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Isolation of indigenous strains of entomopathogenic fungi from rice ecosystems using mycosed cadavers and galleria bait method

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Key Words : Indigenous strains, Entomopathogenic fungi, Rice ecosystems, Using mycosed, Cadavers, Galleria bait method

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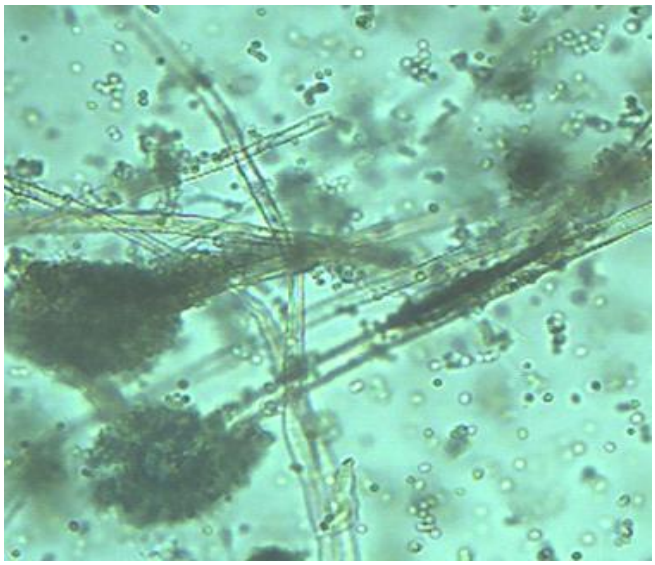
Globally, many technologies have been developed for managing the most noxious pests - the insects that limit agricultural production, of which, many have been forsaken. Today, research converges to biological control by virtue of the effectiveness of the bio agents and their safety to non-targets and environment. The naturally existing and disease causing microbes of insects are applauded as important biocontrolagents world over and research is spinning ahead for exploiting their potential. Soil and insect cadavers present in natural ecosystems are rich sources of entomopathogenic fungi (Ignoffo *et al.*, 1978). The present report focused on the isolation and identification of indigenous strains of entomopathogenic fungi from rice ecosystem.

Periodic surveys were conducted at monthly intervals in thirty rice fields for a period of one year for collecting entomopathogenic fungi from rice ecosystem. Two different methods were followed for the collection of mycosed insects. In the first method, dead insects were collected directly from the rice ecosystem. Insect cadavers collected were brought to laboratory for isolation of fungi, if any. In the second method, sweep net collection of insects were taken and live insects were brought to laboratory, reared and observed regularly for the development of symptoms of fungal infection, if any.

The cadavers collected were kept separately in nine cm Petri plates with moistened filter papers for development of fungal growth. Those cadavers with fungal growth were then surface sterilized for one min in 0.1 per cent mercuric chloride and this was followed by three repeated washings in sterile water under aseptic conditions in a laminar air flow chamber (Hareedranath, 1989). After wards, the specimens were dried by keeping it in sterilized filter paper for two min. The cadavers were then placed in Petri plates with potato dextrose Agar (PDA) for development of mycelia. After the mycelial development, sub culturing was done for thrice, transferred to PDA slants and purified by hyphal tip method (Cloh, 1999). The fungi thus obtained were maintained in PDA slants

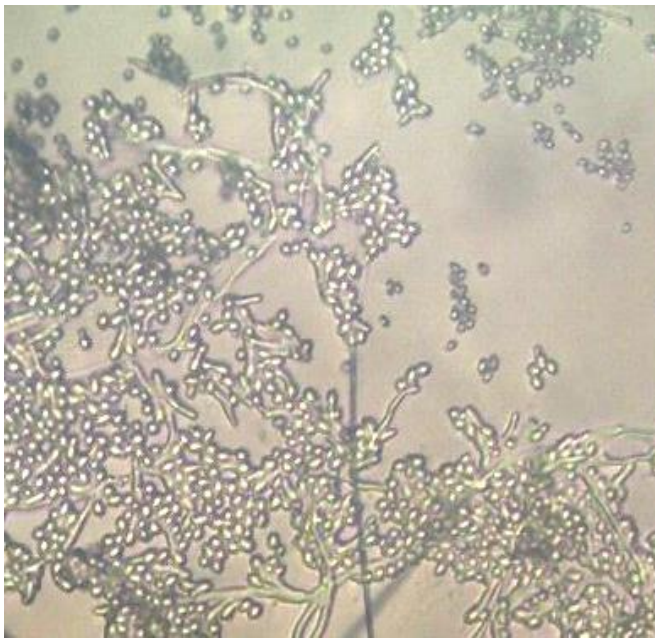
under refrigerated conditions for further studies. Virulence of the fungi was maintained by periodically passing them through adult of rice bug, *Leptocorisaacuta* Thunb.

Soil samples were collected from rice fields of Vellayani, Karamana and Anadu of Thiruvananthapuram district. From each location 10 samples, each weighing 200 g were randomly collected at a depth of 20 cm using an auger (Hasan *et al.*, 2012). The Galleria bait method (Meyling, 2007), that was slightly modified was used to trap entomopathogenic fungi from soil. Eggs of *G. mellonella*, obtained from Department of Agrl. Entomology, College of Agriculture,Vellayani were kept in plastic boxes of dimensions 18 cm x 12 cm. The first instar larvae that emerged were fed with the diet on alternate days until pupation in the rearing containers. The pupae were collected carefully and transferred to

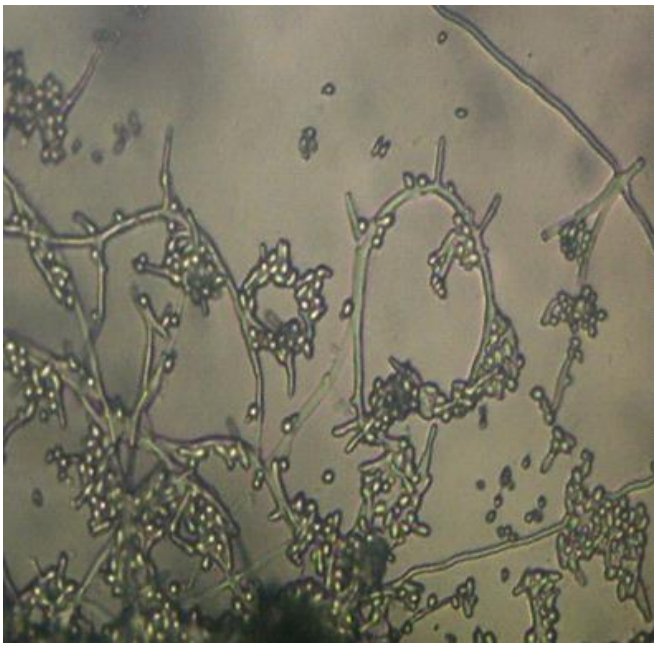


(a) Af-m1

(b) Ma-m1



(c) Bb-m2



(d) Bb-m5

Plate 1 : Photo micro graphs of isolates

plastic boxes of 18 cm x 14 cm for adult emergence. Cotton soaked in 10 per cent honey solution was placed in the bottom of bottles as feed for adults. Folded paper strips (15 cm x 6 cm) were hung inside the bottle containing adults, to facilitate egg laying. Fourth instar larvae obtained from the stock culture were used for trapping fungi from soil.

The soil samples collected were air dried to ward off entomopathogenic nematodes likely to be present in it. The dried samples were then moistened with sterile water @ 10 ml-100 g soil and were taken in medium sized plastic containers of 10 cm x 20 cm. The fourth instar larvae used for trapping entomopathogenic fungi were initially subjected to heat treatment to prevent excessive webbing. For this, they were dipped in hot water at 56°C for one min and afterwards cooled in running water for 30 seconds. The larvae were dried by placing them on a tissue paper and were kept in dark for four h. Subsequently, five larvae were released into each container with 20 g soil sample, and the containers were covered with lids having holes to facilitate aeration. Four replications were maintained for each soil sample. These containers were placed on bricks in a basin with water and covered with towel such that the bottom of the towel got dipped in water in order to maintain sufficient humidity that would favour fungal growth. The containers were examined after 10 days for mycosed larvae. The infected larvae were collected and surface sterilized. The fungus thus isolated from the cadavers was brought to pure culture in PDA slants.

For identification of fungal strains, slide culturing of the fungus was adopted for the morphological studies following the method of Harris (1986) and for molecular identification; Internal Transcribed Spacer (ITS) sequencing was done with the facilities available at Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram.

Aspergillus flavus Link. (Af-m1) was isolated from the cadaver of rice bug, *L. acuta*. Using insect bait method, one isolate of *Metarhizium anisopliae* (Metschnikoff) Sorokin (Ma-m1) and two isolates of *Beauveria bassiana* (Balsamo) Vuillemin (Bb-m2 and Bb-m5 were obtained.

The accession number assigned by National Centre for Biotechnology Information (NCBI) to Af-m1, Ma-m1, Bb- m2and Bb m5 was KP 739825 ,KP 739826, KP 739828 and KP 739831, respectively.

All the isolates tested were pathogenic to larvae of rice leaf roller, *Cnaphalocrocis. medinalis* Guen. and nymphs and adults of rice bug *L. acuta*.

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