POTENTIAL OF ENTOMOPATHOGENIC NEMATODES FOR THE MANAGEMENT OF WEEVIL PESTS OF BANANA (*Musa* SP.)

REMYA, K.R.

Thesis submitted in partial fulfilment of the requirement for the degree of

Master of Science in Agriculture

Faculty of Agriculture
Kerala Agricultural University, Thrissur

2007

Department of Agricultural Entomology
COLLEGE OF AGRICULTURE
VELLAYANI, THIRUVANANTHAPURAM-695 522.

DECLARATION

I hereby declare that this thesis entitled **"Potential of entomopathogenic nematodes for the management of weevil pests of banana (*Musa* sp.)"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

Vellayani,
8-03-2007.

Remya

REMYA, K.R.
(2004-11-41)

CERTIFICATE

Certified that this thesis entitled "**Potential of entomopathogenic nematodes for the management of weevil pests of banana (*Musa* sp.)**" is a record of research work done independently by Ms.Remya, K.R. (2004-11-41) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associate ship to her.

Vellayani,
8-03-2007.

Dr. (Mrs.) M.S. SHEELA
(Chairperson, Advisory Committee)
Associate Professor,
Department of Agricultural Entomology
College of Agriculture, Vellayani,
Thiruvananthapuram-695 522.

APPROVED BY

CHAIRPERSON:

Dr. M.S. SHEELA
Associate Professor,
Department of Agricultural Entomology
College of Agriculture, Vellayani,
Thiruvananthapuram-695522.

MEMBERS :

Dr. T. NALINAKUMARI
Associate Professor and Head,
Department of Agricultural Entomology
College of Agriculture, Vellayani,
Thiruvananthapuram-695522.

Dr. N. ANITHA
Assistant Professor,
Department of Agricultural Entomology
College of Agriculture, Vellayani,
Thiruvananthapuram-695522.

Dr. C. GOKULAPALAN
Associate Professor,
Department of Plant Pathology,
College of Agriculture, Vellayani,
Thiruvananthapuram-695522.

EXTERNAL EXAMINER :

6/7/07

ACKNOWLEDGEMENT

I humbly bow my head before ***God Almighty*** *for all the blessings to complete this arduous endeavour successfully.*

My feeling are always on the look out for proper words to place on record my deep and everlasting obligation to Dr. M. S. Sheela , Associate Professor, Department of Agricultural Entomology and Chairperson of the Advisory Committee, for her superb and superlative extra ordinary consideration with which she always tried to lead me to the gateway of success throughout my postgraduate program. Her sympathetic appreciation of the learners taste and capacity, her erudite and unflinching disposition to conquer and confiscate the unlimited intellectual property has always inspired my life as a scholar.

May I acknowledge with proud gratitude the invaluable help and guidance rendered by Dr. T. Nalinakumari, Associate Professor and Head, Department of Agricultural Entomology and cordial thanks for her wise advice and critical scrutiny of the manuscript of the thesis.

With deep sense of gratitude and respect I express my heart felt thanks and true indebtedness to Dr. N. Anitha, Assistant Professor, Department of Agricultural Entomology and member of my advisory committee for her unfailing and sympathetic attitude towards me as a scholar throughout the course. Her valuable suggestions and encouraging gestures have always been a source of inspiration to me. Her uncompromising and unshaken sense of dedication to duty that has been particularly helpful to create a new ethos of work culture is praise worthy.

Dr. C. Gokulapalan, Associate Professor, Department of Plant Pathology, for his valuable guidance and constructive suggestions. Above all, the moral support rendered by him during the critical period of my work is thankfully remembered.

The whole hearted co operation and constructive suggestions given by the teaching and non teaching staffs of Department of Agricultural Entomology in various stages of the study is gratefully remembered.

I am grateful to Mr. C.E. Ajithkumar, Junior Programmer, Department of Agricultural Statistics for his valuable help in statistical analysis of data.

I am thankful to Kerala Agricultural University for the award of Research Fellowship.

I would like to extend my gratitude to Dr. MohanDas and Sheeba chechi for all the help they rendered me.

My sincere thanks are due to Mr. Biju chettan, ARDRA, for the prompt and timely help rendered in preparing the thesis.

I feel happy to express my immense thanks to my colleagues Vijayasree, Malini, Bharati and Anis and my friends Raji, Shaiju, Malu, Susmin chechi, Sahi chechi, Rani chechi, Resmi and other senior and junior friends.

I am extremely thankful to my dear room mates Renji, Rajani and Julie for their wholehearted help and moral support throughout my P.G. programme.

Words seem to be inadequate to express the sense of gratitude surging in me towards my achan, amma and chechi for their unbounded affection.

Remya, K.R.

CONTENTS

LIST OF TABLES

LIST OF TABLES CONTINUED

LIST OF FIGURES

LIST OF PLATES

INTRODUCTION

1. INTRODUCTION

Banana (*Musa* sp.) is a widely grown fruit crop in India. India is the largest producer of banana in the world. It is used as fruit and vegetable in India. In Kerala, banana is cultivated in an area of about 55906 ha with an annual production of 442220 tons (FIB, 2006). It is rich in easily digestible carbohydrate with calorific value of 67-137 per cent per 100 g fruit. It is also a rich source of vitamin C and minerals. Availability of banana through out the year, irrespective of the season, makes them a popular food –fruit and subsidiary food of the masses in the world.

The soil and climate of Kerala State are ideally suited for banana cultivation. However, there are a number of constraints, which limit the production and productivity of the crop. One of the major constraints is the high incidence of pests and diseases throughout the year in the state. More than thirty insect pests have been recorded on banana. Though the crop is annual, the perennial nature of its cultivation in the garden lands (ratooning) coupled with the practice of clonal propagation results in the persistence of many economically important pests and diseases.

Among the pests of banana, pseudostem weevil *Odoiporus longicollis* Oliv. (Curculionidae:Coleoptera) is very destructive and found very difficult to manage (Dutt and Maiti,1972). This weevil is considered as one of the most serious pests of banana in India (Fletcher, 1916; Kung, 1955; Dutt and Maiti, 1970). Ten to 90 per cent yield loss has been reported due to the pseudostem weevil infestation (Padmanabhan *et al.*, 2001). If the infestation is severe, the plant will topple before harvest and there the loss is cent per cent. However, the occurrence of this pest in Kerala was reported by Visalakshi *et al.* (1989). With in a decade, the pest has spread to all parts of the state causing severe loss to the banana farmers.

The banana weevil, *Cosmopolites sordidus* Ger. is also generally considered the most important insect pest of banana (*Musa* sp.) in many regions of the world. This weevil has been recorded to cause a loss of more than 40 percentage of the banana crop. The weevil grub bore in to the corm and the lower pseudostem causing mortality of suckers, through snapping and toppling. The grubs also interfere with root initiation. Damage is usually greater in ratoon crop (Gold *et al.*, 2004) and sustained attack over several crop cycles may prolong maturation rates and reduced the yield up to 60 per cent.

The present strategy for controlling the above pests is centered on insecticides. It is necessary to evolve effective and ecologically sound alternative management tools, which can be used either alone or in combination with less hazardous insecticides for the management of the pest. In this context, a feasible ecofriendly approach to manage these pests is by advocating effective biocontrol agents.

Biocontrol of crop pests is an ideal alternative and is economical and has long lasting effect without risk to human beings and other non-target organisms. Application of biocontrol agents and their products including entomopathogenic nematodes (EPN) are known to be effective in the management of pests of various crops. Since these biological agents are specific to insects, it will not affect the fauna and flora, hence could be considered as ecofriendly. In order to find out the potentiality of EPN this study was taken up with the following objectives:

1. to isolate native EPN from banana rhizosphere,
2. to standardise artificial rearing of wax moth *Galleria mellonella* L. the trap insect
3. to identify cheap media for the mass production of native EPN,

4. to establish the potential of screening of native isolates of EPN in comparison with standard cultures by laboratory screening and
5. to assess the potential of native isolates for the management of weevils in the laboratory.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

The production of banana and plantain in the tropical and subtropical regions have been adversely affected by the infestation of the weevil pests, rhizome weevil *Cosmopolites sordidus* Ger. and pseudostem weevil *Odoiporus longicollis* Oliv. *O. longicollis* bores into the pseudostem and in severe cases results in toppling of the plant. The grubs of *C. sordidus* bore in to the corm, reducing nutrient uptake and weakening the stability of the plant. Entomopathogenic nematodes (EPN) are potential biocontrol agents of insect pests especially for the control of soil borne pests as well as the pests with cryptic nature. EPN can be easily mass produced and applied using conventional spray equipment. In this study potential of EPN for the management of the pests were assessed.

The literature on distribution of EPN in various ecosystems, isolation and mass production of EPN and potential of EPN for the management of pests with special reference to weevils are collected and presented here.

2.1 DISTRIBUTION OF EPN IN VARIOUS CROPS

EPN belonging to the families Steinernematidae and Heterorhabditidae were soil dwelling lethal parasites of insects that were used for inundative, augmentative or inoculative biological control of crop pests (Bedding *et al.*, 1983., Gaugler and Kaya, 1990., Kaya and Gaugler, 1993., Parkman and Smart, 1996). These were obligate pathogens and have the potential for long term establishment in soil through recycling on infected insects. The only stage found outside the insect body (in soil) was the third stage juvenile. Soil type played an important role in the occurrence and distribution of EPN. The occurrence of EPN was more in sandy loam soil followed by sandy soil and it was low in clay soil (Banu *et al.*, 2005).

2.1.1 Rhizosphere

Survey of Heterorhabditidae and Steinernematidae (Rhabditida, Nematoda) conducted in Western Canada by Mracek and Webster (1994) revealed that out of the 125 samples *Steinernema* sp. was reported in seven samples and these nematodes were found in the top 10 cm of soil. They also reported that most of the nematodes were found in cultivated land.

2.1.1.1 Coconut

Banu *et al.* (1998) collected soil samples from the rhizosphere of coconut and also from cow dung pits in Alappuzha district of Kerala State. Out of 95 samples, one sample from rhizosphere of coconut and one from the cow dung pit showed the presence of the native isolate. Based on the morphometrics they reported that the isolate was *Heterorhabditis indica*. This was the first report of *H. indica* from Kerala. Banu (2001) did an extensive survey through out Kerala and collected 40 isolates of *H. indica* and 16 isolates of *Steinernema* species. Out of 14 districts surveyed EPN were recorded from 12 districts and highest frequency of occurrence was recorded in Ernakulam (16.00 per cent).

2.1.1.2 Paddy

Josephrajkumar and Sivakumar (1997) conducted a random survey of EPN from paddy rhizosphere in Vilavancode and Kalkulam area of Kanyakumari district. They reported prevalence of *Steinernema* species and *H. indica* in 17 samples and one sample respectively out of 163 samples collected.

2.1.1.3 Cotton

Narkhedkar *et al.* (2001) reported 16 isolates of EPN belonging to *Steinernema* and *Heterorhabditis* species from cotton growing areas of north, south and central India using *Corcyra cephalonica* (Stainton) and *Galleria mellonella* L. larvae by trap insect method.

2.1.1.4 Others

Constant *et al.* (1998) collected soils from various habitats in Guadeloupe (Grande terre, Basse terre) and neighbouring islands and they found out both *Heterorhabditis* (34 out of 538) and *Steinernema* species (1 out of 538) from the coastal (91.4 per cent), tropical low lands (5.7 per cent) and tropical middle altitude (2.9 per cent) areas. Yoshida *et al.* (1998) reported EPN from the five climatic regions of Japan. They tested over 1400 soil samples from 266 sites for the presence of nematodes using the *Galleria* baiting technique. Griffin *et al.* (2000) collected soil samples from 79 sites from five islands of Indonesia for isolates of EPN. *Heterorhabditis* and *Steinernema* were equally prevalent and recovered from coastal sites only.

In central north Gujarat 16 different EPN isolates were encountered during the surveys, which comprised four species of *Heterorhabditis,* 11species of *Steinernema* and one unidentified species (Vyas *et al.*, 1998). Kaushal *et al.* (2000) examined 207 soil samples from diverse areas (Assam, Delhi, Gujarat, Himachal Pradesh and Uttar Pradesh). Out of which 17 samples contained EPN, comprising 10 isolates of *Steinernema* and seven isolates of *Heterorhabditis.*

2.1.2 Insect Cadavers

2.1.2.1 Coconut

Sosamma *et al.* (2000) reported the natural occurrence of *H. indica* from cadavers of rhinoceros beetle *Oryctes rhinoceros* Linn.

2.1.2.2 Paddy

Sheela *et al.* (2002) conducted a survey for EPN on paddy crop and they reported 10 isolates of *Heterorhabditis.* These *Heterorhabditis* isolates were found pathogenic to the pests of rice viz., rice bug *Leptocorisa acuta* (Thump), rice hispa *Dicladispa* spp., brown plant hopper *Nilaparvata lugens* (Stal.), grasshopper *Hieroglyphus banian* (Fab.), small rice grasshopper *Oxya chinensis* (Thunberg), green leaf hopper *Nepotettix* spp., rice leptispa *Leptispa pygmaea* Baly, gregarious blue beetle *Haltica cynea* (Web.).

2.1.2.3 Sweet potato

Mohandas *et al.* (2005) reported a pathogenic species of *Rhabditis (oscheius)* from grubs and pupae of sweet potato weevil. The ability to kill many insect pests by this species was also studied.

2.2 ISOLATION OF NATIVE EPN

Southey (1970) and Ayoub (1977) reported a method to recover EPN directly from soil. They used flotation, Baermann funnel and mist extraction techniques for isolation of EPN from soil. Saunders and All (1982) also compared the above techniques for extracting entomophilic rhabditoid nematodes from the soil.

Bedding and Akhurst (1975) reported another method of extracting EPN. They collected the nematodes indirectly from infected hosts with the help of trap insects. Beavers *et al.* (1983) also used the indirect method of collecting nematodes from infected hosts. But they did not use the trap insect technique. They collected the nematodes only from naturally occurring infected host insects.

2.2.1 Trap Insect

Akhurst and Bedding (1986) reported that the last instar *Galleria* larvae were highly susceptible to EPN. So they used these larvae were used to concentrate nematodes in the natural environment.

Progeny production of different *Steinernema* and *Heterorhabditis* species were evaluated in different lepidopteran insect pests. Maximum yield of all the isolates was observed in the final instar larvae of *G. mellonella.* (PDBC, 2001)

2.3 ARTIFICIAL REARING AND MASS PRODUCTION

Plant and animal protein media were evaluated for *in vitro* production of indigenous isolates of *S. carpocapsae, S. bicornutum, S. tami* and *H. indica.* The flasks with foam chips were inoculated with different isolates of *Steinernema* species and incubated at 24^0C for 25 days. Of the eight artificial media evaluated for *Steinernema* species, four media namely Wout's medium, modified egg yolk,

soyflour + cholesterol and modified dog biscuit media yielded highest number of infective juveniles of *S. carpocapsae* (PDBCEN 6.11 and PDBCEN 6.61), *S. tami* (PDBCEN2.1) and *H. indica* (PDBCEN6.71). Maximum yield of *S. carpocapsae* (PDBCEN 6.11 and PDBCEN 6.61) infective juvenile was observed on modified dog biscuit, while *S. tami,* (PDBCEN2.1) gave maximum yield of infective juvenile on modified egg yolk medium. *H. indica* (PDBCEN 6.71) gave maximum yield of infective juvenile on Wout's medium. *S. bicornutum* (PDBCEN 3.2) and *S. abbasi* (PDBCEN 3.1) did not multiply on any of the eight media tried (Hussaini *et al.*, 2002b).

Ponniah (1997) reported that *S. glaseri* can be multiplied on polyurethane foam coated with growth media like yeast extract, beef extract, Bengal gram flour and coconut oil. Modified egg yolk and dog biscuits were also found to give very good yield of *S. carpocapsae, S. biocornutum* and *H. indica* (Hussaini *et al.*, 2002b).

Steinernematids and Heterorhabditis infect and reproduce in a broad spectrum of insects. They can be readily reared *in vivo* in the laboratory. Up to 200,000 *S. feltiae* (Dutky *et al.*, 1964) and 350,000 *H. bacteriophora* infective juveniles (Milstead and Poinar, 1978) have been harvested from one last instar *Galleria*. Karunakar *et al.* (1992) reported that *S. feltiae*, *S. glaseri* and *H. indica* could mass produce in fifth instar larvae of *Chilosacchariphagus indicus*.

Rajkumar *et al.* (2002) conducted an experiment on mass multiplication of EPN from Udaipur on *G. mellonella*. Maximum numbers of infective juveniles of *Steinernema* species (90,945) were recorded in the large sized larvae. Similarly Singh and Yadav (2002) mass produced *H. bacteriophora* on *G. mellonella*. They recorded an average yield of 90945 nematodes per insect for *Steinernema* species and 201520 for *Heterorhabditis* species in *Galleria* larvae.

Banu (2003) standardized the initial inoculum level required for the multiplication of *H. indica*, *S. glaseri* and *Steinernema* sp. in *G. mellonella*. She reported that highest nematode multiplication was observed at an initial inoculum level of 10 IJ per last instar larva of *G. mellonella*.

2.4 MANAGEMENT

Biological control of insects with EPN progressed since 1932 with the discovery of *S. glaseri* (Glaser, 1932) infecting Japanese beetle. In India work on Steinernematids started in 1960's. Use of DD-136 for control of pests of rice, sugarcane and apple was reported by Rao and Manjunath (1966). In the seventies Singh and Bardhan (1974) worked on mortality and field trials of DD-136 (*S. carpocapsae*).

Literature on the management of crop pests with EPN is presented here.

2.4.1 Coleopteran Pests

2.4.1.1 Strawberry black vine weevil Otiorhynchus sulcatus F.

Hanula (1993) conducted field studies in USA to determine the vertical distribution of larvae and pupae of *O. sulcatus*. He reported that in a laboratory bioassay, *H. bacteriophora* was ten times more virulent than *S.carpocapsae,* but both species were equally effective in a field trial at the highest rate tested (210 nematodes/plant). *Heterorhabditis* species controlled larvae of *O. sulcatus* in Netherlands and the efficacy of *Heterorhabditis* species was determined by soil temperature and antagonism in soil (Toll and Van, 1993). Backhaus (1994) carried out an experiment in open field stand of strawberries in Germany to test EPN (*Heterorhabditis* species) as biological control agents against larvae of *O.sulcatus*. He found out that the larvae, pupae and young adults were parasitized by *Heterorhabditis*. He also conducted experiments for the biological control of the *Otiorhynchus* species on ornamentals in the green house using commercially available strains of the EPN *Steinernema* species and *Heterorhabditis* species. Miduturi *et al.* (1994) conducted green house trials and reported 70-95 per cent control of *O. sulcatus* with 1000IJ's per 250 ml pot of *Heterorhabditis* species and 30-35 per cent control by *S. carpocapsae* were observed in 10-20 days. In the field experiments 10,000 IJ per plant of *S. carpocapsae* and *Heterorhabditis* species gave 10-20 per cent and 10-40 per cent control of *O. sulcatus* respectively.

Simser and Roberts (1994) conducted a bioassay of larval and pupal stages of *O. ovatus* with *S. carpocapsae* and with *H bacteriophora* which resulted 62 and 51 per cent mortality. Berry *et al.* (1997) compared the efficacy of *H. marelatus*, a newly described species of EPN with that of *H. bacteriophora* against the root weevils, *O. ovatus* and *O. sulcatus* in strawberry. In the laboratory the effect of *H marelatus* was significantly more virulent than *H. bacteriophora* on the root weevils seven days after nematode application at 14°c. In the field experiments *H. marelatus* with 52 and 136 IJ per cm^2 reduced root weevil larvae and pupae 75.3 and 77.4 per cent respectively at 20 days after nematode application.

Kakouli *et al.* (1997) conducted trials under glass house conditions and reported that *O. sulcatus* larvae were effectively controlled by using *S. carpocapsae* and *H. megidis*. In late summer, field treatment with *S. carpocapsae* induced 49.5 per cent reduction of the early instar larvae and field application of the same nematode species in late spring resulted in 65 percent control of late instar larvae. Schirocki and Hague (1997) conducted an experiment on invasion and pathogenicity of isolates of *S. carpocapsae*, *S. feltiae*, *Heterorhabditis megidis*, and *Heterorhabditis* sp. against black vine weevil, *O. sulcatus*. He reported that *S. feltiae* caused little mortality and *H. megidis* and *Heterorhabditis* species caused greater mortality than *S. carpocapsae*. Wilson *et al.* (1999) conducted two field experiments on the potential of heterorhabditid nematodes to control larvae of the black vine weevil, *O.sulcatus*. He reported that *H. marelatus* reduced the number of weevil grubs.

2.4.1.2 Sweet potato weevil Cylas formicarius F.

The EPN *H. bacteriophora* (Hp88) and *S. carpocapsae* (All and G-13) were evaluated as biological control agents against *C. formicarius*. Field application of Hp88 and All strains of EPN were more effective than chemical insecticides at reducing weevil densities on plants.Hp88 nematodes were more effective than All nematodes and chemical insecticides at reducing weevil damage

to fleshy roots (Jansson *et al.*, 1989, 1991). The heterorhabditids and to a lesser extent, *S.feltiae* and *S. glaseri* were able to locate and infect apionids in roots 15 cm from there original source (Mannion and Jansson, 1992). Laboratory studies were conducted to determine the infectivity of *S. carpocapsae*, *S. feltiae*, *H. bacteriophora* and *Heterorhabditis* species to *C. formicarius*. Root mortality of the sweet potato weevil, *C. formicarius* was caused by six strains of entomophilic nematodes in the families Steinernematidae and Heterorhabditidae. In general, the Heterhabditid nematodes caused higher levels of mortality within the root than the steinernematids but there was no difference in mortality between the two nematode concentrations (Mannion and Jansson, 1993). Janson and Lecrone (1997) conducted an experiment on effect of sweet potato cultivar on efficacy and persistence of EPN for control of *C. formicarius*. Recovery was comparable between nematode strains, *H. bacteriophora* (Hp88) and *S.carpocapsae* (All) in plots planted with jewel cultivar. Recovery of strain All was higher than that of Hp88 in plots planted with Picadito cultivar.

***2.4.1.3 Sugar beet weevil Temnorhynchus mendicus* Gyll.**

Curto *et al.* (1992) conducted an experiment on the effectiveness of two commercial preparations of entomophilic nematodes against *T. mendicus* on sugar beet. He evaluated it in field trials at two farms with clay soils in Italy. The nematodes tested were *Heterorhabditis* species and *S. carpocapsae*. Both nematode species were more effective than the conventional insecticides. *S. carpocapsae* and *Heterorhabditis* species reduced the percentage of weevil infected roots compared with deltametrin. It caused a significant reduction in damage to the roots compared to all treatments. He concluded that one treatment with EPN followed by irrigation was more effective than two chemical applications. (Boselli *et al.*, 1994, 1997)

Curto *et al.* (1996) conducted an experiment on the field effectiveness of EPN against sugar beet weevil larvae. The species tested were *Heterorhabditis* species, *H. bacteriophora* and *S. carpocapsae*. These were applied at different rates and conditions and were compared to conventional chemical control methods. He concluded that biological control of *T. mendicus* with EPN represents a true

alternative to chemical treatments in IPM of sugar beet. The most efficient dose of application against *T. mendicus* was 250,000 IJ per m^2 applied with equipment generally used for applying liquid insecticide. Irrigation of the crop after the treatment was important for the EPN (Heterorhabditis and Steinernematids) to reach the host (Tacconi *et al.*, 1998).

***2.4.1.4 Citrus root weevil Diaprepes abbreviatus* (L.)**

Schroeder (1990) conducted field studies on the use of entomophilic nematodes for the control of *D. abbreviatus* on citrus. He reported that the treatment with *S. carpocapsae* at 100 or 200 IJ per cm^2 or *H. bacteriophora* at 100IJ per cm^2 resulted in 50 per cent reduction of pest emergence. Downing *et al.* (1991) conducted field studies in citrus orchard in Florida to determine the efficacy of the EPN, *S. carpocapsae* and *H. bacteriophora* for the control of *D. abbreviatus*. They also recorded that all the rates of *H. bacteriophora* tested resulted in a statistically significant reduction in the number of adults trapped compared to untreated trees. In another citrus grove in Florida the EPN, *S. carpocapsae* and *H. bacteriophora* were applied to the soil and it significantly reduced the emergence of adults of the *D. abbreviatus*. (70 per cent) compared with an untreated control (Schroeder, 1992).

Duncan and McCoy (1996) conducted experiments in a six year old citrus orchard to test the efficacy of different formulations of the nematodes, *S. riobravis* and *H. bacteriophora* for the control of *D. abbreviatus* and to measure nematode persistence at different soil depths. Nine weeks after application the numbers of *D. abbreviatus* at soil depths of 0-45 cm were reduced by 77 and 90 per cent respectively in plots treated with liquid or granular formulations of *S. riobravis*. Different formulation of *H.bacteriophora* was not affected the population density of *D. abbreviatus*.

McCoy *et al.* (2000) conducted field trials in Florida to test the ant predation and parasitism by nematodes *S. riobrave, H. bacteriophora* and *H.indica.* The main mortality factors of caged sixth instar larvae of the citrus pest were due to the three species of EPN.

2.4.1.5 Red palm weevil Rhynchophorus ferrugineus (Oliver)

Abbas and Hanonik (1999) tested *S. riobrave*, *S. carpocapsae* and *Heterorhabditis* species for their pathogenicity to *R. ferrugineus* in laboratory. These species proved virulent to both larval and adult stages of the insect. The application of EPN (*S. abbasi* and *H. indica*) to leaf axils of coconut palm is recommended for control of red palm weevil. (Sosamma, 2003)

2.4.1.6 Rhizome weevil Cosmopolites sordidus Ger.

Pena *et al.* (1993) conducted laboratory and green house studies on the effects of *Beauveria bassiana* and EPN, *S. carpocapsae*, *S. bibonis* and *Heterorhabditis* species on adults and larvae of *C. sordidus*. Adult weevils were more susceptible to infection by *B. bassiana* than larvae and the larvae were more susceptible to infection by EPN than adult weevils. Smith (1995) conducted four trials over three years to test current and new pesticides and two species of EPN (*H. zealandica* and *S. carpocapsae*). Nematodes were applied in a thickened aqueous solution in to 200 mm deep incisions in the residual rhizomes of harvested plants. But the treatments were not effective possibly because of early nematode mortality caused by free water in the spike holes. More than one or two applications a year would probably be uneconomic. Rosales and Suarez (1998) conducted a survey on EPN found in Aragua and Miranda states (Venezuelan) were carried out and their potential as biological control agent of *C.sordidus* evaluated. Foreign strains including *H. bacteriophora* (FRG-1 and HT1), *H. indica* (FRG -09 and FRG- 15), *S.bibonis* and *S. carpocapsae* and the Venezuelan *Heterorhabditis* spp. strains HV1, HV2, HV3, HV4, HV5 and HV6 strains were compared. HV3 and HV6 strains recorded a mortality range of 16-80 percentages on *C. sordidus*

2.4.1.7 Pseudostem weevil Odoiporus longicollis Oliv.

Jayasree (1992) reported that DD-136 (*Steinernema glaseri*) caused cent per cent mortality of grubs of pseudostem weevil at 10 days after inoculation.

Padmanabhan *et al.* (2002) conducted petridish bioassay using *H. indica* (EN13.3) against third instar grubs of banana pseudostem weevil and he reported that the treatments 10-70 IJ and 80-100 IJ per grub recorded 33.3 and 66.6 per cent mortality respectively at 72 hrs of inoculation.

2.4.1.8White grubs

H. indica and *S. glaseri* were equally efficient in producing high mortality of all three larval instars of *Holotrichia serrata* F. and *Leucopholis lepidophora* Bl. where as *S. feltiae* was less effective (Karunakar, 2000). Bio efficacy of indigenous EPN isolates of *H. indica*, *S. carpocapsae*, *S.abbasi*, *S. bicornutum* and *S. tami* were evaluated against *Holotrichia consanguinea* in the laboratory by soil column assay. *H. indica* isolates were more virulent than *Steinernema* species against *H. consanguinea* (PDBC, 1998).

***2.4.1.9 Rhinoceros beetle Oryctes rhinoceros* Linn.**

Hoy (1954) reported the use of entomopathogenic nematodes *S. glaseri* for the management of *O. rhinoceros* in Wallis Island. Kurian *et al.* (1967) first isolated nematodes from third instar grubs of rhinoceros beetle which when injected in to healthy grubs produced mortality. *Heterorhabditis* species have been reported to occur in diseased *O. rhinoceros* grubs and were found pathogenic to it. Pardede *et al.* (1992) isolated the parasitic nematodes *Heterorhabditis* species from the diseased larvae of *O. rhinoceros* and the surrounding soil obtained from Seipancur experiment station, Malaysia. The nematodes were considered as a potential natural enemy of *O. rhinoceros*. Sosamma (2003) reported that *H. indica* was more virulent to *O. rhinoceros* than *Steinernema* species. When a mixture of *H. indica* and *Steinernema* species was treated against *O. rhinoceros* grub in earthen pots, only *H. indica* could be recovered from the dead cadavers.

***2.4.1.10 Cardamom root grub Basilepta fulvicorne*, Jacoby**

The nematode suspension (*H. indica* @ 100IJ per grub) was dispensed with sufficient amount of water and applied at cardamom root zone during evening

hours for bio- suppression of root grub. LD50 of *H. indica* against cardamom root grub was found to be 49.73 IJ at 120 hours after treatment in soil column bioassay (Josephrajkumar *et al.*, 2005).

2.4.2 Lepidopteran Pests

***2.4.2.1 Helicoverpa armigera* Hb.**

Glazer and Navon (1990) reported that under laboratory conditions, complete mortality of *H. armigera* were achieved with 200 IJ of *S. feltiae* strain All. *S. riobravis* was reported to cause 90 to 100 per cent mortality of *H. zea* on corn @ 2 x 10^9 IJ per ha (Cabanillas and Raulson, 1995). Tahir *et al.* (1995) stated that *H. armigera* was more susceptible to *S. riobravis* than to *Heterorhabditis* species and *S. carpocapsae.*

Narkhedkar *et al.* (2001) reported that *H. bacteriophora* isolates from Nagpur were very effective against all stages of *H. armigera* with 15 IJ per larva causing 88 to 100 per cent mortality. Vyas *et al.* (2002) reported that spray of *Heterorhabditis* species @ 10,000 IJ per m^2 caused 87 per cent mortality of *H.armigera* in pigeon pea and led to increased crop yield.

Hussaini *et al.* (2002a) reported that between the two species strains PDBCEN 6.11 of *S. carpocapsae* and PDBC EN 6.71 of *H. indica* isolates, PDBCEN 6.11 of *S. carpocapsae* was found more effective in reducing the fruit damage in okra by *H. armigera* in terms of number of fruits with borer holes and increase in yields. Under glass house conditions the highest mortality and least fruit damage in okra was observed with *H. indica* after 72 hrs @ 5 X 10^9 IJ per ha followed by *H. indica* @ 2.5 X 10^9 IJ per ha (Umamaheswari *et al.*, 2005).

Ahmed *et al.* (2005) reported that *S. seemae* infected the pupal stage of *H. armigera* and caused 60 per cent mortality at an inoculum level of 500 IJ per larva at 72 hrs.

***2.4.2.2 Cut worm Spodoptera litura* Fabricius**

In Tobacco, DD-136 caused 66 per cent mortality of cut worm, *S. litura* (Gupta *et al.*, 1987). Narayanan and Gopalakrishnan (1987) reported that the

complete mortality of *S. litura* pre- pupae was caused by 10,000 and 1000 IJ of *S. feltiae*. Choo *et al.* (1988) used the entomophilic nematodes *S. feltiae* and *H. heliothidis* for the control of the cut worm, *S. litura.* Vyas and Yadav (1992) reported that *S. glaseri* caused complete mortality of *S. litura* larvae after 72 hrs when applied at the rate of 32 IJ per g of soil in the laboratory.

***2.4.2.3 Diamond back moth Plutella xylostella* L.**

Mason and Wright (1997) reported that the two isolates of *Steinernema* species (M87 and SSL 85) *Heterorhabditits* species and *H. indica* were pathogenic to *P. xylostella* within 24 hrs. Banu *et al.* (1998) also reported that EPN (Heterorhabditis and Steinernematids species) were pathogenic to *P. xylostella.*

Yang *et al.* (1999) reported that the strain of *S. carpocapsae,* A 24 and *H. bacteriophora,* 8406 were able to infect *P. xylostella* second instar larvae, early pupae and adults.

***2.4.2.4 Pink boll worm Pectinophora gossypiella* Saunders**

The mortality of *P. gossypiella* caused by *S. riobravis* was reported by Forlow and Henne (1997). Application of 1.3 billion IJ of *S. riobravis* per acre caused 92.4 per cent mortality at 2 days after application.

***2.4.2.5 Brinjal fruit borer Leucinodes orbonalis* Guen**

The DD- 136 strain caused 100 per cent mortality of brinjal fruit borer (*L.orbonalis*) (Singh and Bardhan, 1974). Hussaini *et al.* (2002a) recorded maximum mortality of *L. orbanalis* by the strains of *S. abbasi,* (PDBCEN3.1) *H. indica,* (PDBCEN 13.3) and *S. carpocapsae* (PDBCEN 6.11), in 48 to 72 hrs.

***2.4.2.6 Castor semi looper Paralellia algera* L.**

The DD-136 strain caused 100 per cent mortality of castor semi looper (*P.algera)* (Gupta *et al.*, 1987).

***2.4.2.7 Red hairy caterpillar Amsacta albistriga* Moore**

In field, DD-136, Burliar, Melur, Cherikunnu strains of *H. bacteriophora* were tested by Bhaskaran *et al.* (1994) on red hairy caterpillar, *A. albistriga,* a pest of

ground nut. They reported that DD-136 was most effective followed by Cherikunnu and Burliar strains.

2.4.2.8 Rice yellow stem borer Scirpophaga incertulas Walker

Rao *et al.* (1971) tested *steinernema* species (DD-136) against yellow stem borer and they reported that spraying of the nematode or application in standing water was effective in reducing the borer incidence. Rahman *et al.* (2000) also reported that the EPN (*Steinernema* and *Heterorhabditis* spp.) could be a safe component of rice stem borer integrated pest management.

2.4.2.9 Rice leaf roller Cnaphalocrocis medinalis Guen.

Srinivas and Prasad (1991) recorded that the natural occurrence of *S.carpocapsae* causing up to 98 per cent mortality in fourth and fifth instar larvae of *C. medinalis*. Prasad *et al.* (2006) reported that an inoculums level of 100 IJ per larva of *H. indica* caused cent per cent mortality of *C. medinalis* with in 18- 20 hrs of exposure while *S. thermophilum* caused cent per cent mortality with in 20 – 36 hrs of exposure in laboratory condition. Sheela *et al.* (2006) isolated five native isolates of *Heterorhabditis* (H1, H2, H3, H4 and H5). Along with this isolates and standards cultures, *H. indica* and *S. glaseri* they conducted a micro plot study in confined condition and *S. glaseri* gave cent per cent mortality of *C. medinalis* at 48 hrs after treatment in shaded condition.

2.4.2.10 Paddy cut worm Pseudoaletia seperata

Israel *et al.* (1969) reported that spraying of DD-136 nematode in the field effectively controlled paddy cut worm and the treatment was on par with parathion spray.

2.4.2.11 Sugar cane borer Eldana saccharina Walker

According to Spaull (1988) larvae of *E. saccharina* were more susceptible to *Heterorhabditis* sp. than *Steinernema*. 50- 200 ml. *Heterorhabditis* infective juvenile per stalk gave moderate control of the pest.

2.4.2.12 Leaf roller Sylepta derogata **L.**

Sheela *et al.* (2002) reported that *Heterorhabditis* species caused 40-100 per cent mortality of leaf eating caterpillar, *S. derogata* at 48 hrs after treatment.

2.4.2.13 Pumpkin caterpillar Diaphania indica **Saund**

Sheela *et al.* (2002) reported that after 48 hrs of treatment *Heterorhabditis* species caused about 40-100 per cent mortality of *D. indica.*

2.4.2.14 Black cut worm Agrotis ipsilon **Hufnagel**

Vyas and Yadav (1992) reported that *S. glaseri* caused complete mortality of *A. ipsilon* larvae after 48 hrs when applied at the rate of 32 IJ per g of soil in the laboratory. Ivanova *et al.* (1994) conducted an experiment on control of potato moth, *A. ipsilon* with aqueous suspensions of *S. feltiae*, *S.bibionis* or *S. carpocapsae* at the rate of 2000 infective larvae per receptacle and these species resulted in 95.5, 93.4 and 93.1 per cent mortality respectively.

2.4.3 Dipteran Pests

2.4.3.1 Mushroom sciarid Lycoriella mali **Fitch**

Rinker *et al.* (1995) evaluated *S. feltiae* and *H. heliothidis* for the larval mortality of mushroom infesting sciarid, *L. mali.* The mortality of *L. mali* larvae ranged from 52 to cent per cent due to *H. heliothidis* and 38 to cent per cent due to *S. feltiae.*

2.4.3.2 Mushroom fly Megaselia halterata **(Wood)**

Scheepmaker *et al.* (1998) reported that *S. feltiae* (30 IJ per larva) caused 78 per cent mortality of mushroom fly, *M. halterata.*

2.4.3.3 Carrot fly Psila rosae **F.**

Jaworska *et al.* (1998) reported that under laboratory conditions EPN (1000 nematodes per insect) caused cent per cent mortality of the early instar larvae of carrot fly, *P. rosae.*

2.4.3.4 Mediterranean fruit fly Ceratitis capitata **(Wiedmann)**

Gazit *et al.* (2000) evaluated the virulence of various EPN strains against the Mediterranean fruit fly, *C. capitata*. The selected nematodes were assessed for their infectivity for the final larval stage of the insect host. Among 12 EPN strains tested *S. riobrave* Texas and *Heterorhabditis* species showed more than 80 per cent mortality.

2.4.3.5 Melon fly Bactrocera cucurbitae **Coq.**

Sheela *et al.* (2002a) reported that *Rhabditis* sp. caused 6- 47 per cent mortality of *B. cucurubitae* at an inoculum level of 50, 100 and 200 IJ per larvae. They also reported that 200 IJ per larva caused 87 per cent and cent per cent mortality at 48 and 72 hrs after treatment respectively.

2.4.3.6 Onion maggot Delia spp.

Choo *et al.* (1988) reported that the entomophilic nematodes *S. feltiae* and *H. heliothidis* were used for the control of onion maggot *D. antique* in the laboratory. They reported that exposure of larvae of *D. antiqua* to *S. feltiae* at 30, 60, 120 or 240 nematodes per larvae caused 80 to 100 per cent mortality.

Shroeder *et al.* (1996) reported that between 100 and 100,000 IJ per plant of *S. feltiae* reduced the number of *D. radicum* pupae and the larvae which caused damage to the roots on potted cabbage.

2.4.4 Hemipteran Pests

2.4.4.1 Coffee mealy bug Planococcus lilacinus **Ckll.**

Rodriguez *et al.* (1997) reported that *H. bacteriophora* strain HC1 caused cent per cent mortality of coffee mealy bug complex (Pseudococcidae) in *in vitro* conditions.

2.4.4.2 Rice bug Leptocorisa acuta Thump

The mortality of *L. acuta* was assessed in *in vitro* condition using four inoculum levels of EPN (isolate 1, 2, 3, 4 and 5 and standard culture, *H. indica* and *S. glaseri*), 10, 25, 50 and 100 IJ per bug. At lowest inoculum level 10 IJ per grub isolate 5 gave cent per cent mortality (Sheela et al., 2006).

2.4.4.3 Areca nut spindle bug Carvalhoia arecae Miller

Mohandas *et al.* (2002) screened *Heterorhabditis* species against areca nut spindle bug, *C. arecae* under laboratory conditions and this caused the mortality of bug within 24 to 72 hrs of treatment.

2.4.5 Hymenopteran Pests

2.4.5.1 Mustard saw fly Athalia proxima Klug

Singh and Bardhan (1974) reported that the DD-136 strain caused cent per cent mortality of mustard saw fly, *A. proxima.*

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The present study was conducted to isolate native entomopathogenic nematodes (EPN) from banana rhizosphere infested with weevil pests, to screen out effective isolates and to find out the potential of native isolates for the management of *Odoiporus longicollis* Oliv. and *Cosmopolites sordidus* Ger. The studies were also conducted to evolve a cheap media for the mass multiplication of EPN. The materials used and methods adopted are given here under.

3.1 DISTRIBUTION OF EPN FROM THE BANANA RHIZOSPHERE

A random survey was conducted during 2005-06 at Instructional Farm, Vellayani to assess the occurrence of native isolates of EPN and further investigations on isolation and screening of native EPN were carried out at Department of Agricultural Entomology, College of Agriculture, Vellayani. One hundred soil samples were collected from the Farm comprised of 17, 18, 18, 15, 18 and 14 samples from A, B, C, D, E and F blocks respectively. Number of soil samples collected from each block was based on the area of banana crop and the infestation of weevils.

3.1.1 Sampling

Soil samples, dead weevils and grubs were collected from the rhizosphere of banana. Top soil from 5 to 10 cm from the base of the plant at a depth of 10-15 cm was removed. Soil sample about 20-25 g was collected in polythene bags of 150 gauge thickness. Since EPN survives only in moist soils, soon after collection the polythene bags were tightly closed with a rubber band to avoid drying.

3.1.2 Isolation of Native EPN

The larvae of *Galleria mellonella* L. were used to concentrate EPN from the soil samples and dead insects. Fifth instar larvae reared in standard medium was used for trapping EPN as described by Woodring and Kaya (1988).

3.1.2.1 Preparation of standard medium

One kg of the medium was prepared by adding the following ingredients.

Corn flour	-	200 g.
Milk powder	-	100 g.
Atta	-	200 g.
Yeast	-	100 g.
Honey	-	100 ml.
Glycerol	-	150 ml.

The corn flour, atta and milk powder were weighed accurately and mixed thoroughly in a container. The honey and glycerol were mixed homogenously in another container. The honey glycerol mixture was then slowly added to the flour ingredients and again mixed well so as to obtain ball break consistency. The medium was then autoclaved at 120°C for 20 minute and then added powdered yeast tablets. The prepared medium was kept for half an hour for fermentation.

3.1.2.2 Rearing of trap insect, wax moth (*G. mellonella*)

Newly emerged adults were collected and placed in a container provided with 50 per cent honey. Strips of folded paper were dispensed from the sides of the container for egg laying (Plate 1). Egg cards obtained from the containers were kept for eclosion. First instar larvae of *G. mellonella* were collected from the containers and released in another container with standard medium. Cleaning, transferring and addition of media were done as and when needed (Plate 2). The fully grown fifth instar larvae were collected from the media and used for trapping EPN.

3.1.2.3 Trapping technique

Five fifth instar larvae of *G. mellonella* were placed at the bottom of a container and packed the moist soil sample lightly over them (Plate 3). It was incubated at room temperature for five to seven days. Dead larvae were collected

Plate 1. Rearing of adult *G. mellonella*

Plate 2. *G. mellonella* larvae in standard medium

from the container and placed in "White trap" advocated by Bedding and Akhurst (1975) using sterilized whatman No. 40 filter paper, watch glass and petridish (Plate 4). Formaldehyde (0.1 per cent) poured in to the petridish and draped the filter paper over the watch glass so that it came in contact with the formaldehyde. Typically infected dead larvae were placed on the filter paper over the edge of the watch glass. Infective juveniles of EPN started to exit six to ten days after infection. After removing the watch glass and dead larvae the infective juveniles were collected in a beaker containing 0.1 per cent formaldehyde.

3.2 SCREENING OF NATIVE EPN

The pathogenic effect of ten native isolates, obtained in the survey, was tested using grubs of pseudostem weevils.

3.2.1 Rearing of Pseudostem Weevil, *O. longicollis*

Fifteen centimeter long fresh pseudostem pieces were used for maintaining the pseudostem weevil culture in the laboratory. Ten grubs were introduced in to each pseudostem and were placed inside a trough. The larvae were taken out and introduced in to fresh pseudostem pieces once in two days. This was continued until pupation. After pupation the cocoons were collected and kept in glass troughs covered with wet muslin cloth for adult emergence. Insects from this culture were used for further experiments.

3.2.2 Preparation of Stock Culture of Native Isolates

Infective juveniles from each isolates multiplied in the *G. mellonella* larvae reared in standard medium were used for the preparation of stock culture. These infective juveniles were stored in coloured tissue culture flasks containing 0.1 per cent formaldehyde and kept in refrigerated condition.

3.2.3 Pathogenic Effect of Native Isolate on *O. longicollis* Grubs

Fifth instar grubs of pseudostem weevil collected from the stock culture were released in to the petridish having moist filter paper containing required concentration of infective juveniles (10, 50, 100 and 200 per grub) of isolates and incubated at room temperature. Each inoculum levels of ten isolates were

Healthy larvae

EPN infected larvae

Plate 3. Trapping of native EPN from soil

Plate 4. White trap method

replicated five times. Mortality of the grubs was recorded at 24, 48 and 72 hrs after treatment. The corrected mortality percentage was worked out by using Abbot's formula. (Abbot, 1925)

3.2.4 Selection of Native Isolates

The isolates which produced maximum mortality of pseudostem weevil grub were selected for further studies.

3.3 REARING OF *G. mellonella* LARVAE IN DIFFERENT ARTIFICIAL MEDIA

In order to find out a cheap medium for rearing of *G. mellonella* four different media (M1, M2, M3 and M4) were prepared with following composition (Plate 5).

3.3.1 Composition of Different Media

M1 medium

Corn flour	-	100 g.
Milk powder	-	100 g.
Atta	-	300 g.
Yeast	-	100 g.
Honey	-	200 ml.
Glycerol	-	50 ml.

M2 medium

Corn flour	-	100 g.
Milk powder	-	100 g.
Atta	-	300 g.
Yeast	-	100 g.
Honey	-	100 ml.

Standard medium

M1 medium

M2 medium

M3 medium

M4 medium

Plate 5. Artificial media for rearing *G. mellonella*

Glycerol	-	50 ml.
Sugar solution	-	100 ml.

M3 medium

Corn flour	-	50 g.
Milk powder	-	100 g.
Atta	-	100 g.
Rice bran	-	400g.
Yeast	-	100 g.
Honey	-	200 ml.
Glycerol	-	50 ml.

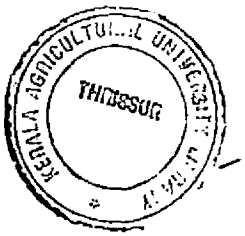
M4 medium

Rice bran	-	600 g.
Atta	-	200 g.
Yeast	-	100g.
Sugar solution	-	200 ml.
Glycerol	-	50 ml.

3.3.2 Preparation of Different Media

M1 medium

First three ingredients were weighed accurately and mixed thoroughly in a container. In another container the honey and glycerol were mixed homogenously. Honey glycerol mixture was slowly added to the flour ingredients and mixed thoroughly so as to obtain ball break consistency. The medium was then autoclaved at 120°c for 20 min. After autoclaving powdered yeast tablets were added to the prepared medium and kept for half an hour for fermentation.



M2 medium

First three ingredients were weighed accurately and mixed thoroughly in a container. In another container the honey and glycerol were mixed homogenously. Here the sugar solution was an additional ingredient. Sugar solution was prepared by adding 100 g sugar in 500 ml water and mixed well and was added to the glycerol honey mixture. This mixture was slowly added to the flour ingredients and mixed thoroughly so as to obtain ball break consistency. The medium was then autoclaved at 120°c for 20 min. After autoclaving powdered yeast tablets were added to the prepared medium and kept for half an hour for fermentation.

M3 medium

First four ingredients were weighed accurately and mixed thoroughly in a container. Honey mixed glycerol was slowly added to the above ingredients and mixed thoroughly. The medium was then autoclaved at 120°c for 20 min. Powdered yeast tablets were added and kept for half hour for fermentation.

M4 medium

Rice bran was weighed accurately and mixed with atta in a container. In another container sugar solution was prepared by adding 100g sugar in 500ml water and mixed well. The sugar solution and glycerol were mixed homogenously. Sugar glycerol mixture was slowly added to the flour ingredients and mixed thoroughly so as to obtain ball break consistency. The medium was then autoclaved at 120°c for 20 min. After autoclaving the powdered yeast tablets were added in to the media and kept for half an hour for fermentation.

3.3.3 Survival of various instars of *G. mellonella* Larvae in Different Media

Twenty first instar larvae were released in separate containers having M1, M2, M3, M4 and standard media. Cleaning, transferring and addition of media were done as per the requirement. The survivals of larvae were observed daily and recorded instarwise.

3.4 MASS PRODUCTION OF EPN

3.4.1 Preparation of Stock Culture

3.4.1.1 Native isolates N1 and N2

The infective juveniles mass multiplied in the *G. mellonella* larvae were used for the preparation of stock culture. The infective juveniles were stored in coloured tissue culture flasks containing 0.1 per cent formaldehyde solution and kept in refrigerated condition.

3.4.1.2 Standard cultures Heterorhabditis indica and Steinernema glaseri

The standard culture was stored in sponge pieces and kept in refrigerated condition. The infective juveniles were extracted either by squeezing the sponge in little amount of water or kept the sponge pieces in a petridish containing little amount of water for half an hour. These infective juveniles mass multiplied in the *G. mellonella* larvae were used for the preparation of stock culture. These infective juveniles were stored in coloured tissue culture flasks containing 0.1 per cent formaldehyde solution and kept in refrigerated condition (Plate 6).

3.4.2 Estimation of EPN in *G. mellonella* Grown in Different Media

Fully grown final instar *G. mellonella* larvae reared in M1, M2, M3 and standard medium were used for the multiplication of EPN. Four isolates of EPN viz., two standard isolates, *H. indica* and *S. glaseri* and two native isolates N1 and N2 were inoculated to the fifth instar larvae of *G. mellonella*. From stock culture of each isolate a small quantity was taken and diluted with formaldehyde to get a suspension having 25 IJ per ml. This suspension was poured in a petri dish with 9 cm whatman No.1 filter paper and evenly distributed. Five *G. mellonella* larvae were placed on the filter paper so as to have five nematodes per larva. For 10 IJ per larva and 25 IJ per larva same procedure was followed with 50 IJ per ml and 125 IJ per ml respectively.

The mortality of *G. mellonella* larvae was observed after 48 and 72 hrs. The dead larvae were placed in to White traps for the extraction of nematodes. Four

EPN juveniles in sponge

EPN juveniles in coloured flask

Plate 6. Storage of infective juveniles

milliliter 0.1 per cent formaldehyde solution was added in to White trap for maximum harvest of nematodes. Within seven to eight days the nematodes started to wriggle out from the *G. mellonella* larvae and migrated all over the petridish. The number of infective juveniles emerged from White trap were recorded at 7, 10, 15 and 20 days intervals.

3.4.3 Selection of Cheap Media for Rearing of *G. mellonella*

Cost of ingredients for the preparation of one kg of various media was calculated. Percentage reduction in the cost of M1, M2 and M3 media over the standard medium was calculated.

3.5 COMPARISON OF ISOLATES FOR THE MANAGEMENT OF WEEVI L PESTS OF BANANA

3.5.1 Assessment of EPN against Weevil Pest in *in vitro* Condition

3.5.1.1 Rearing of pseudostem weevil

Rearing of pseudostem weevil was done as mentioned in 3.2.1 (Plate 7)

3.5.1.2 Rearing of rhizome weevil

Fresh small banana rhizomes were used for rearing the rhizome weevil for maintaining the culture in the laboratory. A rhizome was placed in a trough and ten grubs of rhizome weevil were introduced in to the rhizome. The trough was covered with wire meshes. After two days the introduced grubs were entered in the rhizome. The rhizomes were changed once in four days and the larvae were taken out and introduced in to fresh ones. After pupation the troughs were covered with wet muslin cloth for adult emergence. Insects from this culture were used for further experiments (Plate8).

3.5.1.3 Pathogenicity of EPN against O. longicollis

Grubs

Treatments - 10, 50, 100 and 200 IJ of two standard cultures (*H. indica* and *S. glaseri*) and two native isolates (N1 and N2).

Design - CRD

Replication - 5

Grubs of pseudostem weevil were released in to the petridish having moist filter paper containing required concentration of infective juveniles (10, 50, 100 and 200 per grub) of isolates and incubated at room temperature (Plate 9). Mortality of grubs was recorded at 24, 48 and 72 hrs after treatment. The corrected mortality percentage was worked out by using Abbot's formula.

Adults

Treatments - 10, 50, 100 and 200 IJ of two standard cultures (*H. indica* and *S. glaseri*) and two native isolates (N1 and N2)

Design - CRD

Replication - 5

Same procedure was repeated as in case of grubs of pseudostem weevil also.

3.5.1.4 Pathogenicity of EPN against C. sordidus

Grubs

Treatments - 10, 50, 100 and 200 IJ of two standard cultures (*H. indica* and *S. glaseri*) and two native isolates (N1 and N2).

Design - CRD

Replication - 5

Grubs of rhizome weevil were collected from the stock culture and treated with the different inoculum levels of each isolates collected from the stock culture. Mortality of grubs was recorded at 24, 48 and 72 hrs after treatment. The corrected mortality percentage will be worked out by using Abbot's formula.

Adults

Treatments - 10, 50, 100 and 200 IJ of two standard cultures (*H. indica* and *S. glaseri*) and two native isolates (N1 and N2).

Design - CRD

Plate 7. Rearing of pseudostem weevil

Plate 8. Rearing of rhizome weevil

Plate 9. Filter paper method

Replication - 5

Same procedure was repeated as in case of grubs of rhizome weevil

3.5.2 Assessment of EPN against Weevils Inside the Substrate in the Laboratory

The two native isolates and two standard cultures were used to assess the potential against grubs and adults of weevils of banana inside the substrate in laboratory conditions.

3.5.2.1 *Pseudostem weevil*

Grubs

Treatments - 10, 50, 100 and 200 IJ of two standard cultures (*H. indica* and *S. glaseri*) and two native isolates (N1 and N2)

Design - CRD

Replication - 5 for each four inoculum levels and three exposure periods, (48, 72 and 96 hrs)

Uninfected fresh 15 cm long pseudostem was taken. Trial method was used to standardize the optimum quantity of water for wetting the pseudostem for the survival of EPN in the pseudostem. The 15 cm pseudostem was wet with 5, 10 and 15 ml of water. Ten forth instar grubs of pseudostem were introduced in to the fresh pseudostem and then kept in a trough. At the rate of 10, 50, 100 and 200 infective juveniles of two native isolates N1 and N2 and two standards, *H. indica* and *S. glaseri* were taken from stock culture. Diluted the suspension with 0.1 per cent formaldehyde and the final quantity was 10 ml. The infective juveniles were inoculated in to the pseudostem with the help of pipette. Mortality was taken at 48, 72 and 96 hrs after treatment. Each treatment replicated five times.

Adults

Treatments - 10, 50, 100 and 200 IJ of two standard cultures (*H. indica* and *S. glaseri*) and two native isolates (N1 and N2)

Design - CRD

Replication - 5 for each four inoculum levels and three exposure periods, (48, 72 and 96 hrs)

Same procedure was repeated as in case of grubs of pseudostem weevil

3.5.2.2 *Rhizome weevil*

Grubs

Treatments – 10, 50, 100 and 200 IJ of two standard cultures (*H. indica* and *S. glaseri*) and two native isolates (N1 and N2)

Design - CRD

Replication - 5 for each four inoculum levels and three exposure periods, (48, 72 and 96 hrs)

Uninfected fresh small rhizome was taken. Using the trial method 10 ml water was standardized to wet the rhizome. Ten forth instar grubs of rhizome weevil were introduced in to the fresh rhizome and then kept in a trough. At the rate of 10, 50, 100 and 200 infective juveniles of two native isolates N1 and N2 and two standards, *H. indica* and *S. glaseri* were taken from stock culture. Diluted the suspension with the help of 0.1 per cent formaldehyde and the final quantity was 10 ml. The infective juveniles were inoculated in to the rhizome with the help of pipette. Mortality was taken at 48, 72 and 96 hrs after treatment. Each treatment replicated five times.

Adult

Treatments – 10, 50, 100 and 200 IJ of two standard cultures (*H. indica* and *S. glaseri*) and two native isolates (N1 and N2)

Design - CRD

Replication - 5 for each four inoculum levels and three exposure periods, (48, 72 and 96 hrs)

Same procedure was repeated as in case of grubs of rhizome weevil

3.6 STATISTICAL ANALYSIS

The data generated from the experiments were subjected to analysis of variance (ANOVA) technique (Panse and Suhatme, 1985). The variables which did not satisfy the basic assumption of ANOVA were subjected to logarithmic and angular transformation and then analyzed. The data on percentage mortality was corrected by using Abbot's formula (Abbot, 1925).

RESULTS

4. RESULTS

In the present study native entomopathogenic nematodes (EPN) were isolated from the banana rhizosphere and its potential for the management of pseudostem weevil (*Odoiporus longicollis* Oliv.) and rhizome weevil (*Cosmopolites sordidus* Ger.) infesting banana were studied and the results are given.

4.1 DISTRIBUTION OF EPN IN THE BANANA RHIZOSPHERE OF INSTRUCTIONAL FARM

A random survey was conducted to find out the occurrence of EPN in different stages of banana weevils and in the banana rhizosphere in different blocks of the Instructional Farm, College of Agriculture, Vellayani. Out of the 100 samples collected, ten native isolates were obtained from different blocks covering an area of 75 ha as given in Table 1. Maximum numbers of soil samples were collected from B, C, and E blocks (18 each) followed by A (17) D (15) and F (14). Maximum number of native isolates was obtained from F block (three) and the percentage distribution of EPN was 21.43. From B, C and E two native isolates each were obtained with 11.11 as percentage distribution of EPN. From A block only one native isolate was obtained with a percentage distribution of only 5.88. No native isolate was obtained from D block through 15 samples were collected and proceed. None of insect cadavers of *O. longicollis* and *C. sordidus* recorded EPN association.

Table1. Distribution of native EPN in the rhizosphere of banana in various blocks of the Instructional Farm, Vellayani

Name of Blocks	Number of samples collected	Number of native isolate obtained	Percentage distribution of EPN
A	17	1	5.88
B	18	2	11.11
C	18	2	11.11
D	15	-	0
E	18	2	11.11
F	14	3	21.43
Total	100	10	

4.2 SCREENING OF THE NATIVE ISOLATE OF EPN

The potential of native isolates was assessed by testing their effect on the mortality of *O. longicollis* grubs using different inoculum levels and exposure periods under *in vitro* conditions and the results are presented in Table 2.

10 IJ per grub

The result presented in Table2 showed that there was statistically significant variation in the mean corrected mortality of grubs of *O. longicollis* inoculated with 10 IJ per grub at 48 and 72 hrs after treatment.

48 hrs after treatment

Maximum mortality of grubs was recorded in isolate 1 (N1) (22.36 per cent). The effect of this isolate was significantly superior to other nine native isolates. Isolate 2 (N2) showed a corrected mortality percentage of 12.94 and was on par with isolate 3 (N3) (10.50 per cent) but superior to other seven isolates (N4 to N10). The mortality of grubs treated with isolates 4 (N4), 5 (N5), 6(N6), 7(N7) and isolate 8(N8) were statistically on par and their mortality ranged from 4.18 to 6.90 per cent. Minimum mortality of grubs was recorded in isolate 10 (N10) (0.54 per cent) and it was statistically on par with isolate 9 (N9) (1.03 per cent) (Table 2).

Table2. Effect of ten native isolates of EPN on mortality of grubs of *O. longicollis* under laboratory condition

Native isolates	Percentage mortality at different inoculum levels and periods (hrs)							
	10IJ		50IJ		100IJ		200IJ	
	48hrs.	72hrs.	48hrs.	72hrs.	48hrs.	72hrs.	48hrs.	72hrs.
N1	22.36 *(28.21)	66.03 (54.33)	61.43 (51.59)	74.86 (59.88)	74.33 (59.54)	85.31 (67.44)	76.66 (61.08)	81.69 (64.64)
N2	12.94 (21.07)	42.39 (40.61)	45.79 (42.57)	45.19 (42.22)	62.41 (52.17)	64.42 (53.36)	56.05 (48.45)	60.23 (50.88)
N3	10.50 (18.90)	37.55 (37.78)	34.17 (35.76)	43.39 (41.19)	35.87 (36.78)	38.70 (38.45)	34.97 (36.24)	38.14 (38.12)
N4	6.90 (15.22)	29.55 (32.91)	33.97 (35.64)	42.39 (40.61)	46.99 (43.26)	50.20 (45.10)	38.96 (39.56)	43.95 (41.51)
N5	5.73 (13.84)	29.55 (32.91)	29.94 (33.16)	33.97 (35.64)	39.37 (38.84)	45.55 (40.70)	42.39 (40.61)	46.40 (42.90)
N6	5.87 (14.02)	29.55 (32.91)	29.94 (33.16)	31.79 (34.30)	19.50 (26.19)	23.72 (29.14)	24.37 (29.57)	31.98 (34.42)
N7	5.20 (13.17)	21.77 (27.80)	29.36 (32.80)	32.79 (34.92)	9.18 (17.63)	12.16 (20.40)	34.78 (36.12)	36.55 (37.19)
N8	4.18 (11.80)	22.23 (14.33)	16.77 (24.16)	23.95 (29.29)	12.27 (20.50)	15.07 (22.83)	22.93 (28.60)	34.33 (35.86)
N9	1.03 (5.83)	4.12 (11.70)	2.14 (8.42)	4.40 (12.10)	1.69 (7.46)	4.81 (12.66)	5.99 (14.16)	7.44 (15.82)
N10	0.54 (4.21)	2.14 (8.42)	1.03 (5.83)	3.06 (10.08)	3.06 (10.08)	5.34 (13.36)	6.46 (14.72)	7.44 (15.82)
CD(0.05)	4.474	3.510	4.178	3.691	4.438	3.648	3.412	3.376

*Figures in parenthesis are values after angular transformation

72 hrs after treatment

Maximum mortality of grubs was obtained in N1 with a corrected mortality of 66.03 per cent. This was statistically superior to the other nine isolates (N2 toN10). The effects of N2 and N3 were on par with a mortality percentage of 42.39 and 37.55 respectively. The mortality percentage of grubs treated with N4 (29.55) N5 (29.55) and N6 (29.55) were statistically on par and superior to N7 (21.77), N8 (22.23), N9 (4.12) and N10 (2.14). Minimum mortality of grubs was recorded in N10 and it was statistically on par with N9. (Table 2)

50 IJ per grub

The result showed that the mean corrected mortality of *O. longicollis* inoculated with 50 IJ per grub at 48 and 72 hrs after treatment also vary significantly. (Table 2)

48 hrs after treatment

The maximum mortality of grubs (61.43 per cent) was recorded in N1 and this was statistically superior to other nine isolates (N2 toN10). N2 showed a corrected mortality percentage of 45.79 and it was second only to N1 and significantly superior to other eight isolates (N3 toN10). The isolate N3, N4, N5, N6 and N7 recorded a mortality percentage of 34.17, 33.97, 29.94, 29.94 and 29.36 respectively and were statistically on par. N8 recorded a mortality of 16.77 per cent and it was superior to N9 and N10 and inferior to other seven isolates (N1 to N7). Minimum mortality of grubs was recorded in N10 (1.03) and it was statistically on par with N9 (2.14). (Table2)

72 hrs after treatment

Maximum corrected mortality of grubs was obtained in N1 (74.86 per cent). This was statistically superior to the other nine isolates (N2 to N10). N2 showed a corrected mortality percentage of 45.19 and was on par with N3 and N4 with corrected mortality percentages of 43.39 and 42.39 respectively. The effect

of other isolates N5 (33.97), N6 (31.79), N7 (32.79) were also statistically on par. Minimum mortality of grubs was obtained in N10 (3.06 per cent) and it was statistically on par with N9 (4.40 per cent). (Table 2)

100 IJ per grub

The result showed that the mortality of grubs of *O. logicollis* inoculated with 100 IJ per grub at 48 and 72 hrs after treatment varies significantly. The results are presented in Table 2

48 hrs after treatment

The maximum mortality of grubs was recorded in N1 (74.33 per cent) and was statistically superior to the other nine isolates. The corrected mortality percentage of 62.41 was recorded in N2 and the effect was significantly superior to other eight isolates except N1. The mortality percentage of N3 and N5 were 35.87 and 39.37 and their effect was statistically on par. The mortality percentage of N7 (9.18) and N8 (12.27) were also statistically on par. The minimum mortality was recorded in N9 (1.69 per cent) and it was statistically on par with N10 (3.06 per cent). (Table2)

72 hrs after treatment

The maximum mortality of grubs was obtained in N1 with a corrected mortality of 85.31 per cent and it was statistically superior to other nine isolates. The mortality percentage of N3 and N5 were 38.70 and 45.55 respectively and there was no statistically significant variation. The mortality percentage of N7 (12.16) and N8 (15.07) were also statistically on par. The minimum mortality of the grub was recorded in N9 (4.18 per cent) and it was statistically on par with N10 (5.34 per cent). (Table 2)

200 IJ per grub

The result presented in Table 2 revealed that the mean corrected mortality of grubs of *O. longicollis* inoculated with 200 IJ per grub at 48 and 72 hrs after treatment also showed statistically significant variation.

48 hrs after treatment

The maximum mortality was recorded in N1 (76.66 per cent) and was statistically superior to other nine isolates. Isolate N2 was second only to N1 and gave a corrected mortality percentage of 56.05 and the effect was significantly superior to other eight isolates. The mortality percentage of N3, N4 and N7 were 34.97, 38.96 and 34.78 were statistically on par. The mortality percentage of grubs treated with N4 and N5 (42.39) were also statistically on par. Minimum mortality of grubs was obtained in N9 (5.99) and it was statistically on par with N10 (6.46). (Table2)

72 hrs after treatment

The effect of native isolates on the mortality of *O. longicollis* at 72 hrs after treatment also varies significantly. The maximum mortality was recorded in N1 (81.69 per cent). N2 showed a mortality of 60.23 and was superior to other eight isolates. The mortality of other isolates ranged from 7.44 to 46.40. Minimum mortality was recorded in N9 (7.44) and N10 (7.44) and they were significantly inferior to N3, N4, N5, N6, N7 and N8 with mortality of 38.14, 43.95, 46.40, 31.98, 36.55 and 34.33 per cent respectively. (Table2)

From the over all performance the isolate N1 and N2 were selected for detailed study along with the standards, *Steinernema glaseri* and *Heterorhabditis indica*.

4.3. REARING OF TRAP INSECT, THE WAX MOTH

The result presented in Table 3 showed statistically significant variation in the survival of different stages (first, second, third, fourth and fifth instar larvae) of *Galleria mellonella* L. reared in five media.

First instar larvae

Out of the twenty larvae initially released in different media the mean number of larvae survived varied from 2.81 to 19.19. The maximum percentage survival (95.0) of first instar larvae was observed in standard medium (19.19) followed by M1 and M2 media. In M1 and M2 the survival was above 75

percentage and the mean number being 17.78 and 16.38 respectively. The effect of survival of *G. mellonella* larvae in the standard, M1 and M2 media were statistically on par. In M3 medium the mean number of first instar larvae survived was only 13.96 and this was statistically on par with M2.

Second instar larvae

The results presented in Table 3 revealed that there was statistically significant variation in the survival of second instar larvae also in different media. The maximum survival (95 per cent) was recorded in standard medium (18.99) followed by M1 (17.97) and these two were statistically on par. The mean number of larvae of *G. mellonella* survived in M2 medium was 15.97. The percentage of survival was 85 and 75 respectively in M1 and M2 .These two was statistically on par. In M3 medium mean number of larvae survived was 14.31(70 percentage) and this was statistically on par with M2. Minimum number of larval survival was in M4 medium (2.75).

Third instar larvae

The mean survival of third instar larvae of *G. mellonella* in five different media showed statistically significant variation .The maximum survival of third instar larvae was observed in standard medium (18.99) followed by M1medium (16.79) and these two were statistically on par. The effect of M1 and M2 were statistically on par. In M2 and M3 the survival of third instar larvae were 14.93 and 12.92 respectively revealing more than 50 per cent survival and these were also statistically on par. The minimum number of larvae was survived in M4 medium (1.72) (Table .3)

Fourth instar larvae

There was statistically significant variation in the survival of fourth instar larvae of *G. mellonella* in different media. The results are presented in the Table 3. Maximum survival was observed in standard medium (17.97) and it was significantly superior to M1, M2, M3 and M4. In M1 medium the survival of fourth instar larvae was 15.36 and this was statistically on par with M2 medium

(13.99). In M3 medium fourth instar larvae survived was 11.96 and it was on par with M2. Minimum number of larvae was survived in M4 medium (1.72).

Fifth instar larvae

The results presented in the Table 3 revealed that there was statistically significant variation in the survival of fifth instar larvae of *G. mellonella* in different media. The survival was maximum in standard medium (17.97) followed M1 medium (15.97.) and these two were statistically on par with M2, M3 and the larval survival was 12.96 and 11.92 larvae survival respectively. Minimum number of larvae survived in M4 medium (1.72).

Table 3 Survival of *G. mellonella* larvae (instarwise) in different media

Media	Number of larvae survived (instarwise)				
	First	Second	Third	Fourth	Fifth
Standard	19.19 *(4.38)	18.99 (4.36)	18.99 (4.36)	17.97 (4.24)	17.97 (4.24)
M1	17.78 (4.22)	17.97 (4.24)	16.79 (4.10)	15.36 (3.92)	15.97 (4.0)
M2	16.38 (4.05)	15.97 (4.00)	14.93 (3.86)	13.99 (3.74)	12.96 (3.60)
M3	13.96 (3.74)	14.31 (3.78)	12.92 (3.59)	11.96 (3.46)	11.92 (3.45)
M4	2.81 (1.68)	2.75 (1.66)	1.72 (1.31)	1.72 (1.31)	1.72 (1.31)
CD(0.05)	0.345	0.304	0.325	0.288	0.337

* Figures in parenthesis are values after square root transformation

4.4 Mass production of EPN

Two native isolates, N1 and N2 and two standards, *H. indica* and *S. glaseri* were mass produced in the *Galleria* larvae reared in four different media. The number of infective juveniles of EPN emerged from different initial inoculum levels and period intervals were recorded and are given in Table 4 to 7.

4.4.1 *H. indica*

5 infective juveniles per *G. mellonella* larva (5IJ per larva)

The result presented in Table 4 revealed that the mean number of infective juveniles of *H. indica* emerged from *G. mellonella* larvae, fed with various artificial media, at seven days after inoculation (DAI) varied significantly. *G. mellonella* larva reared in standard medium showed maximum emergence of mean infective juveniles of *H. indica* (196.79). M1 medium recorded 10.74 infective juveniles per larva followed by M2 medium with (8.15) and these two were statistically on par. Minimum emergence of infective juveniles was from M3 medium (1.5 per larva).

At 10 DAI also the effect of various media showed statistically significant variation. Here *G. melonella* larva reared in standard medium showed maximum emergence of infective juveniles (1004.62 per larva) of *H. indica.* M1 medium recorded 233.88 infective juveniles followed by M2 medium (80.35 IJ per larva) and these two were statistically on par. Minimum emergence of infective juveniles was recorded from M3 medium (2.93 per larva). (Table 4)

At 15 DAI also the effects of different media showed statistically significant variation. Maximum emergence of infective juveniles recorded in standard medium (2333.46 per larva). The infective juveniles emerged from *G. mellonella* reared in M1, M2 and M3 media were 574.12, 244.91 and 155.96 per larva respectively. (Table 4)

The results presented in Table 4 revealed that the mean number of infective juveniles of *H. indica* emerged from *G. mellonella* larvae fed with various artificial media varied significantly. Maximum number of infective

Table 4. Effect on the multiplication of *H. indica* in *G. mellonella* fed with different artificial media

Media	Number of *H.indica* emerged at different intervals (days) on a single larva of *G. mellonella* inoculated with											
	5IJ				10IJ				25IJ			
	7DAI	10DAI	15DAI	20DAI	7DAI	10DAI	15DAI	20 DAI	7DAI	10DAI	15DAI	20 DAI
Standard	196.79 *(2.29)	1004.62 (3.00)	2333.46 (3.37)	3111.72 (3.49)	243.78 (2.39)	5597.58 (3.75)	8279.42 (3.92)	14689.3 (4.17)	597.04 (2.78)	1940.89 (3.29)	4395.42 (3.64)	7585.78 (3.88)
M1	10.74 (1.03)	233.88 (2.37)	574.12 (2.76)	1164.13 (3.07)	1051.9 (3.02)	2660.73 (3.43)	3971.92 (3.60)	5754.39 (3.76)	1099.01 (3.04)	2844.46 (3.45)	3467.37 (3.54)	4178.30 (3.62)
M2	8.15 (0.91)	80.35 (1.91)	244.91 (2.39)	582.10 (2.77)	28.38 (1.45)	101.62 (2.01)	481.95 (2.68)	1018.59 (3.01)	21.73 (1.34)	109.90 (2.04)	247.74 (2.39)	645.65 (2.81)
M3	1.5 (0.18)	2.93 (0.47)	155.96 (2.19)	184.50 (2.27)	2.0 (0.30)	5.66 (0.75)	159.22 (2.20)	185.78 (2.27)	1.5 (0.18)	5.90 (0.77)	82.41 (1.92)	191.87 (2.28)
CD (0.05)	0.459	0.468	0.330	0.260	0.259	0.396	0.214	0.262	0.455	0.408	0.345	0.179

DAI – Days after inoculation
*Figures in parenthesis are values after logarithmic transformation

juveniles was emerged from *G. mellonella* reared in standard medium (3111.72 per larva). M1, M2 and M3 recorded 1164.13, 582.10 and 184.50 per larva infective juveniles respectively.

10 IJ per larva

The result presented in Table 4 revealed that the mean number of infective juveniles of *H. indica* emerged from *G. mellonella* larva, fed with different artificial media at 7DAI varied significantly. Larva from M1 medium showed maximum emergence of infective juveniles (1051.96 per larva). Standard, M2 and M3 medium showed 243.78, 28.38 and 2.00 per larva infective juveniles respectively.

At 10DAI also the effect of various media showed statistically significant variation. Larvae from standard medium showed maximum emergence of infective juveniles (5597.58 per larva) followed by M1 medium (2660.73 per larva) and these two were statistically on par. M2 and M3 medium recorded 101.62 and 5.66 infective juveniles per larva respectively. (Table 4)

At 15 DAI also the effect of different media showed statistically significant variation. The larva from the standard medium showed the maximum mean emergence of *H. indica* (8279.42 per larva). M1, M2 and M3 recorded 3971.92, 481.95 and 159.22 infective juveniles per larva. (Table 4)

The mean number of infective juveniles of *H. indica* emerged from Galleria larvae fed with various artificial media varied significantly. The maximum emergence of infective juveniles was seen in standard medium (14689.3 per larva). M1, M2 and M3 recorded 5754.39, 1018.59 and 185.78 infective juveniles per larva. (Table 4)

25 IJ per larva

The mean number of infective juveniles of *H. indica* emerged from *G. mellonella* larva reared in different artificial media at 7 DAI varied significantly. Larvae from M1 medium showed maximum emergence of infective juveniles of *H. indica* (1099.01 per larva) followed by standard

medium (597.04 per larva) and these two were statistically on par. The infective juveniles emerged from the larva reared in M2 and M3 were 21.73 and 1.5 respectively. (Table 4)

In various media, the mean number of infective juveniles emerged from *G. mellonella* larva at 10 DAI showed statistically significant variation. Larvae from M1 medium showed maximum emergence of infective juveniles (2844.46 per larva) followed by standard medium (1940.89 per larva) and these two were significantly on par. M2 and M3 medium recorded 109.90 and 5.90 infective juveniles respectively. (Table 4)

Effect of various media at 15 DAI also showed statistically significant variation. The larva reared in standard medium showed maximum emergence of infective juveniles (4395.42 per larva) followed by M1 medium (3467.37 per larva) and these two were statistically on par. M2 and M3 showed only 247.74 and 82.41 respectively. (Table 4)

At 20 DAI also the effect of different media showed statistically significant variation. The maximum mean number of infective juveniles of *H.indica* was emerged from *G. mellonella* larva reared in standard medium (7585.78 per larva). M1, M2 and M3 medium recorded 4178.30, 645.65 and 191.87 infective juveniles per larva. (Table 4)

4.4.2 *S. glaseri*

5 IJ per larva

The mean number of infective juveniles of *S. glaseri* emerged from *G. mellonella* larva at 7 DAI in various media showed statistically significant variation. The maximum emergence was showed by M1 medium (283.14 IJ per larva) followed by standard medium (157.76 IJ per larva) and these two were statistically on par. M2 and M3 medium recorded 20.14 and 3.00 infective juveniles per larva respectively (Table 5).

At 10 DAI the effect of different media showed statistically significant variation. Larvae from standard medium showed maximum mean emergence of

Table 5. Effect on the multiplication of *S. glaseri* in *G. mellonella* fed with different artificial media

Media	Number of *S. glaseri* emerged at different intervals (days) on a single larva of *G. mellonella* inoculated with											
	5IJ				10IJ				25IJ			
	7DAI	10DAI	15DAI	20DAI	7DAI	10DAI	15DAI	20 DAI	7DAI	10DAI	15DAI	20 DAI
Standard	157.76 *(2.20)	847.23 (2.93)	1737.80 (3.24)	2432.20 (3.39)	317.69 (2.50)	4731.51 (3.68)	6886.52 (3.84)	9840.11 (3.99)	122.46 (2.09)	1896.71 (3.28)	3715.35 (3.57)	5093.31 (3.71)
M1	283.14 (2.45)	517.61 (2.71)	657.66 (2.82)	810.96 (2.91)	57.41 (1.76)	794.33 (2.90)	1717.91 (3.24)	3311.31 (3.52)	26.85 (1.43)	843.33 (2.93)	1367.73 (3.14)	2466.04 (3.39)
M2	20.14 (1.30)	116.68 (2.07)	180.30 (2.26)	417.83 (2.62)	35.89 (1.56)	154.88 (2.19)	364.75 (2.56)	721.11 (2.86)	8.49 (0.93)	71.61 (1.86)	187.07 (2.27)	613.76 (2.79)
M3	3.00 (0.48)	9.59 (0.98)	97.49 (1.99)	184.50 (2.27)	1.88 (0.28)	30.55 (1.49)	128.23 (2.11)	243.78 (2.39)	1.5 (0.18)	2.16 (1.09)	50.23 (1.70)	97.95 (1.99)
CD (0.05)	0.328	0.380	0.309	0.201	0.457	0.427	0.334	0.214	0.287	0.421	0.286	0.224

DAI – Days after inoculation

*Figures in parenthesis are values after logarithmic transformation

infective juveniles (847.23 per larva) followed by M1 medium (517.61 per larva) and these two were statistically on par. M2 and M3 medium recorded 116.68 and 9.59 infective juveniles per larva respectively. (Table 5)

The mean number of infective juveniles of *S. glaseri* emerged from *G. mellonella* larva at 15 DAI in various media were showed statistically significant variation. The larva reared in standard medium showed maximum emergence of infective juveniles (1737.80 per larva). M1 medium recorded 657.66 infective juveniles. M2 and M3 recorded 180.30 and 97.49 infective juveniles respectively and these two were statistically on par (Table 5).

The effect of different media at 20 DAI also showed statistically significant variation. The larva from standard medium showed maximum emergence of infective juveniles (2432.20 per larva). M1, M2 and M3 showed 810.96, 417.83 and 184.50 infective juveniles respectively. (Table 5)

10 IJ per larva

In various media the mean number of infective juveniles emerged from *G. mellonella* larvae at 7 DAI varied significantly. The maximum emergence of infective juveniles was showed by standard medium (317.69 per larva). M1 and M2 recorded the emergence of 57.41 and 35.89 infective juveniles respectively and these two were statistically on par. The minimum emergence of infective juveniles was recorded by M3 medium (1.88 per larva) (Table 5).

The mean number of infective juveniles of *S. glaseri* emerged from *G. mellonella* larva reared in different media at 10 DAI showed statistically significant variation. The maximum emergence of infective juveniles was showed by standard medium (4731.51 per larva) and M1, M2 and M3 medium showed infective juvenile emergence 794.33, 154.88 and 30.55 respectively (Table 5).

The mean number of infective juveniles of *S. glaseri* emerged from *G. mellonella* larva reared in different media at 15 DAI showed statistically significant variation. The maximum emergence was showed by standard medium

(6886.52 per larva). M1, M2 and M3 media showed infective juvenile emergence as 1717.91, 364.75 and 128.23 respectively (Table 5).

The mean number of infective juveniles of *S. glaseri* emerged from *G. mellonella* larvae reared in different media at 20 DAI were showed statistically significant variation. The maximum emergence showed by standard medium (9840.11 per larva). M1, M2 and M3 recorded 3311.31, 721.11 and 243.78 infective juveniles respectively. (Table 5)

25 IJ per larva

The mean number of infective juveniles of *S. glaseri* emerged from *G. mellonella* larva reared in different artificial media at 7 DAI were showed statistically significant variation. Larva from standard medium showed maximum emergence of infective juveniles (122.46 per larva). The media M1, M2 and M3 recorded 26.85, 8.49 and 1.5 infective juveniles respectively. (Table 5)

The number of infective juveniles emerged from *G. mellonella* larva at 10 DAI in various media were showed statistically significant variation. The maximum emergence of infective juveniles recorded from *G. mellonella* larva reared in standard medium (1896.71 per larva) followed by M1medium (843.33 per larva) and these two were statistically on par. The mean values of emergence of infective juveniles from larvae reared in M2 and M3 media were 71.61 and 2.16 respectively. (Table 5)

Number of infective juveniles emerged from *G. mellonella* larvae at 15 DAI was statistically varied. The maximum emergence of infective juveniles was showed by standard medium (3715.35 per larva) followed by M1 (1367.73 per larva). The media M2 and M3 recorded 187.07 and 50.23 infective juveniles respectively. (Table 5)

The effect of different media at 20 DAI also showed statistically significant variation. Larva from standard medium showed maximum emergence of infective juveniles (5093.31 per larva). M1, M2 and M3 media showed the emergence of infective juveniles as 2466.04, 613.76 and 97.95 respectively. (Table 5)

Table 6. Effect on the multiplication of native isolate N1 in *G. mellonella* fed with different artificial media

Media	Number of native isolate N1 emerged at different intervals (days) on a single larva of *G. mellonella* inoculated with											
	5IJ				10IJ				25IJ			
	7DAI	10DAI	15DAI	20DAI	7DAI	10DAI	15DAI	20 DAI	7DAI	10DAI	15DAI	20 DAI
Standard	31.99 *(1.51)	297.85 (2.47)	816.58 (2.91)	937.56 (2.97)	159.96 (2.20)	1300.17 (3.11)	2404.36 (3.38)	5152.29 (3.71)	606.74 (2.78)	1324.34 (3.12)	1625.55 (3.21)	3793.15 (3.58)
M1	170.22 (2.23)	1174.90 (3.07)	2228.44 (3.35)	2454.71 (3.39)	71.78 (1.86)	327.34 (2.52)	1039.92 (3.02)	2018.37 (3.31)	71.29 (1.85)	300.61 (2.48)	1081.43 (3.03)	1892.34 (3.28)
M2	3.13 (0.50)	26.42 (1.42)	115.88 (2.06)	310.46 (2.49)	10.23 (1.01)	45.29 (1.66)	271.02 (2.43)	729.46 (2.86)	20.14 (1.30)	44.98 (1.65)	264.24 (2.42)	721.11 (2.86)
M3	2.35 (0.37)	6.59 (0.82)	103.51 (2.02)	142.56 (2.15)	1.25 (0.10)	6.59 (0.82)	107.15 (2.03)	263.63 (2.42)	1.64 (0.22)	10.79 (1.03)	96.38 (1.98)	147.91 (2.17)
CD (0.05)	0.438	0.299	0.148	0.161	0.453	0.381	0.183	0.106	0.278	0.241	0.161	0.170

DAI – Days after inoculation
*Figures in parenthesis are values after logarithmic transformation

4.4.3 Native isolate, N1

5 IJ per larva

The mean number of infective juveniles emerged from *G. mellonella* larva at 7 DAI in various media showed statistically significant variation and the results were given in Table 6. Larvae from M1medium showed maximum emergence (170.22 per larva). Standard medium recorded only 31.99 infective juvenile emergences. M2 and M3 recorded 3.13 and 2.35 infective juveniles respectively and these two were statistically on par.

The effect of different media at 10 DAI also showed statistically significant variation. Larvae from M1 medium showed maximum emergence of infective juveniles (1174.90 per larva).Standard medium, M2 and M3 recorded only 297.85 26.42 and 6.59 infective juveniles per larva respectively. (Table 6)

The mean numbers of infective juveniles emerged from *G. mellonella* larva reared in different media at 15 DAI showed statistically significant variation. The maximum emergence of infective juveniles was showed by M1 medium (2228.44 per larva).Standard medium recorded only 816.58 infective juveniles per larva. M2 and M3 recorded 115.88 and 103.51 infective juveniles per larva respectively and these two were statistically on par. (Table 6)

The mean number of infective juveniles emerged from *G. mellonella* larva reared in different artificial media at 20 DAI showed statistically significant variation. The maximum emergence of infective juveniles was showed by M1 medium (2454.71 per larva). Standard medium, M2 and M3 recorded 937.56, 310.46 and 142.56 infective juveniles respectively. (Table 6)

10 IJ per larva

The mean number of infective juveniles emerged from *G. mellonella* larva reared in different artificial media at 7DAI showed statistically significant variation. Larvae reared in standard medium showed maximum emergence of infective juveniles (159.96 per larva) followed by M1 medium (71.78) and these

two were statistically on par. M2 and M3 medium recorded 10.23 and 1.25 infective juveniles per larva respectively. (Table 6)

The effect of different media at 10 DAI also showed statistically significant variation. The maximum emergence of infective juveniles was recorded in standard medium (1300.17 per larva). M1, M2 and M3 medium recorded 327.34, 45.29 and 6.59 infective juveniles respectively. (Table 6)

Mean number of infective juveniles emerged from *G. mellonella* larva in different media at 15 DAI showed statistically significant variation. The maximum number of infective juveniles emerged were recorded in standard medium (2404.36 per larva). M1, M2 and M3 recorded 1039.92, 271.02 and 107.15 infective juveniles respectively. (Table 6)

The results presented in Table 6 revealed that the mean numbers of infective juveniles of N1 emerged at 20 DAI in various media varied significantly. Maximum emergence of infective juveniles was recorded in standard medium (5152.29 per larva).M1, M2 and M3 medium recorded 2018.37, 729.46 and 263.63 infective juveniles per larva respectively.

25 IJ per larva

The numbers of infective juveniles emerged the *G. mellonella* larva in various media at 7 DAI, showed statistically significant variation. Larva from the standard medium showed maximum emergence of infective juveniles (606.74 per larva).M1, M2 and M3 recorded 71.29, 20.14 and 1.64 infective juveniles per larva respectively. (Table 6)

The mean numbers of infective juveniles emerged from *G. mellonella* larva reared in different artificial media at 10 DAI showed statistically significant variation. The maximum emergence was showed by standard medium (1324.34 per larva). M1, M2 and M3 recorded 300.61, 44.98 and 10.79 infective juveniles per larva respectively. (Table 6)

The effect of different media at 15 DAI also showed statistically significant variation. The maximum emergence was showed by standard medium (1625.55 per

larva). M1, M2 and M3 recorded 1081.43, 264.24 and 96.38 infective juveniles per larva respectively. (Table 6)

The mean numbers of infective juveniles emerged from *G. mellonella* larva reared in different artificial media at 20 DAI showed statistically significant variation. The maximum emergence of infective juveniles recorded in standard medium (3793.15 per larva). M1, M2 and M3 recorded 1892.34, 721.11 and 147.91 infective juveniles per larva respectively. (Table 6)

4.4.4 Native isolate, N2

5 IJ per larva

The result presented in Table 7 revealed that the mean number of infective juveniles emerged from *G. mellonella* larva reared in different artificial media at 7 DAI showed statistically significant variation. Larva from standard medium showed maximum emergence of infective juveniles (155.59 per larva). M1 medium recorded 32.96 infective juveniles. M2 and M3 medium recorded 3.95 and 2.05 infective juveniles and these two were statistically on par.

The effect of different media at 10 DAI also showed statistically significant variation. The maximum emergence of infective juveniles was showed by standard medium (1076.47 per larva). M1, M2 and M3 recorded 283.14, 14.42 and 9.53 infective juveniles per larva respectively. (Table 7)

The mean numbers of infective juveniles emerged from *G. mellonella* larvae reared in different artificial media at 15 DAI showed statistically significant variation. The maximum emergence showed by standard medium (1905.46 per larva). M1 medium recorded 801.68 infective juveniles. M2 and M3 recorded 116.41 and 100.69 infective juveniles respectively and these two were statistically on par. (Table 7)

The effect of different media at 20 DAI also showed statistically significant variation. The maximum emergence of infective juveniles was showed by standard medium (2735.27 per larva). M1, M2 and M3 recorded 1183.04, 278.61 and 159.22 infective juveniles respectively. (Table 7)

Table7. Effect on the multiplication of native isolate N2 in *G. mellonella* fed with different artificial media

Media	Number of native isolate N2 emerged at different intervals (days) on a single larva of *G. mellonella* inoculated with											
	5IJ				10IJ				25IJ			
	7DAI	10DAI	15DAI	20DAI	7DAI	10DAI	15DAI	20 DAI	7DAI	10DAI	15DAI	20 DAI
Standard	155.59 (2.19)	1076.47 (3.03)	1905.46 (3.28)	2735.27 (3.44)	148.25 (2.17)	966.05 (2.99)	2213.09 (3.35)	3548.13 (3.56)	753.36 (2.88)	1213.39 (3.08)	1963.36 (3.29)	3999.45 (3.60)
M1	32.96 (1.52)	283.14 (2.45)	801.68 (2.90)	1183.04 (3.07)	80.54 (1.91)	325.84 (2.51)	1032.76 (3.01)	1774.19 (3.25)	76.38 (1.88)	276.69 (2.44)	1054.39 (3.02)	1879.32 (3.27)
M2	3.95 (0.60)	14.42 (1.52)	116.41 (2.07)	278.61 (2.45)	18.24 (1.26)	44.36 (1.65)	257.63 (2.41)	644.17 (2.81)	22.13 (1.35)	52.36 (1.72)	252.93 (2.40)	746.45 (2.87)
M3	2.05 (0.31)	9.53 (0.98)	100.69 (2.00)	159.22 (2.20)	3.06 (0.49)	12.33 (1.09)	101.86 (2.01)	195.88 (2.29)	2.77 (0.44)	7.62 (088)	93.76 (1.97)	171.39 (2.23)
CD (0.05)	0.362	0.195	0.123	0.149	0.247	0.197	0.149	0.188	0.319	0.186	0.106	0.173

DAI – Days after inoculation
Figures in parenthesis are values after logarithmic transformation

10 IJ per larva

The mean numbers of infective juveniles emerged from *G. mellonella* larva reared in different artificial media at 7 DAI showed statistically significant variation. Larva reared in standard medium showed maximum emergence of infective juveniles (148.25 per larva).M1, M2 and M3 medium recorded 80.54, 18.24 and 3.06 infective juveniles respectively. (Table 7)

The mean number of infective juveniles emerged from *G. mellonella* larva reared in different artificial media at 10 DAI showed statistically significant variation. The larvae from standard medium showed maximum emergence (966.05 per larva). M1, M2 and M3 medium showed 325.84, 44.36 and 12.33 IJ per larva respectively. (Table 7)

The effect of different media at 15 DAI also showed statistically significant variation. The larvae reared in standard medium showed maximum emergence of infective juveniles (2213.09 per larva). M1, M2 and M3 medium recorded 1032.76, 257.63 and 101.86 infective juveniles respectively. (Table 7)

The mean numbers of infective juveniles emerged from *G. mellonella* larva at 20 DAI in different media showed statistically significant variation. The maximum emergence was recorded in standard medium (3548.13) and the minimum emergence in M3 medium (195.88). (Table 7)

25 IJ per larva

The mean numbers of infective juveniles emerged from *G. mellonella* larva at 7 DAI in various media showed statistically significant variation. Larvae reared in standard medium showed the maximum emergence of infective juveniles (753.36 per larva).M1, M2 and M3 medium recorded 76.38, 22.13 and 2.77 infective juveniles respectively. (Table 7)

The effect of different media at 10 DAI also showed statistically significant variation. The mean number of infective juveniles emerged from the larva in various media are given in Table 7. The maximum emergence of infective

juveniles showed by standard medium (1213.39 per larva).M1 M2 and M3 medium recorded 276.69, 52.36 and 7.62 infective juveniles respectively.

At 15 DAI also the effect of different media showed statistically significant variation. Larvae from standard medium showed maximum emergence of infective juveniles (1963.36 per larva) M1, M2 and M3 medium recorded 1054.39, 252.93 and 93.76 infective juveniles respectively. (Table 7)

Number of infective juveniles emerged at 20 DAI in various media are given in Table 7. The effect of these media showed statistically significant variation. Larvae from standard medium showed maximum emergence of infective juveniles (3999.45 per larva). 1879.32, 746.45 and 171.39 infective juveniles were emerged from M1, M2 and M3 medium respectively.

4.4.5 Interaction of rearing media of *G. mellonella*, and multiplication of EPN

The results of the interaction of EPN with rearing media of *G. mellonella*, the period of exposure and initial inoculum level of EPN are presented in Table 8..

4.4.5.1 Interaction of various EPN and rearing media of G. mellonella

The result showed that there was statistically significant variation in the interaction of EPN and media (standard medium, M1, M2 and M3) for the number of emergence of infective juveniles of EPN.

Standard medium

Maximum multiplication of infective juvenile was recorded in *H. indica* (2139.58) and it was statistically superior to all other isolate. *S. glaseri*, N1 and N2 recorded 1586.16, 820.36 and 1176.61 infective juvenile emergence respectively. (Table8)

M1 medium

In this medium the highest emergence of infective juveniles was recorded in *H. indica* (1141.13 per larva). N1 recorded 604.86 infective juveniles per larva followed by *S. glaseri* (583.54 per larva) and these two were statistically on par. Minimum emergence of infective juveniles was in N2 (401.95 per larva). (Table8)

Table 8. Interaction of rearing media of *G. mellonella* on the multiplication of IJ of different isolates of EPN at varying inoculum levels and periods of harvest

EPN	Media				Period (Days)				Inoculum levels		
	Standard	M1	M2	M3	7	10	15	20	5IJ	10IJ	25IJ
Native isolateN1	820.36 (2.91)	604.86 (2.78)	77.51 (1.89)	22.08 (1.34)	21.25 (1.32)	103.43 (2.01)	451.22 (2.65)	856.39 (2.93)	128.35 (2.11)	189.19 (2.28)	204.88 (2.31)
Native isolateN2	1176.61 (3.07)	401.95 (2.60)	84.04 (1.92)	25.62 (1.41)	26.05 (1.41)	106.84 (2.03)	439.98 (2.64)	831.48 (2.92)	133.53 (2.13)	197.76 (2.29)	215.84 (2.33)
H. indica	2139.58 (3.33)	1141.13 (3.06)	138.67 (2.14)	18.20 (1.26)	33.19 (1.52)	185.90 (2.27)	765.08 (2.88)	1305.08 (3.12)	138.80 (2.14)	446.89 (2.65)	354.52 (2.54)
S. glaseri	1586.16 (3.20)	583.54 (2.77)	127.28 (2.10)	22.69 (1.36)	23.68 (1.37)	219.04 (2.34)	533.53 (2.73)	965.92 (2.98)	181.21 (2.26)	357.07 (2.55)	181.67 (2.26)
CD(0.05)	0.113				0.097				0.079		

M2 medium

In M2 medium *H. indica* recorded maximum number of infective juvenile emergence (138.67 per larva) followed by *S. glaseri* (127.28 per larva) and these two were statistically on par. Mean emergence of infective juveniles of N2 and N1 were 84.04 and 77.51 respectively and these two were also statistically on par. (Table8)

M3 medium

In M3 medium maximum emergence was recorded in N2 (25.63 IJ per larva) followed by *S. glaseri* (22.69 IJ per larva) and N1 (22.08). These three were statistically on par. Minimum emergence was recorded in *H. indica* (18.20 IJ per larva) and this was statistically on par with N1 and *S. glaseri*. (Table8)

4.4.5.2 Interaction of various EPN and period of exposure

The result presented in Table 8 showed that there was statistically significant variation in the emergence of infective juveniles in different exposure period.

7DAI

The emergence of infective juveniles at seven days after inoculation was highest in *H. indica* (33.19 per larva) and it was statistically superior to others. The infective juveniles emerged in N2, *S. glaseri* and N1 were 26.05, 23.68 and 21.25 per larva respectively and these were statistically on par. (Table8)

10 DAI

During this period *S. glaseri* showed maximum emergence (2119.04 per larva) followed by *H. indica* (185.90 per larva) and these two were statistically on par. The infective juveniles emerged from *G. mellonella* larva were 106.84 and 103.43 respectively in N2 and N1 and these two were statistically on par. (Table8)

15 DAI

Maximum emergence of infective juveniles of EPN at 15 days after inoculation was recorded in *H. indica* (765.08 per larva) and it was significantly

superior to all other isolates. The number of juveniles emerged was 533.53 per larva in *S. glaseri* and it was only 451.22 and 439.98 per larva in N1 and N2 respectively. These three were statistically on par. (Table8)

20 DAI

During this period *H. indica* was recorded 1305.08 and it was significantly superior to other EPN. The number of larval emergence of *S. glaseri* was 965.92 IJ per larva but in N1 and N2 it was only 856.39 and 831.48 respectively and these three were statistically on par. (Table8)

4.4.5.3 Interaction of various EPN and initial inoculum levels

The result presented in Table 8 showed that there was statistically significant variation in the emergence of infective juveniles of four EPN and different initial inoculum levels (5, 10 and 25 IJ per larva).

5 IJ per larva

S. glaseri showed maximum emergence of infective juveniles (181.21) from one *G. mellonella* larva initially inoculated with 5 IJ while *H. indica*, N2 and N1 recorded 138.8, 133.53 and 128.35 infective juveniles respectively and this three were statistically on par. (Table8)

10 IJ per larva

The maximum emergence of infective juveniles per *G. mellonella* larva recorded in *H. indica* initially inoculated with 10 IJ per larva (446.89). This was followed by *S. glaseri* (357.07 per larva). The minimum emergence was observed in N1 (189.19 per larva) and it was statistically on par with N2 (197.76 per larva). (Table8)

25 IJ per larva

With an initial inoculum level of 25 IJ per larva maximum emergence was observed in *H. indica* (354.52 per larva) while N2, N1 and *S. glaseri* recorded 215.84, 204.88 and 181.67 infective juveniles respectively and this three were statistically on par. (Table8)

4.4.6 Selection of cheap media for the rearing of *G. mellonella*, the trap insect for EPN

The cost of one kg of different media were computed and presented in Table 9.

Among the four media studied cost of ingredients was highest in standard medium (Rs.155 per kg). In standard medium corn flour and glycerol were the costlier ingredients compared to others. In order to reduce the cost of ingredients in M1 medium the quantity of corn flour was reduced from 200 to 100g and glycerol from 150 ml to50 ml. The cheap ingredients atta and honey in the standard medium was increased from 200 to 300g and the honey from 100 to 200ml. Thus the cost of M1 medium was reduced to Rs. 122 per kg. The percentage reduction of cost was 21.29. (Table 9)

In M2 medium also the quantity of corn flour was reduced from 200 to 100g and glycerol from 150 to50ml. The quantity of atta increased from 200 to 300g.In order to reduce the cost of M2 medium sugar solution was added as an additional ingredient. Here the quantity of honey was reduced from 200 to 100ml and this quantity was substituted by 100ml of sugar solution. The cost of the M2 medium was thus reduced to Rs.100.95 and the percentage reduction of cost was 34.87 over standard medium. (Table 9)

In M3 medium the total cost of ingredients was 121.40 per kg. Rice bran was an additional ingredient to increase the volume of the medium. In order to reduce the cost of ingredients in M3 medium the quantity of corn flour was reduced from 200 to 50g, glycerol from 150 to 50 ml and atta 200 to 100 g and these quantities were substituted by 400g of rice bran. The quantity of honey was increased from 100 to 200 ml. The percentage reduction of cost was 21.67 over standard medium (Table 9).

Table 9 Cost of different artificial media used for the rearing of *G. mellonella*

Media	Quantity and cost of ingredients in 1 kg of media									Total	Per cent reduction in cost of production
	Quantity (g)	Corn flour	Milk powder	Atta	Yeast	Honey	Glycerol	Sugar solution	Rice bran		
Standard		200	100	200	100	100	150	-	-	155/-	-
	Cost (Rs.)	16.4	15.6	3.6	26	21.40	72	-	-		
M1	Quantity (g)	100	100	300	100	200	50	-	-	122/-	21.29
	Cost (Rs.)	8.2	15.6	5.4	26	42.80	24	-	-		
M2	Quantity(g)	100	100	300	100	100	50	100	-	100.95/-	34.87
	Cost (Rs.)	8.2	15.6	5.4	26	21.40	24	0.35	-		
M3	Quantity (g)	50	100	100	100	200	50	-	400	121.40/-	21.67
	Cost (Rs.)	8.2	15.6	3.6	26	42.80	24	-	1.2		

4.5 COMPARITIVE EFFICACY OF ISOLATES FOR THE MANAGEMENT OF WEEVIL PESTS OF BANANA

4.5.1 Effect of EPN on the Mortality of Grubs and Adults of Banana Weevils

4.5.1.1 Pseudostem weevil, *Odoiporus longicollis* Oliv.

Grubs

10 IJ per grub

The result presented in Table 10 showed that the mean corrected mortality of grubs of *O. longicollis* inoculated at 10 IJ per grub at 24 hours after treatment did not vary significantly. However the native isolate N1 and standard, *H.indica* showed maximum mortality (7.94 per cent).

The mortality of grubs recorded at 48 hrs after treatment, revealed that N1 recorded highest mean corrected mortality of 21.44 per cent and this was higher than the corrected mortality observed in two standards *S. glaseri* and *H. indica* with 19.57 and 19.16 per cent respectively. However the mortality at 48 hours after treatment was not enough to get statistical significance. (Table 10)

The mortality of grubs at 72 hours after treatment showed statistically significant variation. The mean corrected mortality percentage was maximum in native isolate N_1 (64.04 per cent) and minimum in native isolate N_2 (43.95 per cent). The effect of N_1 isolate was on par with that of the standard EPN *H. indica* (62.09 per cent) and the mortality of N_2 was on par with standard EPN *S. glaseri* (49.97 per cent). (Table 10)

50 IJ per grub

The result presented in Table 10 showed that the mean corrected mortality of grubs of *O. longicollis* inoculated at 50 IJ/L at 24 hours after treatment did not vary significantly. However the maximum mortality was showed in *H. indica*.

The mortality of grubs observed at 48 hours after treatment showed significant variation. Maximum mortality per cent was recorded in *H. indica* (64.04) and minimum mortality by *S. glaseri* (45.86). The effect of standard EPN

Table 10. Effect of different levels of EPN on the mortality of grubs of *O. longicollis* under laboratory condition

Treatments	Percentage mortality of grubs at varying inoculum levels and periods (hrs)											
	10IJ			50IJ			100IJ			200IJ		
	24	48	72	24	48	72	24	48	72	24	48	72
N1	7.94 *(16.36)	21.44 (27.58)	64.04 (53.15)	11.27 (19.61)	62.09 (52.00)	78.08 (62.08)	29.78 (33.07)	74.12 (59.42)	88.69 (70.35)	33.89 (35.60)	76.13 (60.75)	84.30 (66.66)
N2	3.68 (11.05)	13.64 (21.67)	43.95 (41.52)	13.64 (21.67)	47.96 (43.85)	47.96 (43.82)	23.82 (29.21)	60.05 (50.79)	64.04 (53.15)	27.68 (31.74)	55.99 (48.44)	60.14 (50.84)
H. indica	7.94 (16.36)	19.16 (25.96)	62.09 (52.00)	15.65 (23.30)	64.04 (53.15)	68.22 (55.68)	33.89 (35.60)	74.12 (59.42)	84.30 (66.66)	33.89 (35.60)	76.13 (60.75)	80.38 (63.70)
S. glaseri	6.48 (14.74)	19.57 (26.25)	49.97 (44.98)	7.94 (16.36)	45.86 (42.62)	49.97 (44.98)	23.82 (29.21)	58.16 (49.69)	58.07 (49.64)	29.78 (33.07)	57.99 (49.59)	64.04 (53.15)
CD(0.05)	NS	NS	5.94	NS	6.803	5.860	5.080	6.775	9.188	NS	4.401	6.459

*Figures in parenthesis are values after angular transformation
NS - statistically not significant

H. indica with native isolate N1 (62.09 per cent) was on par and the effect of standard isolate *S. glaseri* was on par with native isolate N2 (47.96 per cent). (Table 10)

The mortality of grubs observed at 72 hours after treatment showed statistically significant variation. Maximum mean corrected mortality was showed by N1 isolate (78.08 per cent) and minimum mortality showed by N2 isolate (47.96 per cent). The mortality showed by N2 was on par with that of *S. glaseri* (45.86 per cent). (Table 10)

100 IJ per grub

The mortality of grubs of *O. longicollis* inoculated with 100 IJ per grub observed at 24 hours after treatment vary significantly. Maximum mean corrected mortality showed by *H. indica* (33.89 per cent) and minimum mortality was showed by *S. glaseri* and N2 (23.82 per cent). The mortality of native isolate N1 (29.78 per cent) was on par with standard isolate *H. indica*. (Table 10)

The mortality of grubs at 48 hrs after treatment varies significantly. Maximum corrected mortality was recorded in N1 and *H. indica* (74.12 per cent). Minimum mortality was showed by *S. glaseri* (58.16 per cent). The effect of native isolate N1 was on par with standard isolate *H. indica* and the effect of N2 isolate (60.05 per cent) was on par with standard culture *S. glaseri*. (Table 10)

The mortality of grubs observed at 72 hrs after treatment showed significant variation. The maximum mean corrected mortality showed by native isolate N1 inoculate (88.69 per cent) and minimum mortality was showed by *S. glaseri* (58.07 per cent). The effect of native isolate N1was on par with *H. indica* (84.30 per cent) and the effect of native isolate N2 (64.04 per cent) was on par with standard isolate *S. glaseri*. (Table 10)

200 IJ per grub

The mean corrected morality of grubs of *O. longicollis* isolated at 200 IJ per grub observed at 24 hours after treatment did not vary significantly. However

the native isolate N_1 showed same mortality as that of *H. indica* (33.89 per cent). (Table 10)

The mean corrected mortality of grubs of *O. longicollis* inoculated at 200 IJ per grub observed at 48 hours after treatment showed statistically significant variation. The maximum mortality was showed by N_1 isolate and *H. indica* (76.13 per cent) and minimum mortality by N_2 isolate (55.99 per cent). The effect of native isolate N1 was on par with *H. indica* and the effect of native isolate N_2 was on par with standard isolate *S. glaseri* (57.99 per cent). (Table 10)

The mean corrected mortality of grubs observed at 72 hours after treatment showed statistically significant variation. The maximum mortality showed by N1 isolate (84.30 per cent) and minimum mortality showed by N_2 isolate (60.14 per cent). The effect of native isolate $N_1$was on par with *H.indica* (80.38 per cent) and the effect of native isolate N2 was on par with standard isolate *S. glaseri* (64.04 per cent). (Table 10)

Adults

10 IJ per adult

When the adults of *O. longicollis* were inoculated at 10 IJ per adult at 24 hrs and 48 hrs after treatment no mortality was observed (Table 11).

The mortality of adults observed at 72 hours after treatment did not vary significantly. The maximum mean corrected mortality of weevil was showed by native isolate N1 (12.29 per cent). (Table 11)

50 IJ per adult

At 24 hrs after treatment no mortality was observed. (Table 11)

The mean corrected mortality of adults observed at 48 hours after treatment did not vary significantly. However the maximum mortality was showed by standard isolate *H. indica* (15.65 per cent) (Table 11).

The mean corrected mortality of adults observed at 72 hours after treatment showed statistically significant variation. The maximum mortality was showed by

Table 11. Effect of different levels of EPN on the mortality of the adults of *O. longicollis* under laboratory condition

Treatments	Percentage mortality of adults at varying inoculum levels and periods (hrs)											
	10IJ			50IJ			100IJ			200IJ		
	24	48	72	24	48	72	24	48	72	24	48	72
N1	0.00	0.00	12.29 *(20.53)	0.00	13.64 (21.68)	17.37 (24.63)	0.41 (3.69)	35.81 (36.76)	66.33 (54.53)	4.82 (12.68)	37.84 (37.96)	66.25 (54.48)
N2	0.00	0.00	6.48 (14.74)	0.00	7.94 (16.37)	13.64 (21.68)	0.00	19.57 (26.26)	49.97 (44.98)	3.68 (11.05)	27.68 (31.74)	58.07 (49.65)
H. indica	0.00	0.00	6.11 (14.31)	0.00	15.65 (23.30)	23.82 (29.21)	2.45 (9.0)	41.78 (40.27)	68.22 (55.69)	9.54 (17.99)	39.89 (39.17)	70.36 (57.02)
S. glaseri	0.00	0.00	1.65 (7.37)	0.00	3.68 (11.06)	11.76 (20.05)	0.00	17.37 (24.63)	43.86 (41.47)	3.68 (11.06)	23.19 (28.79)	60.14 (50.85)
CD(0.05)	–	–	NS	–	NS	6.206	NS	8.295	8.313	NS	7.693	NS

* Figures in parenthesis are values after angular transformation
NS - statistically not significant

H. indica (23.82 per cent) and *S. glaseri* showed the minimum mortality (11.76 per cent). The effect of native isolates N_1 (17.37 per cent) and N_2 (13.64 per cent) were statistically on par with standard isolate *S. glaseri* and the effect of native isolate N1 was on par with standard isolate *H. indica* (Table 11).

100 IJ per adult

The mean corrected mortality of adults of *O. longicollis* inoculated at 100 IJ per adult observed at 24 hours after treatment did not vary significantly. However the maximum mortality was showed by *H. indica* (2.45 per cent) (Table 11).

The mean corrected mortality observed at 48 hours after treatment showed statistically significant variation. The maximum mortality was showed by *H. indica* (41.78 per cent) and minimum mortality by *S. glaseri* (17.37 per cent). The effect of standard EPN *H. indica* and native isolate N1 (35.81 per cent) was on par and the effect of standard EPN *S. glaseri* and native isolate N2 (19.57 per cent) was on par. (Table 11)

The mean corrected mortality of observed at 72 hours after treatment showed statistically significant variation. The maximum mortality showed by *H. indica* (68.22 percent) and minimum mortality was by *S. glaseri* (43.86 per cent). The effect of standard EPN *H. indica* and native isolate N1 (66.33 per cent) was on par and the effect of standard EPN *S. glaseri* and native isolate N2 (49.97 per cent) was on par (Table 11).

200 IJ per adult

The mean corrected mortality of adults of *O. longicollis* inoculated at 200 IJ per adult observed at 24 hrs after treatment did not vary significantly. However the maximum mortality percentage showed by *H. India* (9.54) (Table 11).

The mean corrected mortality of adults observed at 48 hrs after treatment showed statistically significant variation. The maximum mortality was showed by *H. indica* (39.89 per cent). The effect of standard EPN *H. indica* was on par with native isolate N1 (37.84 per cent) and the effect of standard EPN *S. glaseri* (23.19 per cent) was on par with native isolate N2 (27.68 per cent). (Table 11)

The mean corrected mortality at 72 hrs after treatment did not vary significantly. However the maximum mortality showed by *H. indica* (70.36 per cent). (Table 11)

4.5.1.2 Rhizome weevil Cosmopolites sordidus Ger.

Grubs

10 IJ per grub

The result presented in Table 12 showed that the corrected mortality of grubs of *C. sordidus* inoculated at 10 IJ per grub observed at 24 hrs after treatment did not vary significantly. However the maximum mortality was showed by the standard *H. indica* (11.76 per cent).

The mean corrected mortality of grubs at 48 hrs after treatment did not vary significantly. However the maximum mortality was showed by the standard isolate *H. indica* (17.37 per cent). (Table 12)

The mean corrected mortality of grubs at 72 hrs after treatment showed statistically significant variation. The maximum mortality was showed by *H. indica* (41.87 per cent) and minimum mortality by both N2 and *S. glaseri* (23.82 per cent). The effect of the standard EPN *H. indica* was on par with native isolate N1 (41.56 per cent). (Table 12)

50 IJ per grub

The mean corrected mortality of grubs of *C. sordidus* inoculated at 50 IJ per grub observed at 24 hrs after treatment did not vary significantly. However the maximum mortality was showed by *H. indica* (15.65 per cent). (Table 12)

The mean corrected mortality of grubs of *C. sordidus* observed at 48 hrs after treatment did not vary significantly. However the maximum mortality was showed by the standard isolate *H. indica* (25.63 per cent). (Table 12)

The mean corrected mortality observed at 72 hrs after treatment did not vary significantly. However the maximum mortality was showed by *H. indica* (64.34 per cent). (Table 12)

able 12. Effect of different levels of EPN on the mortality of grubs of *C. sordidus* under laboratory condition

Treatments	Percentage mortality of grubs at varying inoculum levels and periods (hrs)											
	10IJ			50IJ			100IJ			200IJ		
	24	48	72	24	48	72	24	48	72	24	48	72
N1	7.94 *(16.37)	11.27 (19.62)	41.56 (40.14)	13.64 (21.68)	19.57 (26.26)	60.05 (50.80)	23.82 (29.21)	29.78 (33.07)	78.50 (62.38)	27.68 (31.74)	37.84 (37.96)	80.38 (63.71)
N2	6.48 (14.74)	13.64 (21.68)	23.82 (29.21)	3.68 (11.057)	13.64 (21.68)	47.96 (43.83)	19.57 (26.26)	23.82 (29.21)	60.05 (50.80)	23.82 (29.21)	33.89 (35.61)	68.51 (55.86)
H. indica	11.76 (20.05)	17.37 (24.63)	41.87 (40.32)	15.65 (23.30)	25.63 (30.42)	64.34 (53.33)	27.68 (31.74)	31.64 (34.23)	78.50 (62.38)	29.78 (33.07)	49.97 (44.98)	82.58 (65.33)
S. glaseri	3.68 (11.06)	7.94 (16.37)	23.82 (29.21)	6.48 (14.74)	17.37 (24.63)	52.07 (46.19)	17.37 (24.63)	21.87 (27.88)	60.05 (50.80)	19.57 (26.26)	25.82 (30.54)	62.39 (52.18)
CD(0.05)	NS	NS	7.679	NS	NS	NS	NS	NS	7.006	NS	6.064	9.750

Figures in parenthesis are values after angular transformation
S - statistically not significant

100 IJ per grub

The mean corrected mortality of grubs of *C. sordidus* inoculated at 100 IJ per grub observed at 24 hrs after treatment did not vary significantly. However the maximum mortality was showed by *H. indica* (27.68 per cent). (Table 12)

The mean corrected mortality observed at 48 hrs after treatment did not vary significantly. However the maximum mortality was showed by *H. indica* (31.64 per cent). (Table 12)

The mean corrected morality of grubs at 72 hrs after treatment showed statistically significant variation. The maximum mortality was showed by both N1 and *H. indica* (78.50 per cent) and the minimum mortality showed by both *S. glaseri* and N2 (60.05 per cent). (Table 12)

200 IJ per grub

The mean corrected mortality of grubs of *C. sordidus* inoculated at 200 IJ per grub observed at 24 hrs after treatment did not vary significantly. However the *H. indica* showed the maximum mortality (29.78 per cent). (Table 12)

The mean corrected mortality of grubs at 48 hrs after treatment showed statistically significant variation. The maximum mortality was showed by *H. indica* (49.97 per cent) and minimum mortality was showed by *S. glaseri* (25.82 per cent). The effect of native isolate N2 (33.89 per cent) was on par with standard isolate *S. glaseri* and native isolate N1 (37.84 per cent). (Table 12)

The mean corrected mortality of grubs of 72 hrs after treatment showed statistically significant variation. The maximum mortality showed by *H. indica* (82.58 percent) and minimum mortality showed by *S. glaseri* (62.39 percent). The effect of native isolate N1 (80.38 per cent) was on par with standard isolate *H. indica* and the effect of native isolate N2 (68.51 per cent) was on par with standard isolate *S. glaseri.* (Table 12)

Table 13. Effect of different levels of EPN on the mortality of adults of *C. sordidus* under laboratory condition

Treatments	Percentage mortality of adults at varying inoculum levels and periods (hrs)											
	10IJ			50IJ			100IJ			200IJ		
	24	48	72	24	48	72	24	48	72	24	48	72
N1	0.00	0.00	11.76 (20.05)	0.00	15.28 (23.01)	19.16 (25.96)	1.65 (7.38)	27.68 (31.74)	64.63 (53.51)	9.54 (17.99)	31.72 (34.28)	76.75 (61.17)
N2	0.00	0.00	3.68 (11.06)	0.00	11.76 (20.05)	15.28 (23.01)	0.00	19.57 (26.26)	58.07 (49.65)	13.64 (21.68)	19.57 (26.26)	58.07 (49.65)
H. indica	0.00	0.00	7.94 (16.38)	0.00	19.57 (26.26)	23.82 (29.21)	2.45 (9.0)	25.17 (30.12)	68.22 (55.69)	17.37 (24.63)	31.43 (34.10)	74.77 (59.85)
S. glaseri	0.00	0.00	3.68 (11.06)	0.00	7.94 (16.37)	17.77 (24.93)	0.41 (3.69)	15.65 (23.30)	52.07 (46.19)	11.76 (20.05)	19.57 (26.26)	58.16 (49.70)
CD (0.05)	_	_	NS	_	NS	NS	NS	NS	NS	NS	NS	9.636

Figures in parenthesis are values after angular transformation
NS - statistically not significant

At the highest inoculum level of 200 IJ per grub isolate N1 and *H. indica* gave a corrected mortality of more than 80 percent.

Adults

10 IJ per adult

No mortality was observed at 24 hours and 48 hours after treatment. (Table 12)

The mean corrected mortality of adults of *C. sordidus* inoculated at 10 IJ per adult observed at 72 hrs after treatment was not enough to get statistical significance. The native isolate N1 showed a highest mean mortality (11.76 per cent) and this was higher than the corrected mortality showed by standard isolates *H. indica* and *S. glaseri* with 7.94 and 3.68 per cent respectively. (Table 13)

50 IJ per adult

The mean corrected mortality of adults of *C. sordidus* inoculated at 50 IJ per adult observed at 24 hours after treatment showed zero mortality (Table 13).

The mean corrected mortality observed at 48 hours after treatment did not vary significantly. However the maximum mortality was showed by *H. indica* (19.57 per cent). (Table 13)

The mean corrected mortality observed at 72 hrs after treatment did not vary significantly. However the maximum mortality was showed by *H. indica* (23.82 per cent) (Table 13)

100 IJ per adult

The mean corrected mortality of adults of *C. sordidus* inoculated at 100 IJ per adult observed at 24 hrs after treatment did not vary significantly. However the maximum mortality was showed by *H. indica* (2.45 per cent). (Table 13)

The mean corrected mortality of adults of *C. sordidus* observed at 48 hrs after treatment did not vary significantly. The native isolate N1 showed a highest mean mortality (27.68 per cent) and this was higher than the corrected mortality

showed by standard isolates *H. indica* and *S. glaseri* with 25.17 and 15.65per cent respectively. (Table 13)

The mortality of adults of *C. sordidus* observed at 72 hrs after treatment did not vary significantly. However the maximum mortality showed by standard EPN *H. indica* (68.22 per cent). (Table 13)

200 IJ per adult

The mean corrected mortality of adults of *C. sordidus* inoculated at 200 IJ per adult observed at 24 hrs after treatment did not vary significantly. However the maximum mortality was showed by the standard isolate, *H. indica* (17.37 per cent). (Table 13)

The mean corrected mortality of adults of *C. sordidus* observed at 48 hrs after treatment did not vary significantly. The native isolate N1 showed a highest mean mortality (31.72 per cent) and this was higher than the corrected mortality showed by standard isolates *H. indica* and *S. glaseri* with 31.43 and 19.57 per cent respectively. (Table 13)

The mortality adults of observed at 72 hrs after treatment showed statistically significant variation. The maximum mortality was showed by N1 isolate (76.75 per cent) and minimum mortality seen in N2 isolate (58.07 per cent). The effect of native isolate N1 was on par with standard isolate *H. indica* (74.77 per cent) and the effect of native isolate N2 was on par with standard isolate *S. glaseri* (58.16 per cent). (Table 13)

Table 14. Effect of different levels of EPN on the mortality of grubs of *O. longicollis* inside the pseudostem under laboratory condition

Treatments	Percentage mortality of grubs at varying inoculum levels and periods (hrs)											
	10IJ			50IJ			100IJ			200IJ		
	48	72	96	48	72	96	48	72	96	48	72	96
N1	1.65 *(7.37)	6.11 (14.31)	23.19 (28.79)	4.82 (12.68)	11.27 (19.62)	27.68 (31.74)	9.54 (17.99)	28.83 (32.48)	56.38 (48.67)	6.11 (14.31)	27.02 (31.32)	43.86 (41.47)
N2	0.41 (3.69)	2.45 (8.99)	13.64 (21.68)	1.65 (7.37)	7.94 (16.37)	17.77 (24.93)	4.82 (12.68)	18.99 (25.84)	48.17 (43.95)	2.45 (8.99)	25.82 (30.54)	39.89 (39.17)
H. indica	1.65 (7.37)	6.11 (14.31)	21.02 (27.29)	3.68 (11.06)	9.54 (17.99)	25.82 (30.54)	9.54 (17.99)	24.54 (29.70)	52.07 (46.19)	6.11 (14.31)	23.82 (29.21)	41.87 (40.32)
S. glaseri	0.41 (3.69)	1.65 (7.37)	9.54 (7.99)	1.65 (7.37)	7.26 (15.64)	23.82 (29.21)	7.94 (16.37)	16.42 (23.90)	39.80 (39.12)	3.68 (11.6)	15.28 (23.01)	27.41 (31.57)
CD(0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

*Figures in parenthesis are values after angular transformation
NS - statistically not significant

4.5.2 Effect of EPN on the Mortality of Banana Weevil Inside the banana pseudostem and rhizome under Laboratory Conditions

4.5.2.1 Mortality of pseudostem weevil inside the pseudostem.

10 IJ per grub

The mean corrected mortality of grubs of *O. longicollis* inoculated at an initial inoculum level of 10 IJ per grub observed at 48 hrs after treatment did not vary significantly between four EPN. However the N1 isolate showed same mortality percentage as that of *H. indica.* (1.65) (Table 14)

The mean corrected mortality of grubs observed at 72 hrs after treatment did not vary significantly. However the N1 isolate showed same mortality percentage as that of *H. indica.* (6.11) (Table 14)

After 96 hrs of treatment also the mean corrected mortality of *O. longicollis* grubs did not vary significantly between EPN. However the maximum mortality was observed in N1 isolate (23.19 per cent). (Table 14)

50 IJ per grub

The mean corrected mortality with an inoculum level of 50 IJ per grub observed at 48 hrs after treatment did not vary significantly. The maximum mortality was observed in N1 isolate (4.82 per cent). (Table 14)

At 72 hrs after treatment the mean corrected mortality did not vary significantly and the maximum mortality was observed in N1 isolate (11.27 per cent). (Table 14)

The mean corrected mortality after 96 hrs also found not varying significantly. However the maximum mortality was showed by N1 isolate (27.68 per cent). (Table 14)

100 IJ per grub

For inoculum level 100 IJ per grub the mean corrected mortality of grubs of *O. longicollis* after 48 hrs of treatment did not vary significantly and the mortality recorded in N1 isolate was same as that of *H. Indica* (9.54 per cent) (Table 14).

After 72 hrs of the treatment also the mortality of grubs showed no significant variation. However the maximum mortality was showed by N_1 isolate (28.83 per cent). (Table 14)

The mortality of grubs of *O. longicollis* observed at 96 hrs after treatment did not vary significantly. The maximum mortality was showed by N1 isolate (56.38 per cent). (Table 14)

200 IJ per grub

The mean corrected mortality of grubs of *O. longicollis* inoculated at 200 IJ per grub observed after 48 hrs treatments did not vary significantly. However the mortality showed by N1 and *H. indica* was same (6.11 per cent). (Table 14)

After 72 hrs of treatment the mortality did not vary significantly. The maximum mortality was showed by N1 isolate (27.02 per cent). (Table 14)

The mean corrected mortality of grubs of *O. longicollis* after 96 hrs treatment did not vary significantly. The maximum mortality was showed by N1 isolate (43.86 per cent) (Table 14)

4.5.2.2 Mortality of *C. sordidus* inside the rhizome.

10 IJ per grub

The mean corrected mortality of grubs of *C. sordidus* inoculated with 10 IJ per grub 48 hrs after treatment did not vary significantly between EPN. The maximum mortality was showed by *H. indica* (13.64 per cent). (Table 15)

Table 15. Effect of different levels of EPN on the mortality of grubs of *C. sordidus* inside the rhizome under laboratory condition

Treatments	Percentage mortality of grubs at varying inoculum levels and periods (hrs)											
	10IJ			50IJ			100IJ			200IJ		
	48	72	96	48	72	96	48	72	96	48	72	96
N1	7.94 *(16.37)	15.28 (23.01)	23.19 (28.79)	3.68 (11.06)	9.54 (17.99)	25.63 (30.42)	13.64 (21.68)	56.38 (48.67)	45.95 (42.68)	10.95 (19.32)	23.19 (28.79)	33.60 (35.43)
N2	3.68 (11.06)	9.54 (17.99)	13.64 (21.68)	1.65 (7.37)	6.48 (14.74)	21.87 (27.88)	7.94 (16.38)	48.17 (43.95)	43.86 (41.47)	10.95 (19.32)	23.19 (28.79)	31.43 (34.10)
H. indica	13.64 (21.68)	17.37 (24.63)	23.19 (28.79)	3.68 (11.06)	13.64 (21.68)	25.63 (30.42)	17.77 (24.93)	52.07 (46.19)	46.16 (42.80)	17.37 (24.63)	27.68 (31.74)	39.8 (39.12)
S. glaseri	3.68 (11.06)	7.94 (16.37)	23.19 (28.79)	1.65 (7.37)	6.48 (14.74)	19.98 (26.55)	6.48 (14.74)	39.80 (39.12)	43.86 (41.47)	7.94 (16.37)	19.57 (26.26)	31.72 (34.28)
CD(0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

*Figures in parenthesis are values after angular transformation
NS - statistically not significant

After 96 hrs also the mortality did not showed statistically significant variation. N1 and *H. indica* showed same mortality (23.19 per cent). (Table 15)

50 IJ per grub

The mean corrected mortality at 48 hrs after treatment did not vary significantly between EPN. The maximum mortality showed by *H. indica* and N1 was same (3.68 per cent). (Table 15)

At 72 hrs after treatment also the mortality did not vary significantly. The maximum mortality was showed by *H. indica* (13.64 per cent). (Table 15)

The mean corrected mortality of *C. sordidus* grubs observed at 96 hrs after treatment did not vary significantly. The mortality showed by *H. indica* and N1 was same (25.63 per cent). (Table 15)

100 IJ per grub

The mean corrected mortality of grubs of *C. sordidus* inoculated at 100 IJ per grub observed at 48 hrs after treatment did not vary significantly. The maximum mortality was showed *H. indica* (17.77 per cent). (Table 15)

The mean corrected mortality of *C. sordidus* grubs observed at 72 hrs after treatment also did not vary significantly. The maximum mortality was showed by N1 (56.38 per cent). (Table 15)

At 96 hrs after treatment also the mean corrected mortality did not vary significantly. However the maximum mortality was showed by *H. indica* (46.16 per cent). (Table 15)

200 IJ per grub

The mean corrected mortality of grubs of *C. sordidus* inoculated at 200 IJ per grub observed at 48 hrs did not vary significantly. The maximum mortality was

recorded in *H. indica* (17.37 per cent). However the mortality showed by N1 isolate was same as that of N2 (10.95 per cent). (Table 15)

72 hrs after treatment also the mean corrected mortality did not vary significantly. The maximum mortality was showed by *H. indica* (27.68 per cent). (Table 15)

The mean corrected mortality of grubs of *C. sordidus* inoculated at 200 IJ per grub observed at 96 hrs did not vary significantly. However the maximum mortality was showed by *H. indica* (39.80 per cent). (Table 15)

DISCUSSION

5. DISCUSSION

Banana is the oldest and most valued fruit crop in India. In Kerala banana is cultivated extensively with an annual production of 8 tons per ha (FIB, 2006). One of the major constraints in banana production is the infestation by pests and diseases. Among the pests, weevil infestation in the pseudostem and rhizome is the two major problems in the production of banana. Chemical pesticides are widely recommended for the control of these pests but they cause hazards like development of pesticide resistance to insects, retention of residues in the edible part of the plant and contamination of soil, air, and water. Biocontrol of crop pests is an ideal alternative and is ecofriendly and sustainable and could be successfully integrated with the management practices. EPN is considered and as one of the biocontrol agents and an excellent candidature against soil insects and insect pests in cryptic environment. With their obligate parasitism and potential of rapid killing of the host insects with in 24 to 48 hrs, their effect could be comparable with insecticides.

The present investigation was taken up to isolate potent native EPN distributed in the banana rhizosphere of Instructional Farm, College of Agriculture, Vellayani during 2005-06. Selection of cheap media for rearing the trap insect, wax moth *G. mellonella*, mass production of EPN and the potential of the native isolates of EPN for the management of grubs and adults of pseudostem and rhizome weevil were studied in detail. The result obtained is discussed below.

5.1 DISTRIBUTION OF EPN IN THE BANANA RHIZOSPHERE

A random survey for EPN was conducted in the weevil infested banana rhizoshpere covering an area of 75 ha. located in Instructional Farm, Vellayani. Only ten native isolates were obtained from 100 samples

processed. Occurrence of native isolates of *H. indica* from Alappuzha district was reported in coconut rhizosphere by Banu *et al.*1998. They obtained one isolate each of *H. indica* from the rhizoshpere of coconut and cow dung pit out of the 95 samples collected. Later Banu (2001) did an extensive survey throughout Kerala and collected 40 isolates of *H. indica* and 16 isolates of *Steinernema* species, out of 597 samples collected revealing 10.6 percentage of natural occurrence of EPN. Present study was in line with the findings of Banu (2001). A state wide survey conducted by Sosamma *et al.* (2001) reported the presence of native isolates of *H. indica* and *Steinernema* sp. from coconut rhizosphere as well as rhinoceros beetle cadaver. But the report of EPN in banana rhizosphere from the state is lacking and the finding of the present investigation is the first of this kind in Kerala. The symptom of infestation of *H. indica, S. glaseri,* native isolates, N1 and N2 in *G. mellonella* are given in Plate 10. The characterizing discolourations independent of each one was evident. This will help to detect the presence easily.

5.2 SCREENING OF NATIVE ISOLATES OF EPN

Screening of ten native isolates of EPN obtained in the survey (N1 to N10) was subjected to pathogenicity trial using grubs of pseudostem weevil *Odoiporus longicollis* Oliv. Among the ten isolates N1 and N2 were selected for detailed investigation based on the mortality rates at different inoculum levels (10, 50, 100 and 200 IJ per grub) and period of exposure (24, 48 and 72 hrs). Highest mortality of 85.31 per cent was recorded by isolate N1 with 100 IJ per grub at 72 hrs after treatment. While N2 isolate recorded only 62.42 per cent mortality at the same inoculum level and period of exposure. These results were in line with that of the reports by Padmanabhan *et al.* (2002) on grubs of pseudostem weevil by *H. indica* in Tamil Nadu. They reported that *H. indica* @ 80-100 IJ per grub gave 66.6 per cent mortality under *in vitro* conditions at 72 hrs after treatment. However

H. indica

S. glaseri

N1 isolate

N2 isolate

Plate 10. Symptoms of EPN on *G. mellonella* larvae

the effect of N1 was better than that of *H. indica* reported from Tamil Nadu against *O. longicollis* grub. The performance of ten different native isolates (N1-N10) was assessed for their immediate kill at 24 hrs after treatment onwards and is presented in Fig. 1. Interestingly there was immediate killing effect of N1 with a corrected mortality of 33 per cent at 24 hrs after treatment itself, indicating that these grubs could be managed to some extent by the N1 isolate.

5.3 IDENTIFICATION OF CHEAP MEDIA FOR REARING OF TRAP INSECT

The survival of different instars of *G. mellonella* was observed in different media and it was found that standard, M1 and M2 media recorded maximum survival of first, second, third, fourth and fifth instars larvae of *G. mellonella*. The survival of first, second, third, fourth and fifth instar larvae in the standard medium were 95.95, 94.95, 94.95, 89.85 and 89.85 per cent respectively.

Among the cheap media studied, the M1 medium recorded maximum survival of first, second, third, fourth and fifth instar larvae with 88.90, 89.85, 83.95, 76.80 and 79.85 per cent survival respectively. The ingredients of M1 medium were corn flour, atta, milk powder, powdered yeast, honey and glycerol @100 g, 300 g, 100 g, 100 g, 200 ml and 50 ml per kg respectively. M1 medium differed from standard medium only in the quantities of the ingredients. The quantities of costly ingredients *viz.*, corn flour and glycerol were reduced from 200 to 100g and 150 to 50 ml respectively and the quantities of cheaper ingredients *viz.*, atta and honey were increased from 200 to 300 g and 100 to 200 ml respectively. Thus there was 21.29 per cent reduction in cost of M1 medium in comparison with standard medium formulated by Hussaini *et al.* (2005). Though the costly ingredients were reduced in M1 medium, the reduction in survival of first, second, third, fourth and fifth instars were only 7.3, 5.4, 11.6, 14.5 and

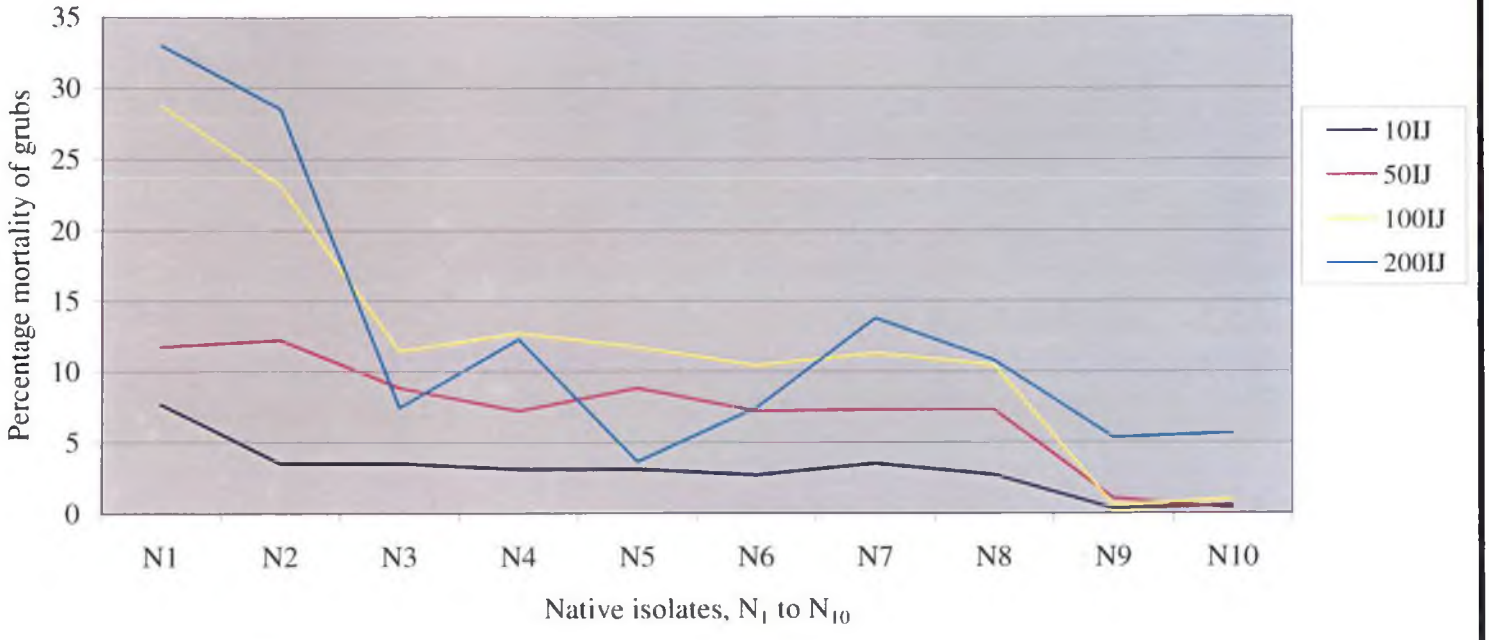


Fig. 1. Effect of native isolates on the mortality of grubs of pseudostem weevil at 24 hrs after treatment

11.1 per cent respectively. Among the different instars maximum reduction was seen in fourth instar (14.5 per cent).

The first, second, third, fourth and fifth instar larvae survived in the M2 medium were 81.90, 79.85, 74.65, 69.95 and 64.80 per cent respectively. The ingredients of M2 medium were corn flour, atta, milk powder, powdered yeast, honey, and glycerol and sugar solution @ 100 g, 300 g, 100 g, 100 ml, 50 ml and 100 ml per kg respectively. Here also the quantities of costly materials *viz.*, corn flour and glycerol from 200 to 100 g and 150 to 50 ml per kg of medium respectively. The cheaper ingredient, atta, increased from 200 to300 g per kg of medium. An additional cheaper ingredient, sugar solution @100 ml per kg medium was added. Thus the cost of M2 was reduced to 34.87 per cent in comparison to the cost of standard medium. Though the costly ingredients were reduced in M2 medium the reduction in survival of first, second, third, fourth and fifth instars were only 14.6, 15.9, 21.4, 22.1, and 27.9 per cent respectively. Thus considering the cost of reduction of media and rate of survival of five different instars of *G. mellonella*, the trap insect of EPN, the cheaper media *viz.*, M1 and M2 were selected. As a part of this study rice bran was also included as an additional cheap ingredient @ 400 and 600 g each in M3 and M4 media respectively. But the survival of different instars was far below the required rate. Hence M4 medium was not selected for detailed studies. There were scanty reports on the studies about the various cheap media for rearing of *G. mellonella* except that of Josephrajkumar (2005). He used an additional ingredient, wheat bran @ 100 g per kg of the medium and also he reduced the quantity of atta, glycerol and yeast @ 100 g, 50 g and 50 ml per kg standard medium respectively. Though he reported that this medium was used for rearing the trap insect, the critical view on the rate of survival of different instars were lacking. Thus this is the first detailed investigation in this aspect about the cheap media.

5.4 MASS PRODUCTION OF EPN

Mass production of selected potent native isolates N1 and N2 along with two standards *H. indica* and *S. glaseri* were done in last instar *G. mellonella* larvae fed with M1, M2 and M3 media along with standard medium. Maximum multiplication of EPN was recorded on *G. mellonella* reared in standard medium and maximum infective juvenile emergence was in case of *H. indica* (14689.3 per larva) than *S. glaseri* (9840.11 per larva). Among the native isolates, N1 recorded maximum emergence of infective juveniles (5152.29 per larva) on *G. mellonella* reared in standard medium and in the same medium N2 recorded 3999.45 infective juveniles per larva. Maximum multiplication of different EPN in four different media with 10 IJ per larva is given in Fig. 2. Similar results were reported by Singh and Yadav (2002). They reported that emergence of infective juveniles from *G. mellonella* was maximum for *Heterorhabditis* sp. (201520 per larva) than *Steinernema* sp. (90945 per larva). The maximum rate of multiplication in the standard, M1, M2 and M3 was given by *H. indica* and *S. glaseri*. The low rate of multiplication of N1 and N2 in these media may be due to some nutritional factors.

Among the cheap media M1 recorded the highest emergence of infective juveniles than M2 and M3 media. Among the three initial inoculum levels tried (5, 15 and 25 IJ per larva), 10 IJ per larva recorded maximum emergence of infective juveniles of *H. indica, S. glaseri* and N1 isolate at 20 days after inoculation. This result is in conformity to that of Banu (2003). She also reported this inoculum level (10 IJ per larva) for highest emergence of *H. indica, S. glaseri* and *Steinernema sp.*

However the native isolate N2 recorded maximum multiplication of infective juveniles in *G. mellonella* initially inoculated with 25 IJ per larva. Maximum multiplication of different EPN in four different media at 25 IJ per larva is given in Fig. 3. In other studies also the performance of N2 was inferior to N1 and others.

5.5 COMPARITIVE EFFICACY OF ISOLATES FOR THE MANAGEMENT OF WEEVIL PESTS OF BANANA

Effect of EPN on the mortality of grubs and adults of weevil pests *in vitro*

Pseudostem weevil

The mortality of grubs and adults of pseudostem and rhizome weevils was assessed with native isolates in comparison with standard cultures in the laboratory. Four inoculum levels used were 10, 50, 100 and 200 IJ per insect.

The mortality of grubs of *O. longicollis* recorded maximum in N1 isolate with 100 IJ per grub at 72 hrs after treatment (88.69 per cent) followed by *H. indica* (84.30 per cent) at the same level and period of exposure. At 200 IJ per grub also N1 recorded maximum mortality (84.30 per cent) than *H. indica* (80.38 per cent) at 72 hrs after treatment. In the case of N2 maximum mortality was recorded in 100 IJ per grub at 72 hrs after treatment (64.04 per cent). To get the same level of mortality *S. glaseri* required an inoculum level of 200 IJ per grub. The effect of EPN on the mortality percentage of grubs of pseudostem weevil at 72 hrs after treatment is presented in Fig. 4. A similar finding was reported by Padmanabhan *et al.* (2002). They conducted petri dish bioassay using *H. indica* against third instar grubs of banana pseudostem weevil and he reported 66.6 per cent mortality of grubs at 72 hrs of inoculation at an inoculum level of 80-100 IJ per grub.

In the case of adults of *O. longicollis* the maximum mortality was recorded in *H. indica* with 200 IJ per adult at 72 hrs after treatment (70.36 per cent) while in N1 isolate the mortality was 66.25 per cent. At 100 IJ per adult also *H. indica* recorded maximum mortality 68.22 per cent than N1 isolate (66.33 per cent) at 72 hrs after treatment. In the case of *S. glaseri* maximum mortality was recorded with 200 IJ per adult at 72 hrs after treatment (60.14 per cent) followed by N2 with 58.07 per cent mortality at the same level and period of exposure. The effect of EPN on the mortality percentage of adults of

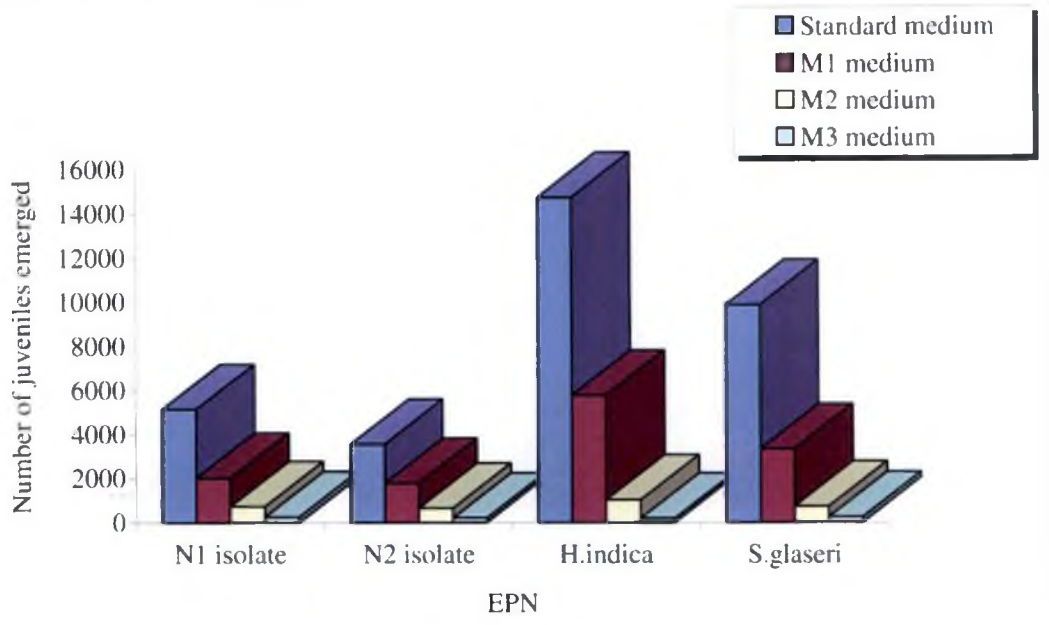


Fig. 2. Multiplication rate of EPN in *G. mellonella* reared in different media inoculated with 10IJ per larva at 20 DAI

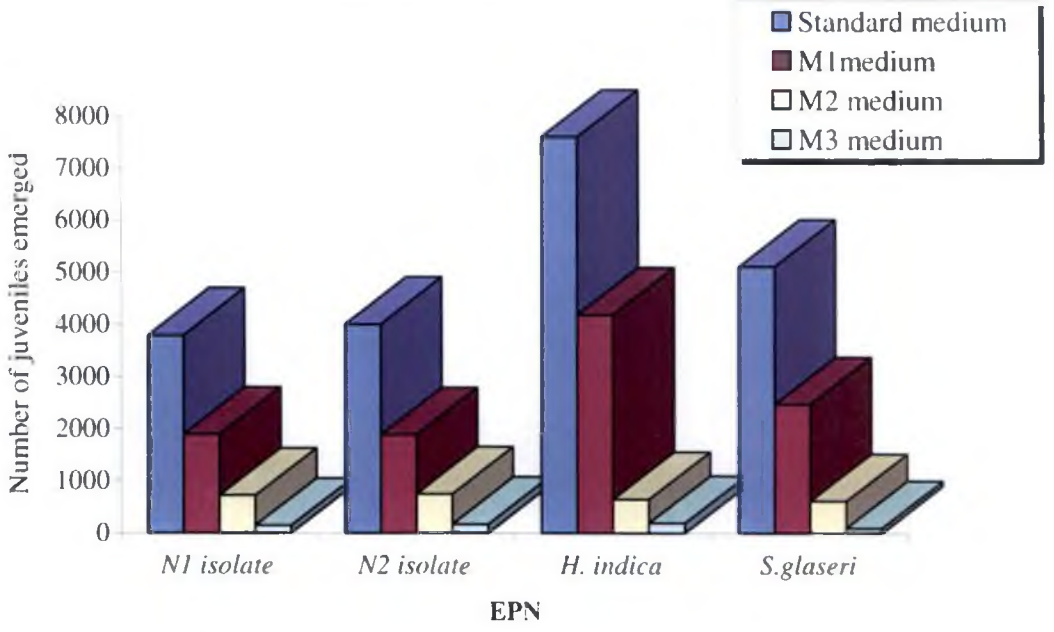


Fig. 3. Multiplication rate of EPN in *G. mellonella* reared in different media inoculated with 25 IJ per larva at 20 DAI

pseudostem weevil at 72 hrs after treatment is presented in Fig. 5. In the above work of Padmanabhan *et al.* (2002), the reports on the mortality of adults of *O. longicollis* was lacking.

Rhizome weevil

Maximum mortality of *C. sordidus* grub was recorded in *H. indica* inoculated with 200 IJ per grub at 72 hrs after treatment (82.58 per cent) followed by N1 (80.38 per cent). When the inoculum level was reduced to 100 IJ per grub N1 and *H. indica* recorded only 78.50 per cent mortality. N2 recorded a maximum mortality of 68.51 per cent with 200 IJ per grub at 72 hrs after treatment than *S. glaseri* (62.39 per cent) at the same level and period of exposure. With 100 IJ per grub both N2 and *S. glaseri* recorded 60.05 per cent mortality at 72 hrs after treatment. The effect of EPN on the mortality percentage of grubs of rhizome weevil at 72 hrs after treatment is presented in Fig. 6. The results agree with the observations of Rosales and Suarez (1998). They assessed the potential of six native isolates along with the standard cultures against the grubs of *C. sordidus*. Among the six isolates two isolates recorded 16-80 per cent mortality of *C. sordidus*.

In the case of adults of *C. sordidus* maximum mortality was recorded in N1 isolate with 200 IJ per adult at 72 hrs after treatment (76.75 per cent) followed by *H. indica* (74.77 per cent). But at the lower level of 100 IJ per adult, *H. indica* and N1 recorded only 68.22 and 64.63 per cent mortality respectively at 72 hrs after treatment. N2 recorded a maximum mortality of 58.07 per cent. The effect of EPN on the mortality percentage of adults of rhizome weevil at 72 hrs after treatment is presented in Fig. 7. The finding obtained in the present study was supported by the findings of Gold *et al.* (2004). They stated that the EPN *Steinernema* and *Heterorhabditis* species attacked both adults and larva of *C. sordidus* in the field. While Pena *et al.* (1993) reported that the grubs of *C. sordidus* were

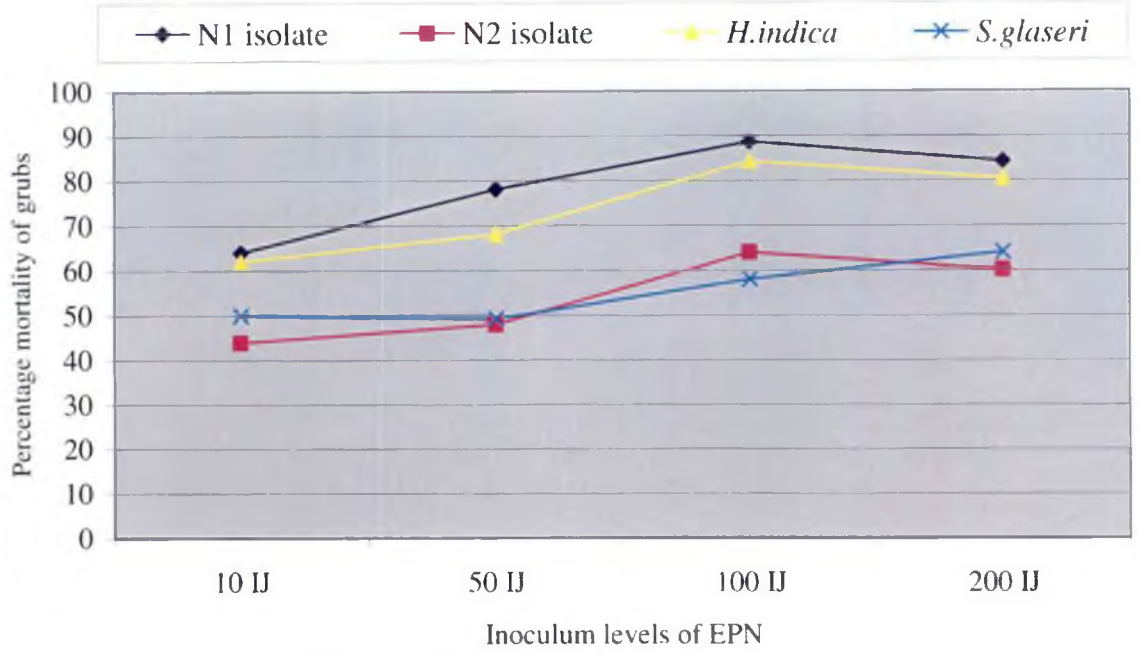


Fig. 4. Effect of EPN on the mortality percentage of grubs of pseudostem weevil at 72 hrs after treatment

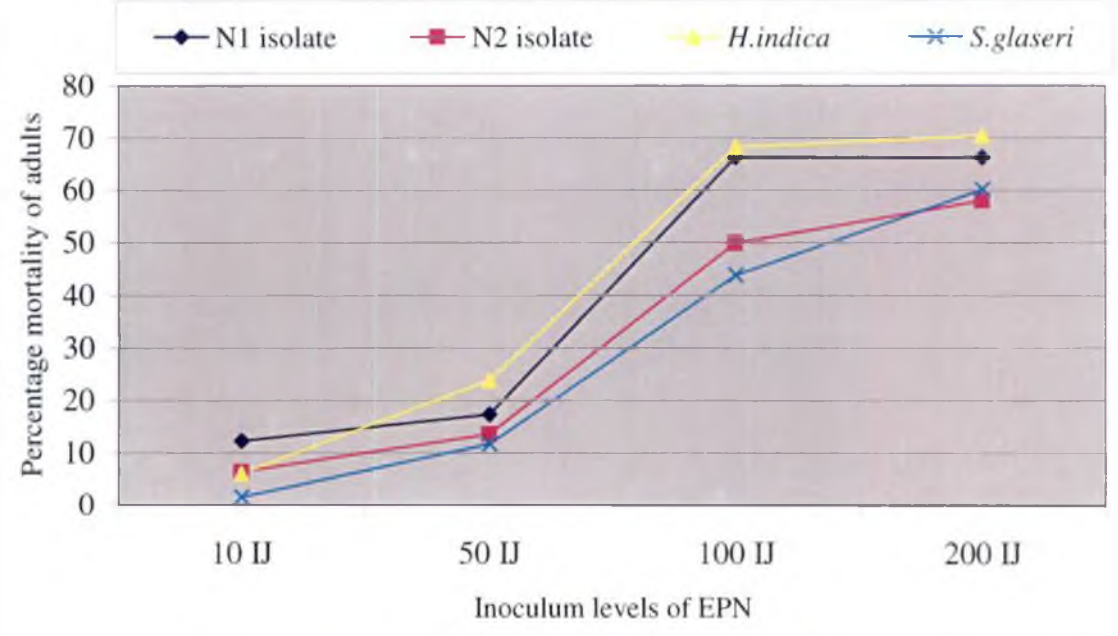


Fig. 5. Effect of EPN on the mortality percentage of adults of pseudostem weevil at 72 hrs after treatment

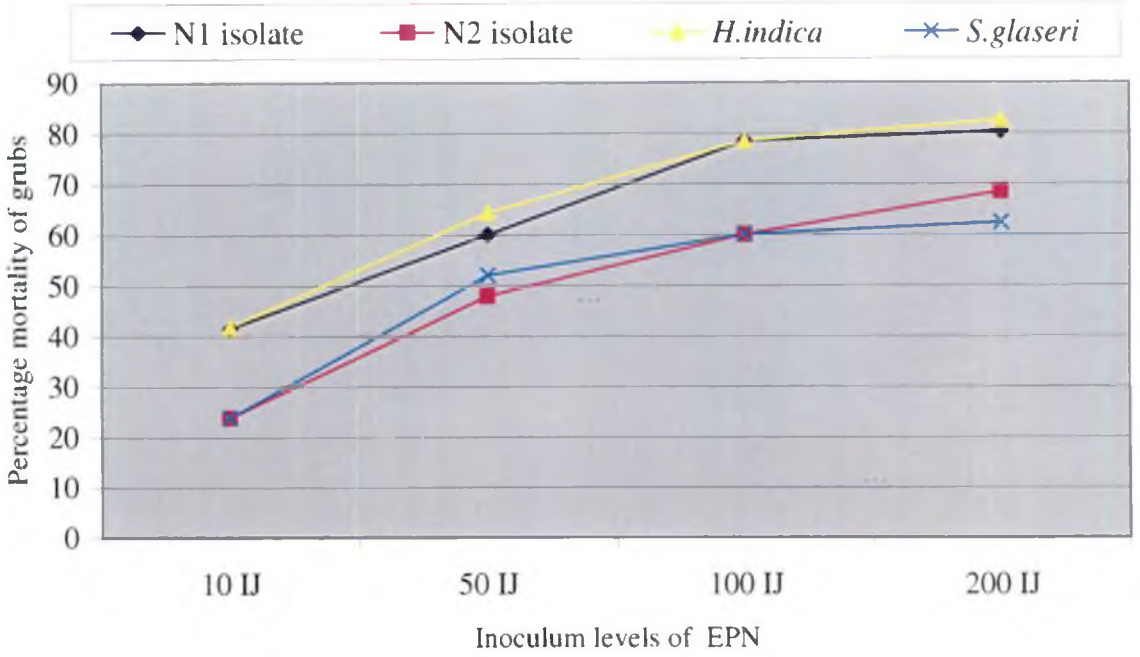


Fig. 6. Effect of EPN on the mortality percentage of grubs of rhizome weevil at 72 hrs after treatment

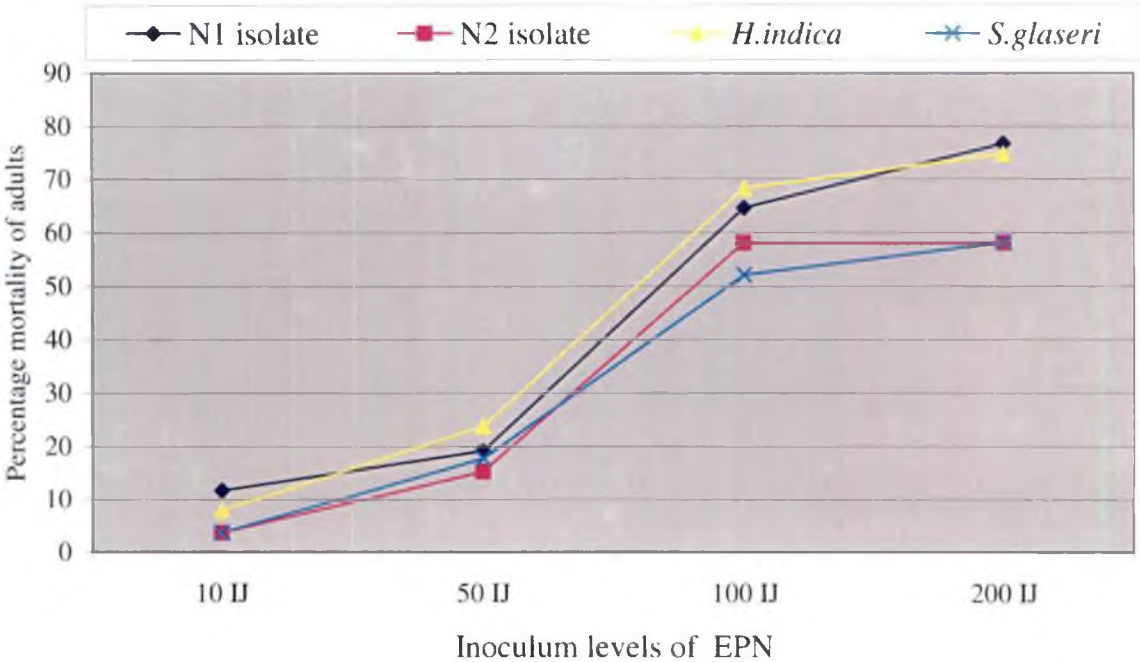


Fig. 7. Effect of EPN on the mortality percentage of adults of rhizome weevil at 72 hrs after treatment

more susceptible to infection by EPN *S.carpocapsae*, *S. bibonis* and *Heterorhabditis* species than adult weevils.

The symptom of infestations of *H. indica*, *S. glaseri*, N1 and N2 in pseudostem and rhizome weevil are presented in Plate11.

Effect of EPN on the mortality of grubs inside the pseudostem and rhizome of banana

Pseudostem weevil

The mortality of grubs of *O. longicollis* inside the pseudostem was highest in N1 isolate treated with 100 IJ per grub at 96 hrs after treatment (56.38 per cent). *H. indica* recorded 52.07 per cent mortality only at this level. N2 recorded 48.17 per cent mortality with 100 IJ per grub at 96 hrs after treatment and *S. glaseri* recorded only 39.80 per cent mortality at the same level and period of exposure. The effect of EPN on the mortality percentage of grubs of pseudostem weevil inside the pseudostem at 96 hrs after treatment is presented in Fig. 8. There was no report on the effect of EPN against the grubs inside the pseudostem. However in straw berry Miduturi *et al.* (1994) conducted field experiments against *O. sulcatus* and they reported that 10,000 IJ per plant of *S. carpocapsae* and *Heterorhabditis* species gave 10-20 and 10-40 per cent control of *O. sulcatus* respectively.

Rhizome weevil

Maximum mortality of *C .sordidus* inside the rhizome was recorded in N1 (56.38 per cent) with 100 IJ per grub at 72 hrs after treatment and *H. indica* with 52.07 per cent at the same level and period of exposure. N2 recorded a mortality percentage of 48.17 with 100 IJ per grub at 72 hrs after treatment and *S. glaseri* recorded 39.80 per cent mortality at the same level and period of exposure. The effect of EPN on the mortality percentage of grubs of rhizome weevil inside the rhizome at 96 hrs after treatment is presented in Fig. 9. The results were in agree with the

N1 isolate on grubs of pseudostem weevil

N1 isolate on grubs of rhizome weevil

N2 isolate on grubs of pseudostem weevil

N2 isolate on grubs of rhizome weevil

H. indica

S. glaseri

Plate 11. Symptoms of EPN on grubs of pseudostem and rhizome weevil

observations of Abbas *et al.* (2001) and they reported that EPN remains the best possible means for managing the red palm weevils which have cryptic behaviour like rhizome weevil. In another report Schroeder (1990) conducted field studies with *S. carpocapsae* at 100-200 IJ per cm^2 and *H. bacteriophora* at 100 IJ per cm^2 against citrus root weevil (*D. abbreviatus)* and he reported that both the nematodes resulted in 50 per cent reduction of population.

The study revealed that EPN could be effectively used as a component in the management of the weevil pests of banana. Further studies are required to find out virulent local isolates or species from different agro climatic condition and their field efficacy. Mass production techniques are available but are to be modified with cheap ingredients to make it cost effective. A number of commercial formulations are available world over but in India two products were evolved but now it is not available in the market hence this aspect also needs emphasis. Based on the literature available and this investigation EPN have been proved as novel biocontrol agent against a number of insect pests especially in soil and cryptic environment. But concentrated efforts are required to develop these organisms as viable option in insect pest management. The biotechnological interventions in scaling up of potential of these organisms will form the need of the millennium.

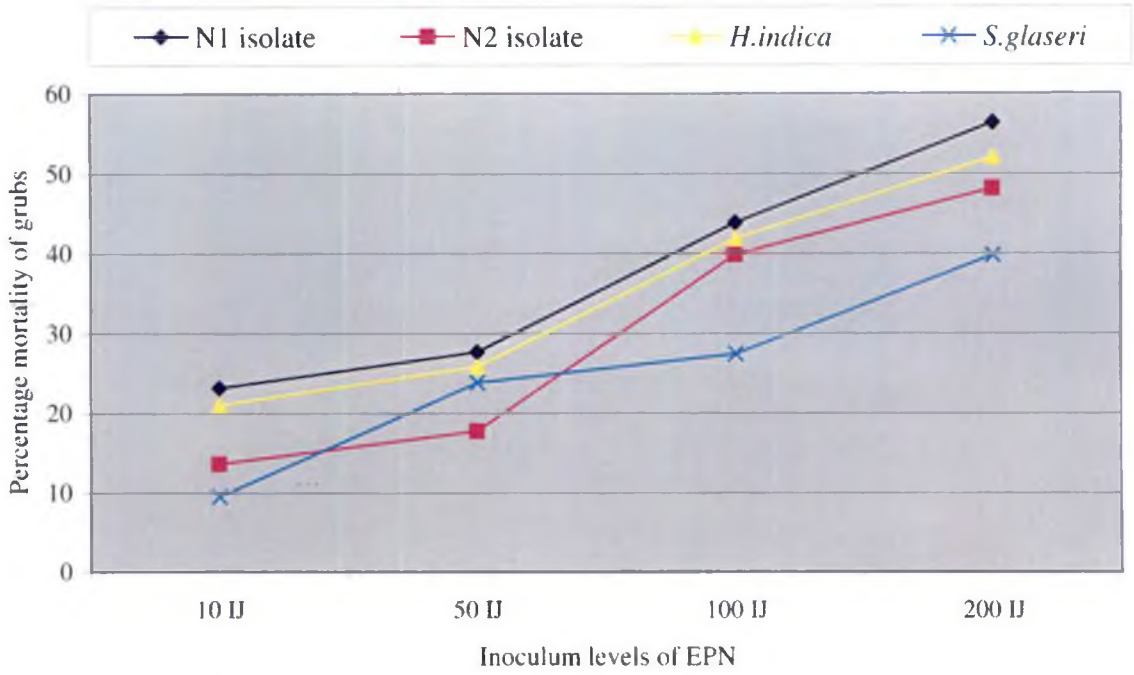


Fig. 8. Effect of EPN on the mortality percentage of grubs of pseudostem weevil inside the pseudostem at 96 hrs after treatment

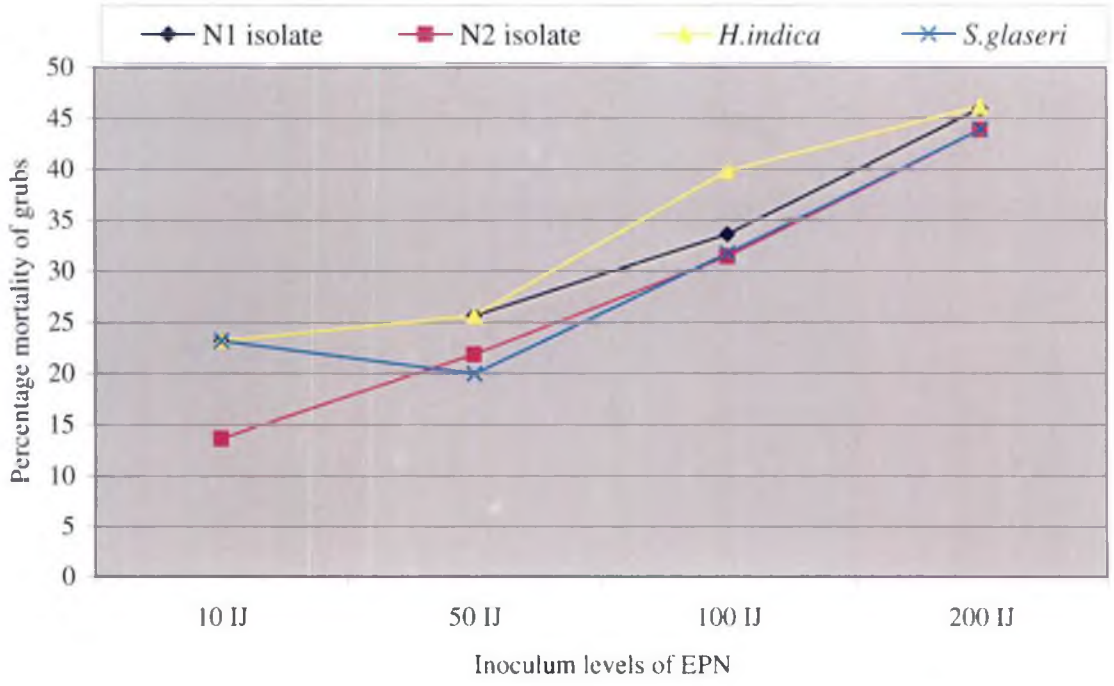


Fig. 9. Effect of EPN on the mortality percentage of grubs of rhizome weevil inside the rhizome at 96 hrs after treatment

SUMMARY

6. SUMMARY

A study was conducted at College of Agriculture, Vellayani during 2005-06 on the potential of entomopathogenic nematodes (EPN) for the management of pseudostem and rhizome weevil infesting banana. A random survey was conducted to isolate the native EPN from different stages of weevil pests and banana rhizosphere. Ten native isolates were obtained from hundred samples collected from banana rhizosphere in different blocks of the Instructional Farm covering an area of 75 ha. The potential of native isolates were assessed by testing their mortality in pseudostem weevil grubs using different inoculum levels and exposure periods *in vitro* conditions. Among the native isolates N1 and N2 were found significantly superior to other isolates recording more than 70 and 60 per cent corrected mortality of grubs respectively at different inoculum levels and period of exposure. From the overall performance, native isolates, N1 and N2 were selected for detailed study along with the standards *Heterorhabditis indica* and *Steinernema glaseri*. Inorder to find out a cost effective rearing media for the wax moth larva *Galleria mellonella* L. the trap insect of EPN, four different media were also tried by reducing the quantity of costly ingredients and substituting some cheap ingredients. The survival of different stages of *G. mellonella* in the above was also studied in laboratory conditions. The cost of different media computed revealed that the total cost of ingredients was reduced to 21.29 and 34.87 per cent respectively in M1 and M2 media. The multiplication of native isolates N1 and N2 and two standards *H. indica* and *S. glaseri* in *G. mellonella* larva reared in different cheap media were also studied. Maximum emergence of infective juveniles of EPN was recorded in *G.mellonella* reared in standard medium. Among M1, M2 and M3 media, M1 medium recorded maximum emergence of infective juveniles.

Among the EPN, *H. indica* recorded maximum emergence of infective juveniles (14689.3 per larva) with 10IJ per larva at 20 DAI in standard medium. In M1 medium the maximum emergence of infective juveniles was recorded with 10 IJ per larva at 20 DAI (5754.39).

In case of *S. glaseri* also, standard medium with 10IJ per larva at 20 DAI (9840.11 per larva) recorded maximum emergence of infective juveniles. In M1 medium maximum infective juveniles was recorded with 10 IJ per larva at 20 DAI (3311.31).

Maximum emergence of infective juveniles of N1 was recorded with an initial inoculum level of 10IJ per larva of *G. mellonella* reared in standard medium at 20 DAI (5152.29 per larva). In the case of larva reared in M1 medium maximum infective juvenile emergence was recorded 2454.71 per larva with an initial inoculum level of 5IJ per larva at 20 DAI.

In standard medium, N2 recorded maximum emergence of infective juveniles initially inoculated with 25 IJ per larva on *G. mellonella* larva reared in standard medium at 20 DAI (3999.45). Among the cheap media the larvae reared in M1 medium recorded maximum emergence of infective juveniles with an initial inoculum level of 25 IJ per larva at 20 DAI (1879.32).

The interaction of EPN and media, EPN and periods of exposure and EPN and initial inoculum levels were also studied. Interaction study revealed that *G. mellonella* reared in standard medium was the best medium for multiplication of all EPN. But among the cheap media M1 medium was the best medium. Among the initial inoculum levels, 25 IJ per larva was best for native isolate N2 and 10 IJ per larva for standard cultures and native isolate N1. Maximum emergence of infective juveniles for all the EPN was recorded at 20 DAI.

The effect of EPN on the mortality of grubs and adults of pseudostem and rhizome weevil were also studied in *in vitro* condition and

also in inside the infested banana pseudostem and rhizome in laboratory conditions.

The mortality of grubs of *O. longicollis* recorded maximum in N1 isolate with 100 IJ per grub at 72 hrs after treatment (88.69 per cent) followed by *H. indica* (84.30 per cent). In case of adults of *O. longicollis*, maximum mortality was recorded in *H. indica* with 200 IJ per adult at 72 hrs after treatment (70.36 per cent). Thus higher inoculum level of *H. indica* and N1 was required to get mortality of adults of *O. longicollis* in *in vitro*.

Maximum mortality of *C. sordidus* grub was recorded in *H.indica* inoculated with 200 IJ per grub at 72 hrs after treatment (82.58 per cent) followed by N1 (80.58 per cent).When the inoculum level was reduced to 100 IJ per grub, N1 and *H. indica* recorded only 78.50 per cent mortality. In the case of adults of *C. sordidus* maximum mortality was recorded in N1 isolate with 200 IJ per adult at 72 hrs after treatment (76.75 per cent) followed by *H. indica* (74.77 per cent). But, at lower level of 100 IJ per adult, *H. indica* and N1 recorded only 68.22 and 64.63 per cent mortality respectively at 72 hrs after treatment.

The mortality of grubs of *O. longicollis* inside the pseudostem was highest in N1 isolate treated with 100 IJ per grub at 96 hrs after treatment (56.38 per cent). *H. indica* recorded 52.07 per cent mortality only at this level.

In the case of *C. sordidus* grub, maximum mortality was recorded in N1 (56.38 per cent) with 100 IJ per grub at 72 hrs after treatment and *H. indica* with 52.07 per cent at the same level.

Thus from the above results, mortality of grubs and adults of the weevil under *in vitro* and inside the banana pseudostem and rhizome required a minimum inoculum level of 200 IJ per grub.

REFERENCES

7. REFERENCES

Abbas, M.S.T. and Hanonik, S.P. 1999. Pathogenicity of entomopathogenic nematodes to red palm weevil, *Rhynchophorus ferrugineus*. *Int. J. Nematol.* 9: 84-86

Abbot, W.S. 1925. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.* 18: 265-267

Ahmed, R., Ali, S.S., Rashid, P. and Hussaini, A.M. 2005. Management of major pests of pulses with EPN – scope and limitations. (eds. Sithanantham, S., Vasanthraj, B.D. and Selvaraj, P.). In: *Biotechnological management of nematode pests and scope of entomopathogenic nematodes*. Sun Agro Biotech Research Centre Publication, Chennai, pp.154-167

Akhurst, R.J. and Bedding, R.A.1986. Natural occurrence of insect pathogenic nematodes (Steinernematidae and Heterorhabditidae) in soil in Australia. *J. Aust. Entomol. Soc.* 25: 241-244

*Ayoub, S. 1977. Plant Nematology : an agricultural training aid. Sacramento, Calif.: California Department of Food and Agriculture 381p.

*Backhaus, G.F. 1994. Biological control of *Otiorhynchus sulcatus* F. by use of entomopathogenic nematodes of the genus *Heterorhabditis*. *Acta Hort.* 364: 131-142

Banu, J.G., Sosamma, V.K. and Koshy, P.K. 1998. Natural occurrence of an entomopathogenic nematode from Kerala. *Proc.Third Int. Symp. on Afro-Asian Soc. Nematologists, April 16- 19, 1998*(ed. Mehta, K.). Sugarcane Breeding Institute, Coimbatore. p.99

Banu, J.G. 2001. Studies on the entomopathogenic nematodes of Kerala. Ph.D. thesis, Tamil Nadu Agricultural University, Coimbatore, India, 180p.

Banu, J.G. 2003. Scope of entomopathogenic nematodes for the management of pests of intercrops in coconut based cropping system In: *Current Status of Research on Entomopathogenic nematodes in India.* (eds. Hussaini, S.S., Rabindra, R.J. and Nagesh, M.). Project Directorate of Biological Control, Bangalore, pp.179-183

Banu, J.G., Nugyen, K.B. and Rajendran, G. 2005. Occurrence and distribution of entomopathogenic nematodes in Kerala, India. *Int. J. Nematol.* 15: 9-16

Beavers, J.B., McCoy, C.W. and Kaplan, D.T. 1983. Natural enemies of subterranean *Diaprepes abbreviatus* (Coleoptera:Curculionidae) larvae in Florida. *Environ. Entomol.* 12: 840-843

Bedding, R.A. and Akhurst, R.J. 1975. A simple technique for the detection of insect parasitic rhabditid nematodes in soil. *Nematologica* 21: 109-110

Bedding, R.A., Molyneux, A.S. and Akhurst, R.J. 1983. *Heterorhabditis* spp., *Neoaplectana* spp. and *Steinernema kraussei*: inter specific and intra specific differences in the infectivity for insects. *Exp. Parasitol.* 55: 249-257

Berry, R.E., Liu, J. and Groth, E. 1997. Efficacy and persistence of *Heterorhabditis marelatus* (Rhabditida: Heterorhabditidae) against root weevils (Coleoptera: Curculionidae) in strawberry. *Environ. Entomol.* 26: 465-470

Bhaskaran, R.K.M., Sivakumar, C.V. and Venugopal, M.S. 1994. Biocontrol potential of entomopathogenic nematodes in controlling red hairy caterpillar, *Amsacta albistriga* (Lepidoptera: Arctiidae) of ground nut. *Indian J. Agric. Sci.* 64: 655-657

Boselli, M., Curto, G.M. and Tacconi, R. 1997. Field efficacy of entomopathogenic nematodes against the sugar-beet weevil *Temnorhinus* (= *Conorrhynchus*) *mendicus* Gyll. (Coleoptera: Curculionidae). *Biocontrol Sci. Technol.* 7: 231-238

Boselli, M., Curto, G.M., Tacconi, R., Pollini, A. and Santi, R. 1994. Field trials with *Steinernema carpocapsae* and *Heterorhabditis* species juveniles against the sugarbeet weevil, *Conorhynchus mendicus* Gyll. in Italy. *Nematologica* 40: 463-465

Cabanallas, H.E. and Raulston, J.R. 1995. Impact of *S. riobravis* (Rhabditida : Steinernematidae) on the control of *Helicoverpa zea* (Lepidoptera : Noctuidae) in corn. *J. Econ. Entomol.* 88: 58-64

Choo, H.Y., Kaya, H.K. and Reed, D.K. 1988. Biological control of onion maggot and tobacco cutworm with insect-parasitic nematodes, *Steinernema feltiae* and *Heterorhabditis heliothidis*. *Korean J. Appl. Entomol.* 27: 185-189

Constant, P. Marchay, L. Fischer, L.S. Briand, P.S. and Mauleon, H. 1998. Natural occurrence of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) in Guadeloupe islands. *Fundamental Appl. Nematol.* 21: 667-672

*Curto, S.M., Boselli, M., Paganini, U. and Ghedini, R. 1992. Control trials against sugarbeet weevil (*Conorhynchus mendicus* Gyll. (Coleoptera Curculionidae) using entomopathogenic nematodes. *Informatore-Fitopatologica* 42: 45-49

*Curto, G.M., Boselli, M., Tacconi, R., Gwynn, R.L., Smits, P.H., Griffin, C., Ehlers, R.U., Boemare, N. and Masson, J.P. 1996. Field effectiveness of entomopathogenic nematodes against the sugarbeet weevil *Temnorhinus* (=*Conorrhynchus*) *mendicus* Gyll. (Coleoptera: Curculionidae) in Italy, 16 - 20 May. *Proc. of the nat. symp.*

entomopathogenic nematodes: application and persistence of entomopathogenic nematodes, Todi, Perugia, Italy.pp.107-115

*Downing, A.S., Erickson, C.G. and Kraus, M.J. 1991. Field evaluation of entomopathogenic nematodes against citrus root weevils (Coleoptera: Curculionidae) in Florida citrus. *Florida-Entomologist* 74: 584-586

Duncan, L.W. and McCoy, C.W. 1996. Vertical distribution in soil, persistence, and efficacy against citrus root weevil (Coleoptera: Curculionidae) of two species of entomogenous nematodes (Rhabditida: Steinernematidae; Heterorhabditidae). *Environ. Entomol.* 25: 174-178

Dutky, S.R., Thompson, J.V. and Cantwell, G.E. 1964. A technique for the mass propagation of the DD-136 nematode. *J. Insect Pathol.* 6: 417-422

Dutt, N. and Maiti, B.B. 1970. Observations on the incidence pattern of banana weevils *Odoiporus longicollis* Oliv. and *Cosmopolites sordidus* Ger. under West Bengal conditions. *Proc. 57th Indian Sci. Congr.* III, 526p.

Dutt, N. and Maiti, B.B. 1972. Bionomics of the banana pseudostem weevil *Odoiporus longicollis* Oliv. (Coleopteran:Curculionidae). *Indian J. Entomol.*34: 20-30

F.I.B. 2006. *Farm Guide - 2006*. Farm Information Bureau, Government of Kerala, Thiruvananthapuram

Fletcher, T.B. 1916. Agricultural Entomology. Reprint from Ann. Rept. Bd. Scientific advice for India. 1914-1915. *Calcutta Econ. Zool.* pp.1-15

*Forlow, J.L. and Henneberry, T.J. 1997. Pink bollworm larval mortality following application of *Steinernema riobravis* entomopathogenic nematodes in cotton furrow irrigation. *Proc. Beltwide Cotton.* Jan. 6-10, 1997. New Orleans, LA, USA. *Abstact*: 1192-1194

Gaugler, R. and Kaya, H.K. 1990. *Entomopathogenic nematodes in biological control.* CRC Press, Boca Raton, FL, USA. 365p.

Gazit, Y., Rossler, Y. and Glazer, I. 2000. Evaluation of entomopathogenic nematodes for the control of Mediterranean fruit fly (Diptera: Tephritidae). *Biocontrol Sci. and Technol.* 10:157-164

Glaser, R.W. 1932. A pathogenic nematode of the Japaneese beetle. *J. Parasitol.* 18: 119

Glazer, I. and Navon, M. 1990. Activity and persistence of EPN tested against *Heliothis armigera* (Lepidoptera: Noctuidae). *J. Econ. Entomol.* 83: 1795-1800

Gupta, M., Desai, P. and Pawar, A. D. 1987. The feasibility of using DD-136 strain of *Steinernema carpocapsae* for the control of insect pests. *Pl. Protection Bull.* 39: 16-19

Gold, C.S., Kagezi, G.H., Night, G. and Ragama, P.E. 2004. The effects of banana weevil, *Cosmopolites sordidus*, damage on highland banana growth, yield and stand duration in Uganda. *Ann. Appl. Biol.* 145: 1744-7348

Hanula, J.L. 1993. Vertical distribution of black vine weevil (Coleoptera: Curculionidae) immature and infection by entomogenous nematodes in soil columns and field soil. *J. Econ. Entomol.* 86: 340-347

Hoy, J.M. 1954. The biology and host range of *Neoaplectana leucaniae*, a new species of insect parasitic nematode. *Parasitology* 44: 392-399

Hussaini, S.S., Singh, S.P. and Nagesh, M. 2002a. *In vitro* and field evaluation of some indigenous isolates of *Steinernema* and *Heterorhabditis indica* against shoot and fruit borer, *Leucinodes orbanalis. Indian J. Nematol.* 32: 63-65

Hussaini, S.S., Singh, S.P., Parthasarathy, R. and Shakeela, V. 2002b. *In vitro* production of entomopathogenic nematodes in different artificial media. *Indian J. Nematol.* 32: 44-46

Hussaini, S.S. 2005. Mass production of entomopathogenic nematodes. Project Directorate of Biological Control, Bangalore 88p.

Israel, P., Rao, Y.R.V. J., Rao, P.P.S. and Varma, A. 1969. Control of paddy cut worms by DD-136, a parasitic nematode. *Curr. Sci.* 16: 390-391

*Ivanova, T.S., Borovaya, V.P. and Danilov, L.G. 1994. A biological method of controlling the potato moth. *Zashchita-Rastenii-Moskva* 2: 39

Jansson, R.K., Lecrone, S.H., Gaugler, R.R. and Smart, G.C. Jr. 1989. Potential of entomopathogenic nematodes as biological control agents of sweetpotato weevil (Coleoptera: Curculionidae). *J. Econ. Entomol.* 83: 1818-1826

Jansson, R.K., Gaugler, R.R., Mannion, C.M., Lecrone, S.H. and Pavis, C. 1991. Recent advances in biological control of *Cylas formicarius* with entomopathogenic nematodes. *Proc. of the Caribbean meetings on biological control.* 5-7 Nov.,1991. Guadeloupe, pp.167-182

Jansson, R.K. and Lecrone, S.H. 1997. Effect of sweet potato cultivar on efficacy and persistence of entomopathogenic nematodes for control of *Cylas formicarius. Nematropica* 27: 41-52

Jayasree, T.V. 1992. Biology of banana pseudostem weevil MSc. (Ag.) Thesis, Kerala Agricultural University, Thrissur

Jaworska, M., Simoes, N., Boemare, N. and Ehlers,.R.U. 1998. Susceptibility of carrot fly *Psila rosae* F. to entomopathogenic nematodes. *Proc. Symp. Pathogenicity of entomopathogenic nematodes versus insect defence mechanisms: impact on selection of virulent strains, March 17 - 20, 1996.*Universidade dos Acres, Ponta Delgada, Acores, Portugal, pp. 267-270

Josephrajkumar, A. and Sivakumar, C.V. 1997. A survey for entomopathogenic nematodes in Kanyakumari district, Tamil Nadu, India. *Indian J. Entomol.* 59: 45-50

Josephrajkumar, A., Devi, A.D., Murugan, M. and Vasanthakumar, K. 2005. Entomopathogenic nematodes - mass production and application in cardamom root grub, *Basilepta fulvicornre* Jacoby management. *Indian J. Arecanut, Spices and Medicinal plants* 7: 54-59

Kakouli, D.T., Labuschagne, L. and Hague, N.G.M. 1997. Biological control of the black vine weevil *Otiorhynchus sulcatus* (Coleoptera: Curculionidae) with entomopathogenic nematodes (Nematoda: Rhabditida). *Ann. Appl. Biol.* 131: 11-27

Karunakar, G., David, H. and Easwaramoorthy, S. 1992. Influence of dosage of *Steinernema carpocapsae*, *S. glaseri* and *Heterorhabditis indica* on mortality of the host and multiplication of infective juveniles in sugarcane internode borer *Chilo sacchariphagus indicus. J. Biol. Control* 6: 26-28

Karunakar, G., Easwaramoorthy, S. and David, H. 2000. Pathogenicity of Steinernematid and Heterorhabditid nematodes in white grubs infesting sugar cane in India. *Int. J. Nematol.* 10: 19-26

Kaushal, K.K., Rawat, R., Naved, S.H. and Siyanand, N. 2000. Survey of entomopathogenic nematodes in India. *Ann. Pl. protection Sci.* 8: 119-121

Kaya, H.K. and Gaugler, R. 1993. Entomopathogenic nematodes. *Ann. Rev. Entomol.* 38: 181-206

Kung, K.S. 1955. The bananas stem borer weevil *Odoiporus longicollis* Oliv. in Taiwan. *J. Agric. Taiwan* 4: 80-113

Kurian, C. 1967. Progress Report- Central Coconut Research Station, Kayangulam, Kerala 101p.

Mannion, C.M. and Jansson, R.K. 1992. Comparison of ten entomopathogenic nematodes for control of sweetpotato weevil (Coleoptera: Apionidae). *J. Econ. Entomol.* 85: 1642-1650.

Mannion, C.M. and Jansson, R.K. 1993. Within-root mortality of *Cylas formicarius* (Coleoptera: Apionidae) by entomopathogenic nematodes. *J. Econ. Entomol.* 86: 722-729

McCoy, C.W., Shapiro, D.I., Duncan, L.W. and Nguyen, K. 2000. Entomopathogenic nematodes and other natural enemies as mortality factors for larvae of *Diaprepes abbreviatus* (Coleoptera: Curculionidae). *Biol. Control* 19: 182-190

*Miduturi, J.S., Clercq, R., Grisse, A. 1994. Greenhouse and field control of black vine weevil *Otiorhynchus sulcatus* F. with *Steinernema carpocapsae* and *Heterorhabditis* sp. *Bull.OILB-SROP.* 7: 148-154

*Milstead, J.E. and Poinar, G.O. 1978. A new entomogenous nematode for pest management systems. *Calif. Agric.* 32(3): 12

Mohandas, C. and Sheela, M.S. 2002. Isolation of Entomopathogenic Nematodes and their pathogenicity on insect pests. *Natl. Symp. Bio Control of Insect Pests.* 7-8 Feb., *Abstract* p.80

Mohandas, C., Rajamma, P. and Sheela, M.S. 2005. *Rhabditis* (osheius)sp. (Nematoda:Rhabditis) a new pathogenic nematodes of crop pests. *In Green pesticides for insect pest management.* pp.200-202

Mracek, Z. and Webster, J.M. 1994. Survey of Heterorhabditidae and Steinernematidae (Rhabditida, Nematoda) in Western Canada. *J. Nematol.* 25: 710-717

Narayanan, K. and Gopalakrishnan, C. 1987. Effect of entomogenous nematode *Steinernema feltiae* (Rhabditida: Steinernematidae) to the pre pupa, pupa and adult of *Spodoptera litura* (Noctuidae: Lepidoptera). *Indian J. Nematol.* 17: 273-276

Narkhedkar, G.N., Lavhe, N.V. and Mayee, C.D. 2001. Entomopathogenic nematodes- Potential agent against American boll worm of cotton *Helicoverpa armigera SAIC Newsl.* 11: 4

Padmanabhan, B., Sundararaju,P., Cannayane,I. and Hussaini, S.S. 2002. Effect of entomopathogenic nematode, *Heterorhabditis indica* (PDBCEN 13.3) on banana stem weevil, *Odoiporus longicollis in vitro. Indian J. Nematol.* 32: 207

Panse, V.G. and Suhatme, P.V. 1985. *Statistical methods for agricultural workers*. Fourth Edition. Indian Council of Agricultural Research, New Delhi, 381p.

*Pardede, D., Djamin, A. and Utomo, C. 1992. Nematode *Heterorhabditis* sp. (Rhabditida: Heterorhabditidae) as a natural enemy of *Oryctes rhinoceros* L. (Coleoptera: Scarabaeidae) *Bull. Perkebunan* 23: 167-176

*Parkman, J.P. and Smart, G.C. 1996. Entomopathogenic nematodes, a case study: Introduction of *Steinernema Scapterisci* in Florida. *Biocontrol Sci. and Tecnnol.* 6: 413-419

PDBC. 1998. *Survey, identification and utilization of Entomopathogenic nematodes against some lepidopterous and coleopterous insect pests.* Annual Report, Project Directorate of Biological Control, Bangalore 203p.

PDBC. 2001. *Survey, identification and utilization of entomopathogenic nematodes against some lepidopterous and coleopterous insect pests.* Annual Report, Project Directorate of Biological Control, Bangalore, India, 209p.

*Pena, J.E., Duncan, R., Martin, R. and Gold, C.S. 1993. Biological control of *Cosmopolites sordidus* in Florida. *Proc.on A Res. Coordination Meeting, Biological and integrated control of Highland banana and plantain pests and diseases*.12-14 Nov Cotonou, Benin, pp.124-139

Prasad, J.S., Gururaj, K., Padmakumari, A.P and Sankar, M. 2006. Bioefficacy of indigenous entomopathogenic nematodes against leaf folder, *C. medinalis* in rice. *Second Int. rice congress* 9-13 Oct. 2006. Indian Agricultural Research Institute, New Delhi. *Abstract*: p.418

Ponniah, T. 1997. *Mass multiplication of entomopathogenic nematodes Steinernema glaseri and Heterorhabditis indica.* MSc. (Ag) thesis, Tamil Nadu Agricultural University, Coimbatore, 140p.

Rahaman, P.F., Sharma, S.B. and Wightman, J.A. 2000. A review of insect parasitic nematodes research in India, 1927-1997. *Int. J. pest mgmt.* 46: 19-28

Rajkumar, M., Parihar,A. and Siddiqui, A.U. 2002. Effect of entomopathogenic nematode, *Heterorhabditis* sp. against tobacco caterpillar, *Spodoptera litura Proc. Nat. Symp. Biodiversity and management of nematodes in cropping systems for sustainable agriculture.* 15-18 Feb,2002. ARS, Durgapura, Jaipur, p.77

Rao, V. P. and Manjunath, T.M. 1966. DD-136 nematode that can kill many insect pests. *Indian Fmg.* 16: 43-44

Rao, Y.R., Prakash V.J., Rao, P.S., Verma, A. and Israel, P. 1971. Tests with an insect parasitic nematode DD-136 against the rice stem borer, *Tryporyza incertulas. Indian J. Entomol.* 33: 215- 217

Rinker, D.L., Olthof, T.H.A., Dano, J. and Alm, G. 1995. Effects of entomopathogenic nematodes on control of a mushroom infesting sciarid fly and on mushroom production. *Biocontrol Sci. and Technol.* 5: 109-119

*Rodriguez, I., Rodriguez, M.G. Sanchez, L. and Angeles, M.M. 1997. Effectivity of *Heterorhabditis bacteriophora* (Rhabditidae : Heterorhabditidae) against coffee mealy bugs (Homoptera : Pseudococcidae). *Revista de protection- Vegetal* 12(2): 119-122

*Rosales, A.L.C. and Suarez, H.Z. 1998. Entomopathogenic nematodes as possible control agents of the banana root borer weevil *Cosmopolites sordidus* (Germar)(Coleoptera: Curculionidae). *Boletin-de-Entomologia-Venezolana,-Serie-Monografias.* 13: 123-140

Saunders, M.C. and All, J.N. 1982. Laboratory extraction methods and field detection of entomophilic rhabditoid nematodes from soil. *J. Environ. Entomol.* 11: 1164-1165

Scheepmaker, J.W.A., Geels, F.P., Griensven, L.J., Van, L.D., Smits, P.H. and Griensven, L.J.L.D. 1998. Susceptibility of larvae of the mushroom fly *Megaselia halterata* to the entomopathogenic nematode *Steinernema feltiae* in bioassays. *Bio Control* 43: 201-214

*Schirocki, A. and Hague, N.G.M. 1997.Evaluation of UK heterorhabditids and steinernematids for the control of the black vine weevil *Otiorhynchus sulcatus*. *Tests-of-Agrochemicals-and-Cultivars.* 18: 46-47

Schroeder, W.J. 1990. Entomopathogenic nematodes for root weevil control in Citrus. Proc. *fifth Int. Colloquium Invertebrate Pathol. and Microbial Control,* 20-24 August1990. Adelaide, Australia. *Abstracts*: 267

Schroeder, W.J. 1992. Entomopathogenic nematodes for control of root weevils of citrus. *Florida-Entomologist*. 75: 563-567

Schroeder, P.C., Ferguson, C.S., Shalton, A.M., Wilsey, W.T. Hoffmann, M.P. and Petzoldt, C. 1996. Green house and field evaluations of entomopathogenic nematodes (Nematoda: Heterorhabditidae and Steinernematidae) for control of cabbage maggot (Diptera: Anthomyiidae) on cabbage. *J. Econ. Entomol.* 89: 1109-1115

Sheela, M.S., Mohandas, C., Arunrajkumar, K.R., Nisha, M.S. 2002. Potential of entomopathogenic nematodes for the management of lepidopteran pests. *Proc. XII Swadeshi Sci. Cong.* pp. 85-87

Sheela, M.S., Mohandas, C., Nisha, M.S. and Manu, C.R. 2002a. Potential of *Rhabditis* sp. for the management of pests of bitter gourd, *Momordica charantia* L. In Green pesticides for insect pest management pp. 203-205

Sheela, M.S., Mohandas, C., Nalinakumari, T. and Susmin S.C. 2006. Potential of entomopathogenic nematodes in rice BIMP strategy.

Second Int. rice congress, 9-13 Oct. 2006. Indian Agricultural Research Institute, New Delhi. *Abstract:* 1361 415-416

Simser, D. and Roberts, S. 1994. Suppression of strawberry root weevil *Otiorhynchus ovatus*, in cranberries by entomopathogenic nematodes (Nematoda: Steinernematidae and Heterorhabditidae *Nematologica* 40: 456-462

Singh, J. and Bardhan, V. 1974. Effectiveness of DD-136, an entomophilic nematode against insect pests of agricultural importance. *Curr. Sci.* 43: 662

Singh, V. and Yadava, C.P.S. 2002. Mass production technique of entomopathogenic nematode, Heterorhabditis bacteriophora on greater wax moth, *Galleria mellonella* for insect control. *Proceedings Nat. Symp. Biodiversity and management of nematodes in cropping systems for sustainable agriculture,* 15-18 Feb. ARS, Durgapura, Jaipur, p.80

Smith, D. 1995, Banana weevil borer control in south-eastern Queensland. *Australian J. Experimental Agri.* 35: 1165-1172

Sosamma, V.K. 2003. Current Status of Research on Entomopathogenic nematodes in India. *Utilization of Entomopathogenic Nematodes in plantation crops* (eds. Hussaini, S.S., Rabindra, R.J. and Nagesh, M.) Project Directorate of Biological Control, Bangalore pp.109-120

Sosamma, V.K., Jaijo, M.J. and Rasmi, B. 2000. Occurrence of entomogenous nematodes and entomopathogenic fungus, *Metarhizium flavoviride* associated with rhinoceros beetle, a pest of coconut. *Nat. Nematol. Symp. Integrated Nematode Management for Sustainable Agriculture in the changing Agro ecological and Economic Scenario in the New Millenium*, Nov 23-24, 2000, Bhuvaneswar, Nematological Society of India. pp. 188-191

Southey, J.F. 1970. Laboratory methods for work with plant and soil nematodes. Ministry of Agriculture, Fisheries and Food. *Technical Bull. 2*. London : Her Majesty's Stationery Office

Spaull, V.W. 1988. A preliminary evaluation of entomogenous nematodes to control the African sugarcane stalk borer *Eldana saccharina* (Lepidoptera: Pyralidae). *Proc. the Sixty-second Annual Congress of the South African Sugar Technologists' Association,* 6 - 9 June. Durban and Mount Edgecombe, pp.123 24

Srinivas, P.R. and Prasad, J.S. 1991. Record of DD-136 nematode infection of rice leaf folder, *Cnaphalocrosis* medinalis. *Indian J. Agri. Sci.* 61: 348-349

*Tacconi, R., Boselli, M., Curto, G.M. and Santi, R. 1998. Entomopathogenic nematodes against the sugarbeet weevil (*Temnorhinus mendicus* Gyll. (Coleoptera, Curculionidae) *Notiziario-sulla-Protezione-delle-Piante* 8: 213-218

Tahir, H.I., Otto, A.A. and Hague, N.G.M. 1995. The susceptibility of *Helicoverpa armigera* and Ereas vitella larval to EPN. *Afro Asian J. Nematol.* 5: 161-165

*Tol, R.W.H.M-van and Van-R.W.H.M. 1993. Control of the black vine weevil (*Otiorhynchus sulcatus*) with different isolates of *Heterorhabditis* sp. and *Metarhizium anisopliae* in nursery stock. *Proc. on Section Experimental and Applied Entomology, Nov. 12-15, 1993.* Netherlands Entomological Society, Netherland, 181-186

*Treverow, N., Bedding, R.A., Dettmann, E.B. and Maddok, C. 1991. Evaluation of entomopathogenic nematodes for control of *Cosmopolites sordidus* Ger.(Coleoptera:Curculionidae) a pest of banana in Australia. *Ann. Appl.Biol.* 119: 139-145

Umamaheswari, R., Sivakumar, M. and Subramanian, S. 2005. Bioefficacy of native entomopathogenic nematodes against *Helicoverpa armigera*

Hub. in Tamil Nadu. In: *Biotechnological management of nematode pests and scope of entomopathogenic nematodes* (eds. Sithanantham, S., Vasanthraj, B.D. and Selvaraj,P.). Sun Agro Biotech Research Centre Publication, Chennai, pp.175-177

Visalakshi, A. Nair, G.M., Beevi, S.N. and Amma, A.M.K. 1989. Occurrence of *Odoiporus longicollis* Oliv. (Coleoptera: Curculionidae) as a pest of banana in Kerala. *Entomon* 14(3): 367-368

Vyas, R.V. and Yadav, D.N. 1992. Infectivity of entomopathogenic nematode to the two soils dwelling lepidopterous. *Ann.Biol.* 8: 59-63

Vyas, R.V., Yadav, P. Chaudhary, R. and Patel, N.S.1998. Natural incidence of entomopathogenic nematodes in central North Gujarat. *Proc. Nat. Symp. on Rational approaches in nematode mgmt. for sustainable agriculture, Nov. 5- 7,1998.* (eds. Dhawan, S.C. and Kaushal, K.K.). Gujrat Agricultural University, Anand, Gujarat, India, pp.86-88

Vyas, R.V., Patel, N.B., Patel, P. and Patel, D. J. 2002. Efficacy of entomopathogenic nematodes against *Helicoverpa armigera* on pigeon pea. *International chickpea and pigeon pea Newsl.* 9: 43-44

Wilson, M., Nitzsche, P. and Shearer, P.W. 1999. Entomopathogenic nematodes to control black vine weevil (Coleoptera: Curculionidae) on strawberry. *J. Econ. Entomol.* 92: 651-657

Woodring, J.L. and Kaya, H.K. (1988). Steinernematid and Heterorhabditid Nematodes: *A Hand Book of Techniques* Southern Cooperative Series Bulletin 331. Arkansas Agricultural Experiment Station, Fayetteville, USA, 30p.

*Yang, P., Xin, L.N. and Ying, L.J. 1999. Study on application of entomopathogenic nematodes to control the diamond back moth *(Plutella xylostella). Natural Enemies of Insect* 21: 107- 112

*Yoshida, M., Reid, A.P., Briscoe, B.R. and Hominick, W.M. 1998. Survey of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) in Japan. *Fundamental and Appl. Nematol.* 21: 185-198

---

*Original not seen

POTENTIAL OF ENTOMOPATHOGENIC NEMATODES FOR THE MANAGEMENT OF WEEVIL PESTS OF BANANA (*Musa* SP.)

REMYA, K.R.

Abstract of the
thesis submitted in partial fulfilment of the requirement
for the degree of

Master of Science in Agriculture

Faculty of Agriculture
Kerala Agricultural University, Thrissur

2007

Department of Agricultural Entomology
COLLEGE OF AGRICULTURE
VELLAYANI, THIRUVANANTHAPURAM-695 522.

ABSTRACT

A study was taken up, to isolate native entomopathogenic nematodes from banana rhizosphere, to standardize artificial media for the wax moth *Galleria mellonella* L., the trap insect of EPN, to identify cheap medium for the mass production of native EPN, to establish the potential of native isolates of EPN in comparison with the standard cultures by screening and to assess the potential of native isolates for the management of two major weevil pests of banana *viz.* , pseudostem weevil *Odoiporus longicollis* Oliv. and rhizome weevil *Cosmopolites sordidus* Ger. in the laboratory of Department of Agricultural Entomology, College of Agriculture, Vellayani during 2005-06.

Native isolates of EPN were collected from banana rhizosphere in six blocks of the Instructional Farm, Vellayani covering an area of 75 ha. Out of the hundred samples processed, ten native isolates were obtained. Based on the mortality of grubs of pseudostem weevil in the laboratory, the native isolates N1 and N2 were selected for further studies.

The *G. mellonella* larvae were fed with standard medium along with cheap media *viz.*, M1, M2, M3 and M4. The result revealed that among the cheap media, maximum survival of larvae was recorded in M1 medium. The cost of preparation of standard medium used for the rearing was high as compared to other three media. The percentage reduction of cost of ingredients of M1, M2 and M3 media from the standard medium was 21.29, 34.87 and 21.67 respectively. Among the three media, minimum reduction in cost of ingredient was computed for M2 medium. But considering the number of larvae survived and mass multiplication rate M1 medium was promising.

For mass multiplication of native EPN, the rate of multiplication of infective juveniles was assessed in *G. mellonella* reared in different

cheap media along with standard medium. The result showed that all the EPN (*H. indica, S. glaseri,* N1 and N2) reared in standard medium recorded maximum emergence of infective juveniles.

The studies on the rate of multiplication of native isolates along with standards revealed that initial inoculum of 10 infective juveniles per *G. mellonella* larva recorded maximum emergence of infective juveniles of all EPN except N2. But the native isolate N2 required an initial inoculum level of 25 IJ per *G. mellonella* larva for maximum emergence of infective juveniles of EPN. The results of the study revealed that maximum emergence of EPN including native isolates were recorded at 20 days after inoculation rather than 15, 10 and 7 DAI.

In order to establish the potential of native isolates on the mortality of grubs and adults of pseudostem and rhizome weevil, trials were conducted *in vitro* condition and using infested pseudostem and banana rhizome in the laboratory condition. Maximum mortality of grubs and adults of the pseudostem and rhizome weevils were recorded at 72 hrs after treatment.

Under *in vitro* conditions the inoculum required for getting maximum mortality of grubs of pseudostem weevil was 100 IJ per grub, while it was 200 IJ per grub in rhizome weevil. In the case of adult weevils, the inoculum level required to get maximum mortality was 200 IJ per adult in both the cases. But inside the pseudostem and banana rhizome both the grubs of pseudostem and rhizome weevils recorded maximum mortality with 100 IJ per grub.

From this investigation it was evident that native EPN strains are available in different agro ecosystems and are more virulent than the standard ones. The rearing media for trap insect, *G. mellonella* was modified with cheap ingredients and there by reducing the cost of production to 21.29 per cent with out affecting the nutritional status and survival of different instars. Comparative efficacy of native isolates (N1

and N2) and standards were worked out for the management of grubs and adults of weevil pests of banana. An inoculum level of 100 IJ per insect recorded a mortality of above 80 per cent in vitro condition in rhizome and pseudostem weevils but it was reduced to 52 to 56 per cent inside the rhizome and pseudostem. Thus from these studies, mortality of grubs and adults of the weevils inside the banana pseudostem and rhizome required a minimum inoculum level of 200 IJ per insect.