

# ON FARM PRODUCTION OF GREEN MUSCARDINE FUNGUS TO COMBAT RHINOCEROS BEETLE

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## Introduction

Ever since rhinoceros beetle (*Oryctes rhinoceros* Linnaeus) was reported damaging coconut palms (*Cocos nucifera*) in 1889, it has spread widely in over 37 countries of Africa, Asia, Australia and Pacific Islands (Singh and Rethinam, 2004. *Cocoinfo Int.* 11 (2): 19-24). In Sri Lanka, the damage of rhinoceros beetle is prevalent in all parts of the country. Although it damages palms of all ages, its attack to seedlings and young palms is economically important. According to the recent diagnostic survey conducted by the Coconut Research Institute of Sri Lanka 72% of coconut growers have reported rhinoceros beetle damage in their plantations, but only 52% of them were aware of its severity (Peris *et al.* 2006).

Rhinoceros beetle damage can be identified by geometric cuts on opened leaves, breaking of flag leaf, holes on the petiole bases, and choking of developing leaves and formation of crooked and malformed leaves in seedlings. Severe and repeated damage to seedlings could lead to death.

Use of an integrated management package is recommended for the management of rhinoceros beetle. These include destruction of breeding grounds (cultural method), extraction of beetles using a hook (physical method), and application of used engine oil or coal tar and use of naphthalene balls or carbofuran granules (chemical method). Green muscardine fungus (GMF) *Metarhizium anisopliae*



Rhinoceros beetle damage: Left - Geometric cuts on fronds; Right - A crooked malformed seedling

(Metch.) Sorokin var. *major* and *Oryctes rhinoceros* Virus (OrV) are the key components in biological control. Since use of biological methods are sustainable and low in cost, coconut growers are encouraged to employ these methods.

The Coconut Research Institute, Sri Lanka has developed and introduced a convenient method for on-farm production of GMF, so that growers could produce their own GMF or even generate income by sale of it to others.

## Green Muscardine Fungus (GMF)

GMF (*Metarhizium anisopliae*) is a common soil-borne entomofungal pathogen, which infects was rhinoceros beetles, grubs and pupae of rhinoceros beetle and some other insects, is a widely used entomofungal pathogen ever since it was reported in 1879. Although GMF is known for many years as an entomofungal pathogen of rhinoceros beetle it is less popular and hardly used as a tool of management. The main reason for this is that GMF is not readily available at farmer level. To

overcome this difficulty and to popularize GMF as a management tool of rhinoceros beetle, two simple methods of GMF production, which could be undertaken by the farmers are described.

## Methods of production

1. Production on maize grains
2. Production on rhinoceros beetle

For both methods a local isolate of GMF collected from infected *O. rhinoceros* larva was used. Mother culture of GMF is maintained on Saboraud Dextrose Agar. After every four sub culturing the cultures are renewed by passing through *Oryctes* larvae to maintain high virulence of the fungus.

### 1. Production on maize grains

Materials required:

- a. Dried and broken maize grains (medium size). Maize grains can be crushed either mechanically in a grinder or manually.

- b. Poly propylene bags (gauge 150, size 12"x 6")
- c. Pressure cooker / steamer
- d. GMF Inoculum
- e. Pieces of PVC (1" diameter and 1" length)
- f. Surgical sprite (Isopropyl alcohol)
- g. Cotton wool (absorbent)
- h. Rubber bands
- i. Candles

## Method

### Step 1 - Preparation of bags maize with grains

Wash the crushed maize grains and drain off the excess water. Fill polypropylene bags with 150-200 g of maize grain. Insert the open end of the polypropylene bags in to the PVC ring and turn over. Plug the ring with cotton wool and place a piece of polypropylene over the cotton wool with the aid of rubber band. Air in the bag should remove as much as possible before plugging the cotton wool.

### Step 2 - Sterilization of bags containing maize grains

Two methods can be used



GMF inoculated bags after 2 weeks

depending on the resources of the farmers. Place bags containing maize grains either in a pressure cooker or a steamer and cook, as follows

- a. In a pressure cooker 1 hour and 20 minutes (at the pressure of 15 lbs per inch)
- b. In a steamer for 3 hours

### Step 3 - Inoculation of maize grains

The GMF inoculum (maize grains) need to be provided to the

farmers.

This step should be done on clean table/bench in a clean room. First wipe the table top with surgical sprite (Isopropyl alcohol). Then fix 3 lighted candles on table in half circle to create aseptic working area for about 10-15 minutes before starting the inoculation.

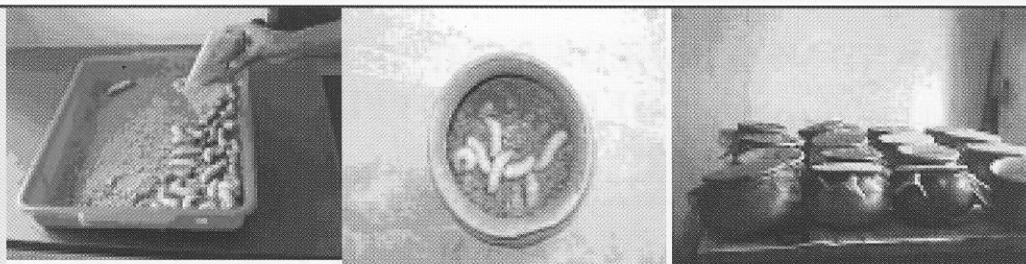
Wash hands with soap and rub with surgical sprite. Wear mask and gloves. Open the GMF inoculum source on the aseptic area. Remove cotton plug of the sterilized maize bag and hold between fingers. Put 1/5 GMF inoculum of (approximately 15 g) in to the maize bags return the plug.

Massage the bags well to mix with GMF spores and maize grain pieces. Keep the inoculated bags in a ventilated dark place for incubation.

After 4-5 days white colour mycelium can see on maize. Massage the bags for second time to contact GMF spore with maize grain. Olive green colour maize grain can be seen within 8-10 days. Shelf life of GMF on maize grains is about one month at 4°C temperature and two weeks at room temperature. However it



Inoculation of maize grains by GMF



GMF production on grubs

should not be exposed to direct sunlight.

## 2. Production on *Oryctes* larvae

### Materials required

- Oryctes* larvae (Collect the *Oryctes* larvae as many as possible)
- GMF inoculum (dried formulation of GMF)
- Medium for breeding larvae such as cow dung, coir dust, saw dust
- Clay pots /containers

### Method

GMF inoculum need be provided to the farmers. The inoculum is produced by air drying the GMF culture of maize grains in the laboratory for 10 days to reduced moisture content 8%. This dry formulation is packed into 100g packets.

Allow *Oryctes* grubs to crawl in small amount of inoculum (depending on the number of grubs) for 20-30 minutes in contact with GMF spores. Transfer infected grubs to clay pots filled breeding medium. The number grubs per pot may depend on the size of the pot. Keep the medium moistened by adding water. Collect only healthy grubs to avoid cross contaminations.

Grubs shows brown colour patches on the skin with in 12-15 days. Subsequently, white colour mycelium appears on the body and later turn to green colour in 20-25 days.

The dead grubs (olive green colour) can be used as inoculum source for re-infect more grubs. One infected grub could be used to infect 5 healthy ones. Allow healthy grubs to contact with infected grubs for about 20

minutes. Transfer to clay pots containing breeding medium. Clay pots should be checked regularly and removed dead grubs due to virus or other reason.

### Application of GMF (maize grins, infected grubs and air dried formula)

GMF could be a applied to the breeding sites such as cow dung heaps, decaying coconut logs, manure heaps, coir dust heaps, and rafter processing site etc. Also it could be used in artificially prepared impregnation pits. It is important that the breeding medium be moist for the persistence of the fungus.

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GMF application in the fields

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