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# Occurrence of entomogenous nematodes and an entomopathogenic fungus, *Metarhizium flavoviride* associated with Rhinoceros beetle, a pest of coconut.

V. K. Sosamma, Jaijo M. Jose\* and B. Rasmi

Nematology Laboratory, Central Plantation Crops Research Institute,  
Regional Station, Kayangulam, Krishnapuram P. O. 690 533, Kerala, India.

**Abstract :** The role of microbial control in plant protection has expanded considerably with the discovery and development of new microbial control agents. Entomopathogenic nematodes belonging to families Heterorhabditidae and Steinernematidae expands options for control of insect pests especially those with soil inhabiting stages. Fungi continues to offer control options using entomopathogens against insects. Survey on micro organisms (nematodes, bacteria and fungi) associated with Rhinoceros beetle, *Oryctes rhinoceros* L. (Scarabaeidae : Coleoptera), a pest of coconut, *Cocos nucifera* L. and natural distribution of entomophilic nematodes occurring in soil collected from different Districts in Kerala was carried out. Entomophilic nematodes belonging to *Rhabditis* sp. and an unidentified nematode were isolated from *Oryctes* grubs and pupae. For extraction of entomophilic nematodes baiting was done using last instar *Corcyra cephalonica* Staint./*Galleria melonella* L. (Galleridae : Lepidoptera) larvae. The nematodes isolated were multiplied on *G. melonella* in modified White traps. Nematodes isolated and identified were *Heterorhabditis indicus* (28%), *Rhabditis* sp. (58%) and *Aphelenchus* sp. (2%). *Metarhizium flavoviride* Gams and Rozsypal, an entomogenous fungus was isolated from dead *Oryctes* from a cow dung pit out of 42 samples collected. The other associated fungi were *Aspergillus* sp and *Penicillium* sp. Fungal isolations were made using PDA. This is the first report of *M. flavoviride* on rhinoceros beetle. *Eridersia khallikottosus*, an earth worm could also be isolated from 40% of the insect cadavers used for baiting EPN. The work was undertaken with a view to identify promising bio control agents and to promote their use for the sustainable bio management of pests of coconut.

**Key words :** *Cocos nucifera*, *Oryctes rhinoceros*, entomophilic nematodes, earthworm.

## Introduction.

Microbial control of insect pests employing bacteria, fungi and nematodes is a fast expanding field with the development of new potential bio-control agents. Entomogenous fungi cause natural infection among many insect and they combine the attributes of host specificity, proven safety and direct penetration through insect cuticle. Entomopathogenic nematodes (EPN) belonging to the genera *Heterorhabditis* and *Steinernema* are ideal bio-control agents due to their broad host range, high virulence, safety to non-target organisms, ability to search for hosts, high efficiency in favourable habits, high reproductive potential, ease of production and application and combatibility with other control agents (Lacey and Goettel, 1995).

The rhinoceros beetle, *Oryctes rhinoceros* L is one of the major pests of coconut, *Cocos nucifera* L. The adult beetle causes damage, burrows and remains between leaf sheath near the crown and thus cuts the leaf in folded stage and causes 10% reduction in annual yield (Nirula, 1956). The present paper deals with the natural occurrence of entomogenous nematodes and fungi on various stages of *Oryctes* collected from fields and occurrence of entomophilic nematodes in soil.

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\*Cochin Export Processing Zone,  
No. 349, 7<sup>th</sup> Main, Vivek Nagar,  
Bangalore- 560 047.

## Materials and Methods.

*Oryctes* larvae, pupae and adults were collected from cow dung pits from areas near CPCRI, Kayangulam. Dead insects collected were kept in moist chambers for 3- 5 days. Isolations were made from dead beetle with fungal infection by PDA plating. The dead grubs and pupae were dissected to detect the presence of nematodes after washing with water and 0.1% formalin.

Soil samples were collected from root zone of coconut palms, both healthy and those attacked by *Oryctes*. Each composite sample consisting of three random sub samples collected 1m away from the bole of the palm from a 15cm deep stratum was mixed thoroughly and taken in polythelene bags. The EPN present in soil was baited using last instar *Corcyra cephalonica* Staint/ *Galleria melonella* L. (Galleridae : Lepidoptera) larvae. For this 5 – 7 live *Corcyra/ Galleria* were placed at the bottom of 500ml plastic jars and the soil was lightly packed into it. A little moistening was done and kept for 5 – 7 days at 25°C in the laboratory. After 5 days the insect cadavers were taken out, washed in distilled water and 0.1% formalin and then kept in moist chambers. After 3 days they were examined under stereoscopic binocular microscope. The nematodes were picked up and multiplied on last instar *Corcyra/Galleria* larvae in White traps. (White, 1927). It consists of an inverted watch glass placed at the bottom of 9cm petri plate and over which a moistened 9 cm Whatman No:1 filter paper is placed. The infected larvae were placed at the periphery of the trap. The nematodes multiplied were collected and transferred to sterile 100ml conical flasks and aerated once in two days.

## Results and Discussions.

The fungus isolated from dead beetles was identified as the entomopathogenic hyphomycete, *Metarhizium flavoviride*\* Gams and Rozsypal. This is the first report of this fungus from *Oryctes*. Other fungi isolated were *M. anisopliae*, *Aspergillus* sp. and *Penicillium* sp. Earlier *M. anisopliae* was reported from *Oryctes* and was found pathogenic to it (Prior and Arura, 1984). *Metarhizium* species was frequently isolated from insects in research conducted on fungal micro organisms associated with mycosis of phytophagous insects collected from different Sicilian environments (Burruano et. al., 1999). *M. flavoviride* was found to be highly infective (> 86% mortality) to locusts and the grass hopper *Schistocerca pallens* (Xavier et. al., 1999 and Caudwell and Gatehouse 1996). This fungus was found to be most resistant to irradiation by stimulated sunlight than *Beauveria bassiana* and *M. anisopliae* (Fargues et. al., 1996).

On dissection of dead grubs and pupae, it was found that 70% of them carried the entomophilic nematode *Rhabditis* sp. in their haemocoel. In addition an unidentified nematode could also be isolated. There was a report of *Rhabditis* sp. from the larvae of Rhinoceros beetle on west coast of Ceylon (Surany 1960).

\* Indian type culture collection No. 4027.2K by Dr. P. N. Choudary, IARI, New-Delhi.

Nematodes isolated from soil samples included were *Heterorhabditis indicus* (28%), *Rhabditis* sp. (58%) and *Aphelenchus* sp. (2%). The soil types covered included laterite, alluvial, sandy loam and clay. (Tables 2 and 3).

Along with the nematodes 40% of the soil samples yielded an earthworm, *Erioderesia khallikottosus*. (identified by A. V. Thampi). This is an indication of the harmlessness of EPN to non target organisms (Visa Nuutinen et. al.,1991). This earth worm was obtained from 5-7 days old dead insect cadavers (*Corcyra/ Galleria*) used for baiting EPN .

**Table. 1** Microbes isolated from various stages of *Oryctes rhinoceros*.

Stage	Nematodes	Fungi
Adult	<i>Rhabditis</i> sp.	<i>M. flavoviride</i> <i>M. anisopliae</i> <i>Aspergillus</i> sp. <i>Penicillium</i> sp.
Pupae	<i>Rhabditis</i> sp.	<i>M. anisopliae</i> <i>Aspergillus</i> sp. <i>Penicillium</i> sp
Larvae	<i>Rhabditis</i> sp. Unidentified	-

**Table 2. Distribution of entomophilic nematodes isolated from soil from different Districts in Kerala.**

District	No. of samples collected	No. of samples positive for EPN			
		<i>Rhabditis</i>	<i>H. indicus</i>	<i>Aphelenchus</i>	Total
Alappuzha	180	126	36	6	168
Kottayam	20	8	4	-	12
Palakkad	59	20	12	-	32
Trivandrum	10	4	2	-	6
Trissur	31	16	10	-	26
Total	300	174	64	6	244

**Table 3 Distribution of EPN in different soil types.**

Soil Type	No. of samples collected	No. of samples positive for <i>Heterorhabditis</i>
Laterite	90	24
Alluvial	12	2
Sandy loam	175	36
Clayey	23	2

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