



Farmer friendly production technology of the green muscardine fungus for the management of rhinoceros beetle

Chandrika Mohan, P. Rajan, C.P.R. Nair, Sunny Thomas and P. Anithakumari*

Pest infestation causes breaking of spear leaf and drying of inflorescence. The damaged spear leaf when unfurls exhibit the characteristic 'V' shaped cuts on the lamina. Repeated attack by the pest on young seedlings results in stunted growth or even mortality.

Rhinoceros beetle (*Oryctes rhinoceros*) is a major pest of coconut and oil palm and is ubiquitous in distribution. The adult beetle causes injury to the palms by boring into the spear leaf, young petioles and developing spathes. The beetle feeds on the soft tissues and the chewed up fibrous material protruding through the bore hole is one of the major diagnostic symptoms of infestation on coconut palm. Pest infestation causes breaking of spear leaf and drying of inflorescence. The damaged spear leaf when unfurls exhibit the characteristic 'V' shaped cuts on the lamina. Repeated attack by the pest on young seedlings results in stunted growth or even mortality. Damage on the spathe causes direct crop loss extending up

to 10%. Rhinoceros beetle attack in majority of cases leads to infestation by red palm weevil, fungal infections *etc.* also leading to death of the palms, if untreated.

Accumulation of organic materials like cattle dung, compost, coir dust, decaying coconut logs *etc.* in the gardens serve as suitable breeding grounds for the rhinoceros beetle. Proper and timely removal of decaying organic debris and other farm sanitary measurements are essential to minimise the pest incidence. An Integrated Pest Management (IPM) strategy is recommended to manage this pest. Farm sanitation, prophylactic leaf axil filling with chemical/botanical repellents and biological suppression



GMF grown on rice grains

*Central Plantation Crops Research Institute, Regional Station, Kayamkulam, Krishnapuram -690 533, Kerala



Grubs of rhinoceros beetle infected by GMF

using entomopathogens are the important components in IPM (Rajan *et al.*, 2009).

Among the entomopathogens *Metarhizium anisopliae* (Metsch.) Sorokin commonly termed as the green muscardine fungus (GMF) is one of the most effective and successful biocontrol agents employed against the pest (Nirula *et al.*, 1955, Pillai, 1993). *M. anisopliae* var. *major* (spore size 10-14 μm) is highly infective to larva, pupa and adults of the black beetle and causes green muscardine disease. The fungus gains entry through membraneous joints of the cuticle of the host and multiplies inside the body by extracting nutrients from the tissues and kills the insect. Production of toxins like destruxin and desmethyldestruxin and swainsonine inside the host by the fungus results in the death and mummification of the insect. After the death of the insect, white mycelial mat of the fungus appears externally turning green a few days later because of production of spores.

Controlling the immature stages of the pest in the breeding grounds is less labour oriented than the other

components of IPM. Treatment of breeding sites of rhinoceros beetle with GMF is one of the most ecofriendly and sustainable methods of IPM of this pest. Generally the fungus can be located in the breeding sites of the black beetle, but the population is very meagre for an effective natural biosuppression. Green muscardine fungus (GMF) needs moisture for its growth and multiplies well in the field during low temperature (28-32p C) and high humidity (>80%).

In field demonstrations on IPM of black beetle, farmers rated GMF as one of the most acceptable farmer friendly technologies with its easiness of application and low cost. However, one of the main constraints for adoption of the technology by farmer is the non availability of the material. The production of fungus in the laboratory is costly involving sophisticated equipment. Hence there is a need for developing a farm level production technology of green muscardine fungus. CPCRI has developed production of GMF in liquid media using cheaper substrate (coconut water). Using this method, the fungus was multiplied in flat sided glass bottles. Rhinoceros beetle being an ubiquitous pest of coconut palm throughout the coconut growing countries of the world, several methods were developed for production of GMF on varying substrates. CRI (2007) has developed a methodology for production of GMF with maize grains as substrate. These methods were modified according to needs and a farm level production technology was developed for



Opening the PP bags containing media for inoculation



localised production by farming community targeting the farm women groups, so that in addition to fulfil the needs of IPM in their farm, they could get an additional income.

Production of GMF

Materials needed

1. Grains of rice/wheat or dried tapioca chips
2. Polypropylene bags -12"x6"
3. Pressure cooker
4. Candles
5. Cotton wool
6. Aluminium foil
7. PVC tube pieces (1' diameter, 1' long)
8. Rubber bands
9. Surgical ethanol/rectified spirit/disinfectant solution
10. Disposable rubber gloves
11. Disposable mask
12. Inoculum of *M. anisopliae*

Method

Preparation of media and sterilization

1. Soak the media (grains/tapioca ships) in the water for 30 minutes, wash and strain.
2. Cook it in pressure cooker/open pan so that the grains become

partially cooked but still firm on touch. Tapioca also may be cooked partially to attain semisoft texture.

3. Allow to cool
4. Using a spoon take about 100 g of the medium and transfer in each PP cover.
5. Insert the PVC ring through the open end of the PP cover and position it with rubber band, plug with cotton and cover with aluminium foil.
6. Sterilize the packets along with media in cooker 121°C for 20 minutes.
7. After cooling to room temperature this material is ready for inoculation.

Inoculation with GMF

Introduction of GMF into the sterilized medium is called inoculation. This has to be performed in a clean environment with care.

1. Choose a clean and dry surface (table/wooden plank/concrete plain surface). Wipe out dust, if any, from the surface with a clean cloth.
2. Wipe again with cotton wool soaked in spirit/disinfectant

solution so that it will sterilize the surface properly.

3. Light the candles and place in a semicircle for about 10-15 minutes in order to sterilize the air in the surrounding area.
4. Materials such as inoculation needles, scissors etc. should be sterilized by holding in the flame for 1-2 minutes.
5. Wash hands thoroughly with soap and water. Wipe dry and wipe with disinfectant solution and wear the surgical gloves and a disposable mask.
6. Carry out the inoculation in the area surrounded by the lit candles.
7. Remove the cotton wool plug of the bag close to the flame and hold it with a finger (do not place it on the table).
8. Make a hole in the inoculum packet with the sterilized scissors.
9. Transfer 5 gm of inoculum (grain with sporulated fungus) to the bag using an inoculation needle.
10. Plug it with cotton plug properly and cover the mouth area over the plug with aluminium foil.
11. Mix the inoculum thoroughly with the medium in the bags.
12. Place the bags in a cool dark place for growth of the fungus.
13. After 3-4 days a white mycelium could be seen on the medium, which gradually turns dark green.
14. After 10-12 days the medium appear totally dark green in colour.
15. When culture sporulate well, seal the PP cover and remove PVC ring along with cotton plug.



Rhinoceros beetle infected by GMF



Plugging the PP cover with cotton



Sterilizing inoculation needles

16. The sporulated medium can be applied in the breeding sites or may be stored under refrigeration.

Application of GMF in the breeding sites of black beetle

The sporulated medium showing uniformly dark green colour indicates a good GMF growth. Yellowish or black patches in the medium may be due to contamination. GMF packets should be used within 7-10 days. GMF could be directly applied to the natural breeding grounds of black beetle. Mix the sporulated medium with water. Dig shallow holes in the breeding grounds and add the fungal culture. Cover the pits to protect from direct sunlight. One packet of 100 g of sporulated medium (containing 3.6×10^8 spores/g of the medium) is enough to treat an average breeding area (compost / cowdung pit) of 1m^3 size.

Where to apply

GMF can be applied to any type of breeding grounds of Rhinoceros beetle like farm yard manure, coir pith heaps, decaying coconut logs, compost tanks etc. The entomopathogen had no toxic effect

on the earthworms (Gopal *et al.*, 2006). The fungus can be applied in the rhinoceros beetle traps (PVC traps) in the base along with the coirpith/cowdung so that the trapped beetles get infection and the fungus infected dead beetles can be used in the breeding grounds to spread the infection to other immature stages.

Know the effectiveness against Rhinoceros beetle

1. Monitor the breeding sites at regular intervals for various pest stages. The treated breeding pits were found free of the pest stages during monitoring at regular intervals in the field evaluation trials.
2. Reduction in leaf damage on palm crown- visual effect could be noticed in the palm crown in newly emerged leaves (without pest damage, no fresh spindle or spathe damage).

References

CRI, 2007. *On farm production of Green muscardine fungus for the management of Black beetle*. Crop protection Division, Coconut

Research Institute, Sri Lanka. March 2007. 10pp.

Gopal Murali, Alka Gupta and George V. Thomas. 2006. Prospects of using *Metarhizium anisopliae* to check the breeding of insect pest, *Oryctes rhinoceros* L. in coconut leaf vermicomposting sites. *Bioresource technology* 97(15): 1801-1806.

Nirula, K.K., Radha, K. and Menon, K.P.V. 1955. The green muscardine disease of *Oryctes rhinoceros* L.I. Symptomatology, epizootology and economic importance. *Indian Coconut Journal* 9: 3-10.

Pillai, G.B. 1993. Biological control of insect pests of plantation crops 235-252 In: *Organics in soil health and crop production* (Ed. P.K. Thampan) Peekay Tree Crops Development Foundation, Cochin 254pp.

Rajan, P., Chandrika Mohan, Nair, C.P.R. and Joseph Rajkumar, A. 2009. Integrated Pest Management in Coconut. *Technical Bulletin* No 55. Central Plantation Crops Research Institute, Regional Station, Kayamkulam, Kerala. 20pp.