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Metarhizium:
Microbial control agent for rice black bug

Rice Technology Bulletin Series

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FOREWORD

Rice black bug (*Scotinophara coarctata* Fabricius) or RBB remains as one of the most damaging insect pests especially in the Visayas and Mindanao rice producing areas. At high levels of infestation, it can cause severe damage by sucking the plant sap, resulting in deadheart during the vegetative stage and empty grains or whiteheads during the reproductive stage. Complete crop loss could occur due to bugburn.

The existing control measures for RBB include synchronous planting, use of resistant varieties, light trapping during a full moon period, flooding or increasing the water level to submerge and prevent eggs from hatching, herding of ducks, and the use of biological control (biocon) agents. Among the biocon agents, *Metarhizium anisopliae* is the most effective and can be easily produced using locally-available substrates. It is also economical, compatible with other pest management components, and is environment-friendly.

This bulletin presents how *Metarhizium* works. It provides a step-by-step procedure on how to mass produce the fungus and how to properly apply it in the field. We hope that agricultural technicians and other rice stakeholders interested to multiply the fungus and use it in RBB stricken areas would be able to manage the pest more effectively.

LEOCADIO S. SEBASTIAN
Executive Director

The Microbial Agent:

Metarhizium anisopliae

Metarhizium anisopliae is a green muscardine fungus which attacks rice black bugs (RBB). It is a microbial control agent which is environment-friendly and easy to mass produce using locally available substrates. It is safe to humans and animals and is effective against the RBB. It is easy to apply and is compatible with other pest management components.

How *Metarhizium* works

Metarhizium attacks the RBB with its green spores. These infectious spores germinate once they come in contact with the RBB's skin. They form extensive hyphal growths within the RBB's body that penetrate into its blood vessels and invade its body cavity. They produce toxins that paralyze the pest and then kill it.

White hyphal growths on an RBB's body surface

Then the fungus that develops on the surface of the dead pest's body initiates a new round of infection for other insects. This remarkable chain reaction effectively reduces RBB populations in the field.

Advantages of using *Metarhizium*

One preparation of *Metarhizium* contains 2.5 trillion spores. Since the fungus can multiply by itself, one planting season requires only two to three applications, whereas five to six applications are required when chemical pesticides are used.

Metarhizium is also cheaper than chemical pesticides. A farmer would have to spend only P250 per hectare. Chemical pesticides, on the other hand, cost around P800 to P1,200 per hectare.

Metarhizium is commonly applied through spraying, which should be done late in the afternoon because the active spores are killed by direct sunlight. Unlike chemical pesticides, it does not leave any residue on the crops that may be harmful to human health.

How to isolate *Metarhizium* from infected RBB

Farmers, technicians, and interested individuals can isolate the fungus from infected RBBs using the following procedures:

1. Collect *Metarhizium*-infected RBB from the field.
2. Prepare plated Potato Dextrose Agar *(see steps for preparation on page 5)*. Mark the bottom of the plate as your guide for inoculation.
3. Using the tip of the inoculating needle, slightly touch the spores on the body surface of the infected insect and prick it into the marked portion of the plate.
4. Incubate the inoculated plated agar for 1-2 weeks or until the colony turns green.
5. Transfer the fungus isolate to tubes with culture medium.

**PhilRice offers technical assistance for the isolation and mass production of *Metarhizium*.

How to mass produce *Metarhizium*

Materials for mass production:

Equipment:

- Pressure cooker/autoclave
- Inoculating chamber/Laminar Flow Hood

Supplies:

- Artificial culture media
- Polypropylene plastic bag (9 x 16) or any heat-resistant plastic bag
- Inoculating loop/inoculating needle
- Glass syringe, 10 ml (dispenser)
- Detergent (all-purpose)
- Ethyl alcohol
- Denatured alcohol
- Glass stirring rod
- Chlorox
- Cotton
- Rubber bands
- Alcohol lamp
- Spatula
- Hand sprayer
- Lighter/match
- PVC Ring
- *Palay*/corn

Glassware:

- 500 ml and 1000 ml beakers
- 250 ml and 500 ml flasks
- Test tubes
- Petri dishes

A. Preparation and inoculation of artificial culture media

1. Prepare any of the following artificial culture media to be used throughout the mass production process:

A. Potato Dextrose Agar (dehydrated)

a.1) Suspend 39 g of commercially available dehydrated potato dextrose agar in 1 liter of water.

a.2) Mix thoroughly using a stirring rod.

a.3) Heat with frequent agitation until the powder is completely dissolved.

a.4) Dispense 5 ml of the culture medium in the culture tube.

a.5) Cover the culture tube with cotton plug.

a.6) Sterilize the culture tube in pressure cooker at 121°C or at 15 psi for 15 minutes.

B. Potato Sucrose Agar

Peeled potatoes, cubes	200 g
Sucrose (refined sugar)	10 g
Agar	15 g
Water	1000 ml

b.1) Place 500 ml water and 200 g peeled potato cubes in beaker.

b.2) Boil potato cubes for 15 minutes or until done.

b.3) Filter potato juice with cheese or muslin cloth.

b.4) Add agar and allow to boil until agar is thoroughly melted.

b.5) Add 10 g sugar and stir thoroughly until thick.

b.6) Dispense 5 ml of the culture medium in the culture tube.

b.7) Cover the culture tube with cotton plug.

b.8) Sterilize the culture tube in pressure cooker at 121°C or at 15 psi for 15 minutes.

C. Oatmeal Agar

Oats	60 g
Agar	12 g
Water	1000 ml

c.1) Blend any ordinary oats in 600 ml water for 5 minutes.

c.2) Filter the blended oats using a mesh cloth.

c.3) Add 400 ml of the melted agar and allow to boil.

c.4) Dispense 5 ml of the culture medium in the culture tube.

c.5) Cover culture tube with cotton plug.

c.6) Sterilize the culture tube in pressure cooker at 121°C or at 15 psi for 15 minutes.

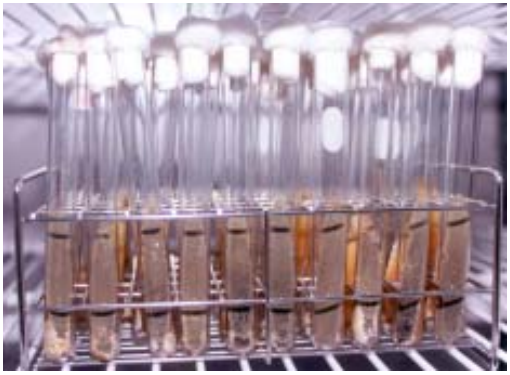
2. Dispense 5 ml of the prepared culture media in each culture tube.

3. Autoclave at 121°C or at 15 psi for 15 min.

4. Allow the slants to cool before inoculation.

5. Using 1-2 week old cultures of *Metarhizium*, slightly touch the spores with the tip of the inoculating loop and streak it onto the agar slant.

6. Incubate the inoculated agar slants for 1-2 weeks or until the colony turns green.



B. Preparation of *palay* substrates

1. Place 200 g *palay* in a 9 x 16 polypropylene or any heat-resistant plastic bag.

2. Add 200 ml water and mix thoroughly.

3. Place PVC ring at the open end of the plastic bag and secure with a rubber band.

4. Seal the open end with cotton plug.

5. Sterilize the *palay* substrate in pressure cooker/ autoclave at 15 psi for 1 hour.
6. Allow the sterilized *palay* to cool before inoculation.

C. Inoculation of *palay* substrates

1. Using the 1-2 week old cultures of *Metarhizium*, add 5 ml of sterile soap solution (0.05%) to each culture tube from the prepared culture media.

2. Scrape off the conidia from the agar surface using the inoculating wire loop.

3. Inoculate *palay* substrate with five (5) ml conidial suspension of *Metarhizium*.

4. Mix *palay* and conidial suspension thoroughly then incubate for 1-2 weeks.

Metarhizium *cultures in plastic bags*

How to apply *Metarhizium*

A. Spray Application

1. Prepare conidial suspension by adding 200 ml of 0.05% soap solution (a mixture of 0.5 g all-purpose detergent and 1 liter water) to each bag of *palay* substrate.
2. Mix the fungal culture and soap/detergent solution thoroughly to dislodge the spores. Strain the suspension to remove the *palay*.

> Metarhizium spores are like dust particles that are easily blown at the slightest movement. When adding soap / detergent solution, extra care should be given in such a way that escape of the spores could be prevented.

3. Place 1000 ml of the conidial suspension in the spray tank and bring the water level to 16 liters per tank load.

   Five bags containing 200 g palay *substrate yields 1 li spore suspension. Fifty bags of* palay *substrates with the fungus are recommended per hectare. This contains approximately 1x10^{13} spores per hectare.*

4. Spray early in the morning (around 6 - 8) or late in the afternoon (around 4 - 6).

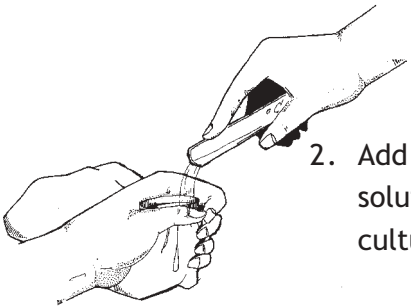
Spray at the base of the plants early in the morning or late in the afternoon

5. Apply the formulation at the base of the plant to ensure contact between the fungus and the RBB.

6. *Metarhizium*-infected RBBs can be observed 4-7 days after application.

B. Through irrigation water

1. Drain the field before application (usually in the early hours of the day of application).



2. Add 200 ml soap/detergent solution to *Metarhizium* cultures in plastic bags.

3. Mix the fungal cultures thoroughly.
4. Apply the fungus in the afternoon when most of the bugs have settled at the base of the plant.
5. Place the *palay* substrates with the fungus at the water inlets while water is conveyed onto the paddy.
6. Bring the water level to 6 cm.
7. Allow the water level to go down through natural evaporation until soil moisture is at saturation point.

8. Irrigate the field and maintain the normal water level to 2 cm.

Characteristics of *Metarhizium*-infected RBB

In the early stages of infection, RBB may show ill effects such as cessation of feeding and weakness.

Metarhizium mummifies the RBB and white conidiophores grow out through the cuticle within 24 hours. Profuse green spores follow 1-2 days later.

☛ **When applied, *Metarhizium* can reduce 30-68% of the RBB population in 7 days. Under controlled conditions, 88% mortality of RBB can be obtained.**

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PhilRice

The Philippine Rice Research Institute (PhilRice) is a government-owned and controlled corporation created through Executive Order 1061 approved on Nov. 5, 1985, which was amended by EO 60 dated Nov. 7, 1986 and EO 76 dated March 4, 2002 to help develop high-yielding technologies so that farmers can produce enough rice for all Filipinos. PhilRice accomplishes this mission through research, technology promotion, and policy advocacy, which are implemented through a network that includes 57 agencies and 95 seed centers strategically located nationwide.

Its interdisciplinary programs include the following: (1) direct-seeded and (2) transplanted irrigated lowland rice; (3) hybrid rice; (4) rice for adverse environments; (5) rice-based farming systems; (6) policy research and advocacy; and (7) technology promotion. With these programs, PhilRice aims to develop and promote technologies that are ecosystem-based, location- and problem-specific, and profitable to the Filipino farmers.

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