The Green Muscardine Fungus *'Metarhizium anisopliae'* as Mycoinsecticide for Control of Rhinoceros Beetle of Coconut Palm

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Introduction

Among the environmentally benevolent pest control agents, the fungi play an important role. More than 700 species of fungi, mostly belonging to order Entomophthorales and class Deuteromycetes from about 90 genera, are pathogenic to insects. Genera that have been most intensively investigated for mycoinsecticides include *Beauveria, Metarhizium, Hirsutella, Verticillium, Paecilomyces, Entomophthora, Nomuraea, Aspergillus, Tolypoclaidium, Aschersonia, Leptolegnia, Culicinomyces, Caelomomyces* and *Legenidium*. The best studied insect pathogens among this groupings are the species belonging to the two genera *Metarhizium* (green muscardine fungus) and *Beauveria* (white muscardine fungus). There are three species of *Metarhizium* known to infect insects, amongst which *M.anisopliae* is the most studied. This species have been identified from about 300 species of Lepidoptera, Coleoptera, Orthoptera, Hymenoptera and Hemiptera, indicating a wide host range. Latch (1965) gave 166 insect species attacked in nature and Veen (1968) listed 204 hosts under their respective orders. But the most preferred hosts are found in the order Coleoptera.

Description of the fungus

Metarhizium mycelium is composed of hyaline, septate, branched hyphae. Conidiophores are short, hyaline, septate, erect, simple or branched, terminating in single or a cluster of phialides. Conidia are single - celled, hyaline, smooth, long-ovoid to cylindrical, abstracted from the tips of the phialides and forming long basipetal chains sometimes columns. The conidial mass is dark green in colour and hence, it is called "Green muscardine" fungus. Based on the spore size. *M.anisopliae* is differentiated into two varieties : *M. anisopliae* var. *anisoplae* (spore size - 5-8μ) and *M.anisopliae* var. *major* (spore size 10-14μ).

History of work on this fungal pathogen

Agostino Barsi (1835), an Italian scientist, was the first to demonstrate that a fungus can cause a disease of insects and he was the one who initially noticed the "Green and white muscardine" fungus which caused disease of silk worms. Metchnikoff (1879) reported 'green muscardine" disease of *Anisoplia austrica,* the wheat cockchafer. *M.anisopliae* was first seen infecting *Oryctes rhinoceros* in Western Samoa by Friederichs in 1913.

In India, Nirula and co-workers (1955) first reported the incidence and conducted detailed experiments of work on control of rhinoceros beetle using the "green muscardine fungus". Field application of the fungus gave successful suppression of the coconut rhinoceros beetle population in South Pacific Island (Catley, 1969; Marschall, 1969). Treatments with *Metarhizium* was also done in Tonga Islands by Young in 1974. Ferron and coworkers (1975) also used the fungal agent to control rhinoceros beetle in the Pacific Islands. Detailed investigations on *M.anisopliae* mass - multiplication on cheap substrates, time and dose of application and field trials have been carried out at this station from 1980 onwards.

Mode of infection of the fungus

The infection cycle begins with attachments of fungal spores to the insect epicuticle, followed by germination of spores, the success of which is apparently determined by prevailing temperature and relative humidity (RH) (27-28^{0}C temperature and 92% RH is optimum). The adhesion of the conidia to the host is probably due to hydrophobic forces exerted by the rodlets covering the conidia. Under conducive micro environment spores germinate and produce germ tubes which with mechanical and enzymatic (proteinases, lipoprotein lipases, chymoelastases, etc.) actions penetrate both the epicuticle (cross-linked protein and lipid) and the inner procuticle (made of chitin and protein). Once inside, the fungal hyphae spreads into the haemocoel and internal tissues leading to the death of the host. The *Metarhizium* produces toxic mycochemicals like destruxins, desmethyl destruxins and swainsonine that arrest the host metabolism. Under suitable environmental conditions (essentially high humidity) death is followed by external sporulation, which helps to spread the fungus and establish an epizootic resulting in very high levels of kill.

Susceptibility of *Oryctes Rhinoceros* to *M. anisopliae*

All the stages of O.rhinoceros are infected by *M.anisopliae.* The first

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instar larva is most sensitive and dies within 10-12 days. IInd, IIIrd instar and pupae take about 2 weeks to die. The adults are relatively less susceptible.

Diagnostic symptoms in the host

The grubs of *O.rhinoceros* infected by fungus become sluggish, lose appetite, and slowly get mummified. Some white powdery fungal colonies can be seen growing at all the integument joints. After 4-7 days these white colonies turn green in colour and finally the whole grub becomes black and decomposed.

Mass multiplication techniques

The fungus can be multiplied in laboratory using various media (Czapek dox agar, potato dextrose agar, etc.), but for multiplying in huge quantity, cheaper substrates and methods of culturing are necessary. Some of the common substrates are agricultural products like grains of different cereals. Till date, the cheapest medium used for mass multiplication was cassava chips mixed with rice bran supplemented with urea or waste fish meal extract as nitrogen source (Mohan & Pillai, 1982). Coconut water, a still cheaper and easily available agricultural waste from copra making industry is also used for mass production of the fungal spores (Danger *et al.*, 1991). The incoculum from cassava chips can be mixed at 1:1 ratio with sterilised cowdung and can be stored for a week at 4^0C. The method for mass production is very simple and it needs few kitchen utensils, coconut water and entomopathogen for multiplication; thus it can be carried out in any farm household.

Field application

The fungus could be applied at the breeding sites of the rhinoceros beetle like the farmyard manure or compost pits. The application is effective during the monsoon season when the temperature is low and relative humidity is high. Further efficiency can be obtained if the "green muscardine" is applied during the moulting time of the grubs.

The fungal spores mixed in ordinary water can be sprayed on the pits or can be mixed with the breeding site materials. About $5x10^{11}$ spores/m^3 are required for successful establishment of the fungus to provide effective destruction or killing of the grubs in manure pits.

Storage

The entomofungal pathogen can be stored as dry mycelium or as blastospores or conidia. The 'pellets' of *M.anisopliae* dried to granules can be packed into vacuum plastic bags and if held at low temperature, remains viable for a relatively longer period. Long-term conidial viability of *M.anisopliae* (upto 3 years) can be obtained on the silica gel crystals at - 20^0C.

Field efficiency and persistence

In laboratory trials, 100% mortality results in the grubs of *O. rhinoceros*, whereas adults (beetles) showed an average of 38% mortality. In field application, the mortality is more than 75% if the pathogen is applied at optimum dose during the favourable conditions (Abad *et al.*, 1992 ; Fernando *et al.*, 1995; Moslim *et al.*, 1999). Once applied , the fungus persists in the breeding sites for at least 2 years as fungal spores are resistant to unfavourable hot conditions.

Safety aspects

Latch (1976) reported that *M.anisopliae* is not pathogenic or toxic to warm blooded animals and mammals. Some *M. anisopliae* isolates produce destruxins which are implicated in insect mortality. But there have been no records of toxicity caused by ingestion of these or any other toxic compounds produced by fungus to vertebrates. Major reviews have suggested mycoinsecticides like *M. anisopliae* present minimal risks to humans, domestic animals and wildlife and non-target invertebrates (Goette *et al.* 1990, Siegel and Shadduck, 1990

How *M. anisopliae* is used ?

(i) Collection of diseased materials
Host specimens showing the symptoms of fungal disease are collected from the breeding sites and brought to laboratory.

(ii) Culturing the fungus : Spores from the dead specimens are cultured in medium (potato dextrose agar) and identified. After identification, *M.anisopliae* is mass cultured.

(iii) Pathogenicity test : The fungus thus cultured is then applied to various stages of the host. Death of the host confirms the pathogenicity and virulence of the fungus. The optimum dosage causing maximum mortality is also ascertained.

(iv) Storage : The fungal culture is stored at 4^0C in refrigerator for further use.

Advantages of using *M.anisopliae*

1. It controls *O.rhinoceros* during the monsoons when no pesticide can be either applied or proved effective.
2. It can be used individually or can be integrated with other methods as a part of Integrated Pest Management (IPM).
3. It is safe to humans and animals.
4. It can be easily mass multiplied in farm household.
5. It is cheaper when compared to other pest protection measures.

Future outlook

1. Collection of new material of the fungus and screening for virulence, specificity and resistance to adverse environmental conditions and agricultural factors such as pesticides, to get better genotypes for field use.

2. Improvement of the already available strains by parasexual recombination or by direct gene manipulation to suit different environmental conditions.
3. Development of pesticide resistant strains for use along with the common pesticides.
4. Refinement of formulation and application technology.

The research into non-chemical methods of pest control has brought us to the stage where the biological potential of entromopathogenic fungi for their use in practical insect control can no longer be ignored. As the problems associated with conventional chemical insecticides are on the increase, the stage is set to look for biotic crop protection products and mycoinsecticides like *Metarhizium anisopliae* hold a bright future to become an essential part of pest control strategies.

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