March- August) and 73-111 days during second generation (September-February). Cardamom root grub (*Basileptafulvicorne*: Chrysomelidae) is a major pest damaging roots of small cardamom (*Elettariacardamomum* Maton) causing 29 to 66 percent crop loss under various levels of infestation. Chemical control with agrochemicals viz. Chlorpyrifos and Phorate, though help in managing the pest, repeated application of such chemicals is deleterious to soil microbes that contribute to the health of soils. The application of agrochemicals disrupts the agroforestry ecosystem in the Western Ghat region that support the cultivation of several spices including small cardamom. Moreover, these chemicals are banned for use in cardamom cultivation. Biological control of root grub is a suitable alternative; Entomopathogenic fungi such as Beauveria bassiana (on beetles of root grub) and Metarhizium anisopliae (on grubs) are pathogenic to the pest, but controlling under field condition is not highly effective. Entomopathogenic nematode (EPN), a native isolate *Heterorhabditis indica* (ICAR-NBAIR EPN) is highly effective in controlling the pest under field conditions. The technology of mass production and application of EPN, which are user-friendly, in the field has been standardized. Several farmer groups, NGOs, a commercial firm, and KVK were trained on the technology; more than 500 farmers used EPN in their fields, and root grub problem were effectively controlled in Kerala.

Failure of chemical insecticides: Several synthetic chemicals, viz. organophosphates, carbamates, neonicotinoids and fumigants are used indiscriminately with little effect on the target pest. Farmers are desperately looking for ecologically safe, effective, sustainable and on-farm recyclable green technologies which can be an alternative to soil contaminating synthetic chemicals in order to secure the crop, soil health and productivity and their livelihood.

Entomopathogenic nematodes – a boon in root grub management: Entomopathogenic nematodes (EPN) belonging to the families Heterorhabditidae and Steinernematidae are microscopic, non-segmented roundworms that are obligate parasites of insects and have become important in biological control and integrated insect pest management as bio-pesticides. EPN occur naturally in soil environments, locate their host in response to carbon dioxide and chemical cues from hosts. EPN infect many different types of soil insects and their life stages, larval, pupal and adult forms of lepidopteran, coleopteran and dipteran pests, as well as adult crickets and grasshoppers. These nematodes working with their symbiotic bacteria (Xenorhabdus and Photorhabdus) in their gut, can kill their insect hosts within 24–48 hours.

Mass production of EPN: Nematodes are amenable to mass production and their application is compatible with standard agrochemical equipment, including various sprayers and irrigation systems. Under in vivo mass production, wax moth (*Galleria mellonella*) larvae are inoculated with the infective juveniles (IJs) of respective EPN and are allowed for infection and mass multiplication. The IJs are harvested later from the dead cadavers of *G. mellonella*

Challenges in utilization of EPN in insect pest management: The utilization and transformation of the ecological services of natural, soil-dwelling beneficial nematodes into an IPM component or bio-product with marketsuitable shelf-life, ease of handling, storing, transport and field application are challenging tasks. NBAIR's role in production and commercialization of EPN technology The production of EPN was scaled–up in vivo on the larvae of wax moth to the tune of 1,00,000 larvae/ batch/3 days that led to the production of 1,200 tonnes of WP formulation (patented). The product and process developed encompasses a novel WP formulation for infective juveniles of beneficial entomopathogenic nematodes which confers a shelf–life of 10–12 months at normal temperature and pressure, easy

application and safety during transport, storage and application for biological control of white grubs and other soil insect pests.

Method of application: Soil application EPN power formulation @ 5gm per L of water in soil at the base of small cardamom plant (@5L of EPN Liquid per plant which mean two lakh to four lakh IJs of EPN) the IJs of EPN comes out from the cadaver in the soil, search for root grub and kill them.

Horizontal Spread of the technology:

S. No.	Villages	Area under EPN technology before KVK intervention in ha	Increase in area EPN technology after KVK intervention in ha
1.	Senapathy	20	1250
2.	Konnathady	135	360
3.	Santhanpara	89	210
4.	Bison valley	85	1745
5.	Nedumkandam	180	990
6.	Vandanmedu	35	1425
7.	Kumily	121	725

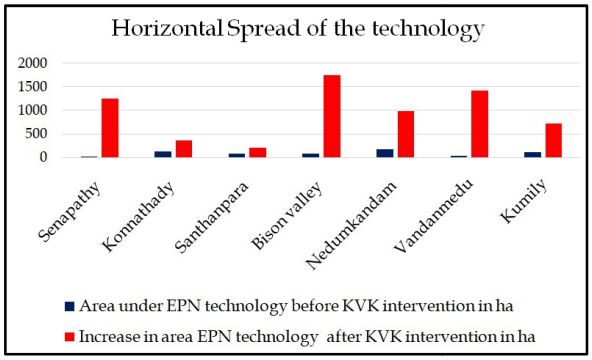

Figure 1. Horizontal Spread of the technology

Vertical Spread of the technology

S.no	Year	Production in kg(ha)	Productivity (qtl./ha)
1	2011-12	824	3.94
2	2012-13	912	4.68
3	2013-14	1022	4.90
4	2014-15	1120	5.50
5	2015-16	904*	4.38
6	2016-17	1175	5.61
7	2017-18	1282	5.72
8	2018-19	650**	2.10
9	2019-20	1481	5.91
10	2020-21	1502	6.20

**Productivity decreased in 2015-16 due to increase in day temperature and acute drought and ** Productivity decreased in 2018-19 due to heavy rainfall.*

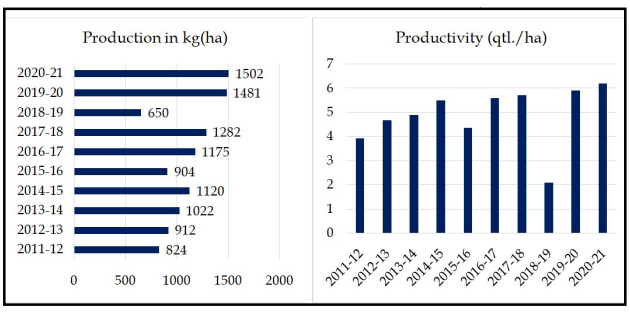

Figure 2. Vertical Spread of the technology

Economic analysis

Parameters	Demonstration	Farmers Practice
% reduction in root grub attack	98	22
Number tiller/plant	56	42
Gross cost (Rs/ha)	358000	310000
Gross Return (Rs/ha)	776000	488000
BCR	2.16	1.57

Impact of the EPN technology: An area of 7335 ha of small cardamom was covered using EPN for the management of root grub and other soil-borne insect pests. EPN WP formulation to the tune of 1,200 tonnes was produced during 2017 through private stakeholders. The WP formulation was found very effective in root grub management that reduced the cost of production and mitigated the use of pesticides (fipronil, chlorpyriphos, phorate) in cardamom.

CONCLUSION

Entomopathogenic nematodes and their mutuallistic bacteria are commercially used as safe alternatives to chemical insecticides. Opportunities that enabled their development and implementation for the control of insect pests should be better exploited. EPN application involves implanting novel powder formulation at plant base that can be done with ease and fast and saves a lot of labour. There are still important factors such as their cost and efficacy which relegate EPNs to niche pesticide markets. Therefore, the relatively inexpensive and wider practical use of EPN liquid culture could offer them a unique opportunity to be sold at fairly low costs. Further improvement of production, formulation, and application technology could be practiced. Molecular handling of EPNs should examine non-stability of beneficial traits preferably via detecting chemical markers of beneficial genes. Awareness-raising of growers and stakeholders for broader dissemination of conditions under which the EPNs constitute a cost-effective, value-added approach to IPM should be better addressed. Further, the technology could meet the enormous demand-supply-requirement in the Idukki for EPN by successfully establishing supply chain through Department of agriculture and Different FPOs.

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