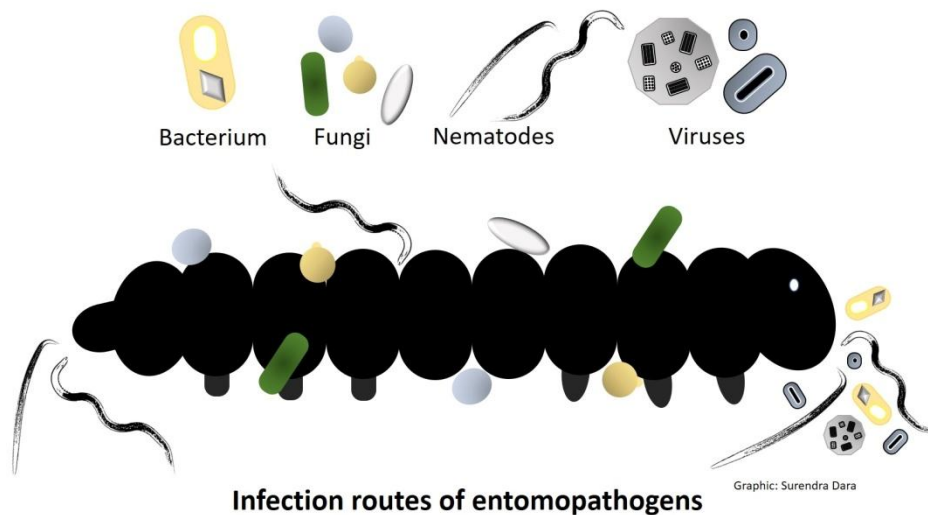


## Mass production of Entomophaga and entomopathogens invading coconut pests

Sujithra M and Rajkumar

Entomopathogens are microorganisms that are pathogenic to arthropods such as insects, mites, and ticks. Several species of naturally occurring bacteria, fungi, nematodes, and viruses infect a variety of arthropod pests and play an important role in their management. Some entomopathogens are mass-produced in vitro (bacteria, fungi, and nematodes) or in vivo (nematodes and viruses) and sold commercially. Using entomopathogens as biopesticides in pest management is called microbial control, which can be a critical part of integrated pest management (IPM) against several pests.



### Entomopathogen groups

Important entomopathogen groups and the modes of their infection process are described below:

#### Entomogenous fungi:

Entomogenous fungi have long been known to attack insect pests. They are potentially the most versatile biological control agents, because they have wide host ranges, infect at different ages and stages of their hosts and often cause natural epizootics. An attractive feature of these fungi is that infectivity is by contact and active penetration. Therefore ingestion is not required to initiate infection whereas; most other entomopathogens such as bacteria, protozoa and viruses have to be necessarily eaten, to be infective. Entomogenous fungi comprise a heterogenous group of over 100 genera with approximately 750 species, reported from different insects, many of which offer great potential in pest management. They belong to Zygomycotina, Ascomycotina, Basidiomycotina and Deuteromycotina. Major

entomopathogenic fungi are presented in Table 1. Several of these genera are principally or exclusively associated with a single family, genus or a few species of insect pests.

**Table 1: List of major taxa containing entomopathogenic species**

**Zygomycotina:** *Conidiobolus*, *Entomophaga*, *Entomophthora*, *Erynia*, *Massospora*, *Mueor*, *Neozygites*, *Pandora*, *Rhizopus*, *Strongwellsea*, *Tariehium*, *Triplosporium*, *Zoophthora*, *Zygaenobia*

**Ascomycotina:** *Aseosphaera*, *Caloneetria*, *Cordyceps*, *Hypoerella*, *Massaria*, *Neetria*, *Sphaerostilbe*, *Torrubiella*, *Laboulbeniales*

**Basidiomycotina:** *Septobasidium*, *Stereum*, *Uredinella*

**Deuteromycotina:** *Aerostalagmus*, *Aegertia*, *Akanthomyces*, *Asehersonia*, *Aspergillus*, *Beauveria*, *Cephalosporium*, *Cladosporium*, *Culicinomyces*, *Derexia*, *Fusarium*, ***Hirsutella***, *Isaria*, ***Metarhizium***, *Nomuraea*, *Paecilomyces*, *Penicillium*, *Sorosporella*, *Spicaria*, *Verticillium*

Some of the most widely used species include *Beauveria bassiana*, *Metarhizium anisopliae*, *Nomuraea rileyi*, *Paecilomyces farinosus* and *Verticillium lecanii*. Many of them have been commercialized globally. Some species such as *B. bassiana* and *M. anisopliae* cause muscardine insect disease and after killing the host, cadavers become mummified or covered by mycelial growth. For about 130 years, entomopathogenic fungi especially *Metarhizium anisopliae* (Metschn.) Sorokin, have been used for biocontrol of pest insects. Besides the two other entomopathogenic fungi *Beauveria bassiana* and *Beauveria brongniartii*, *M. anisopliae* is one of the most widely used fungus and mycoinsecticide throughout the world, mainly as an inundative control agent. *Metarhizium anisopliae* is a well-known entomopathogenic fungus active against many insect pests. The species *M. anisopliae* was originally described by Metschnikoff (1879) as *Entomophthora anisopliae* and later transferred to the new genus *Metarhizium* by Sorokin (1883). The genus is defined on the basis of the arrangement of the phialides bearing chains and columns of dry and generally green, cylindrical or slightly ovoid conidia. The columns are formed by aggregation of the conidial chains. In most cases, the fungus has been isolated from soil using the so-called *Galleria* bait method or by special selective media.

### **Mode of action**

The infective unit in most of the entomopathogenic fungi is a conidium or spore which when lands on a susceptible host, put forth 'germ tubes' or 'infection pegs' from appressoria. These structures secrete a complex of cuticle degrading enzymes viz., chitinases, proteases and lipases, which are capable of hydrolysing corresponding cuticular constituents namely chitin,

protein and lipid in the epicuticle. The infection process of *M. anisopliae* is similar to other entomopathogenic fungi, i.e. the infection pathway consists on the following steps: (1) attachment of the spore to the cuticle, (2) germination and formation of appressoria, (3) penetration through the cuticle, (4) overcoming of the host response and immune defence reactions of the host, (5) spreading within the host by formation of hyphal bodies or blastospores, and (6) outgrowing the dead host and production of new conidia. The destruxins are the most important metabolites/toxins produced by *M. anisopliae* after invading the host and chemically, destruxins are cyclic hexadepsipeptides containing five amino acids, i.e. b-alanine, alanine, valine, isoleucine and proline, and an  $\alpha$ -hydroxy acid.

The entomopathogenic fungus, *Metarhizium anisopliae* has been used widely as a mycopesticide to control many insect pests including the major palm insect pest, *Oryctes rhinoceros*. The effective isolate was identified in length as *M. anisopliae* variety major with the spore dimension between 9-15  $\mu\text{m}$ . The pathogenicity tests showed that at the concentration of  $10^8$  spores per mL, the fungus killed 100% the third instars larvae of *Oryctes rhinoceros* between 12 – 14 days after treatment, causing 70 -75% mycosis. Field application by directly drenched the fresh spore solution and broadcasting of solid substrate with spores onto the breeding sites was significantly reduced the *Oryctes* population, especially the larvae. Inoculate the breeding sites with green muscardine fungus, *Metarhizium anisopliae* ( $5 \times 10^{11}$ ) spores/ $\text{m}^3$  of breeding area effectively manages the rhinoceros beetle. *Metarhizium* has a number of advantages as a mycoinsecticide: it is relatively easy to mass produce, strains can be found with appropriate levels of virulence and specificity, and the conidia can be dried to ensure good persistence in the field and storage for over one year in cool conditions.



**Fig:** *Metarhizium* infested *Oryctes* cadavers

Since 1998, the coconut mite, **Aceria guerreronis**, has been a chronic constraint to coconut farming in India, the third largest producer of coconuts in the world. A mite-specific fungal pathogen, **Hirsutella thompsonii**, as the most important natural regulator of the coconut

mite was later identified. *Hirsutella thompsonii* var. *synnematos*a is an important fungal parasite of phytophagous mite, especially those belong to families Eriophyidae and Diptilomiopidae. The fungus invades the internal tissues of the host and eventually kills it within 2-3 days. Subsequently, mycelia penetrate from the cadaver through mite cuticle where spores are produced along the mycelium. It is known that spore is the only infective unit of this fungus.

Mycohit, a powder formulation of the fungus was evaluated as a short-term as well as a long-term biocontrol agent. Spraying of the product on young bunches resulted in high fungus-associated mortality of the mite. The fungus was found to be capable of bringing down the mite population up to 90%, resulting in considerable reduction in pre-harvest nut damage. In several trials, the fungal treatment was superior to azadirachtin, dicofol, triazophos, and/or wettable sulphur.

**Bacteria:** The bacteria that are used as biopesticides can be divided into four categories: crystalliferous spore formers (such as *Bacillus thuringiensis*); obligate pathogens (such as *Bacillus popilliae*); potential pathogens (such as *Serratia marcesens*); and facultative pathogens (such as *Pseudomonas aeruginosa*). Out of these four, the spore formers have been most widely adopted for commercial use because of their safety and effectiveness. The most commonly used bacteria are *B. thuringiensis* and *Bacillus sphaericus*. *B. thuringiensis* is a specific, safe and effective tool for insect control. It is a Gram-positive, spore-forming, facultative bacterium, with nearly 100 subspecies and varieties divided into 70 serotypes. The insecticidal property of *B. thuringiensis* resides in the Cry family of crystalline proteins that are produced in the parasporal crystals and are encoded by the cry genes. The Cry proteins are globular molecules (65–145 kDa, depending on the strain) with three structural domains connected by single linkers. The 200 Cry proteins belong to a single family that contains about 50 subgroups.

**Mode of Action:** When **Bt** is ingested, alkaline conditions in the insect gut (pH 8-11) activate the toxic protein (delta-endotoxin) that attaches to the receptors sites in the midgut and creates pore in midgut cells. This leads to the loss of osmoregulation, midgut paralysis, and cell lysis. Contents of the gut leak into insect's body cavity (hoemocoel) and the blood (hoemolymph) leaks into the gut disrupting the pH balance. This includes starvation and lethal septicemia of the host insect.

**Virus:** Over 700 insect-infecting viruses have been isolated, mostly from Lepidoptera (560) followed by Hymenoptera (100), Coleoptera, Diptera and Orthoptera (40). About a dozen of these viruses have been commercialized for use as biopesticides. The viruses used for insect control are the DNA-containing baculoviruses (BVs), Nucleopolyhedrosis viruses (NPVs), granuloviruses (GVs), acoviruses, iridoviruses, parvoviruses, polydna- viruses, and poxviruses and the RNA-containing reo- viruses, cytoplasmic polyhedrosis viruses, nodaviruses, picorna-like viruses and tetraviruses. The introduction of *Oryctes* virus into outbreak areas of the rhinoceros beetle, *Oryctes rhinoceros* (Coleoptera: Scarabaeidae), has been a major success for "classical" biocontrol with a virus and has led to a dramatic

reduction in palm damage in many areas of the Asia/Pacific region. In size and structure, the *Oryctes* virus very much resembled the baculoviruses of the many nuclear polyhedrosis and granulosis diseases of insects known at that time. But the main difference was that, with minor exceptions, it is not occluded in paracrystalline proteinaceous bodies. Therefore, this first rod-shaped, non-occluded insect virus was assigned to a new genus and originally described as *Rhabdionvirus oryctes*. When the classification and nomenclature of insect viruses was completely revised by the International Committee on Taxonomy of Viruses (ICTV), the *Oryctes* virus became the type species of Subgroup C of the family Baculoviridae. Thus, for a long time it was cited in literature as ‘baculovirus of *Oryctes*’ or briefly as ‘*Oryctes* baculovirus.’ Recently, Evans and Shapiro (1997) have assigned the virus to a new *Oryctes* virus family. Release of *Oryctes rhinoceros* (OrV) virus infected adult beetles @ 10 -15 per ha.

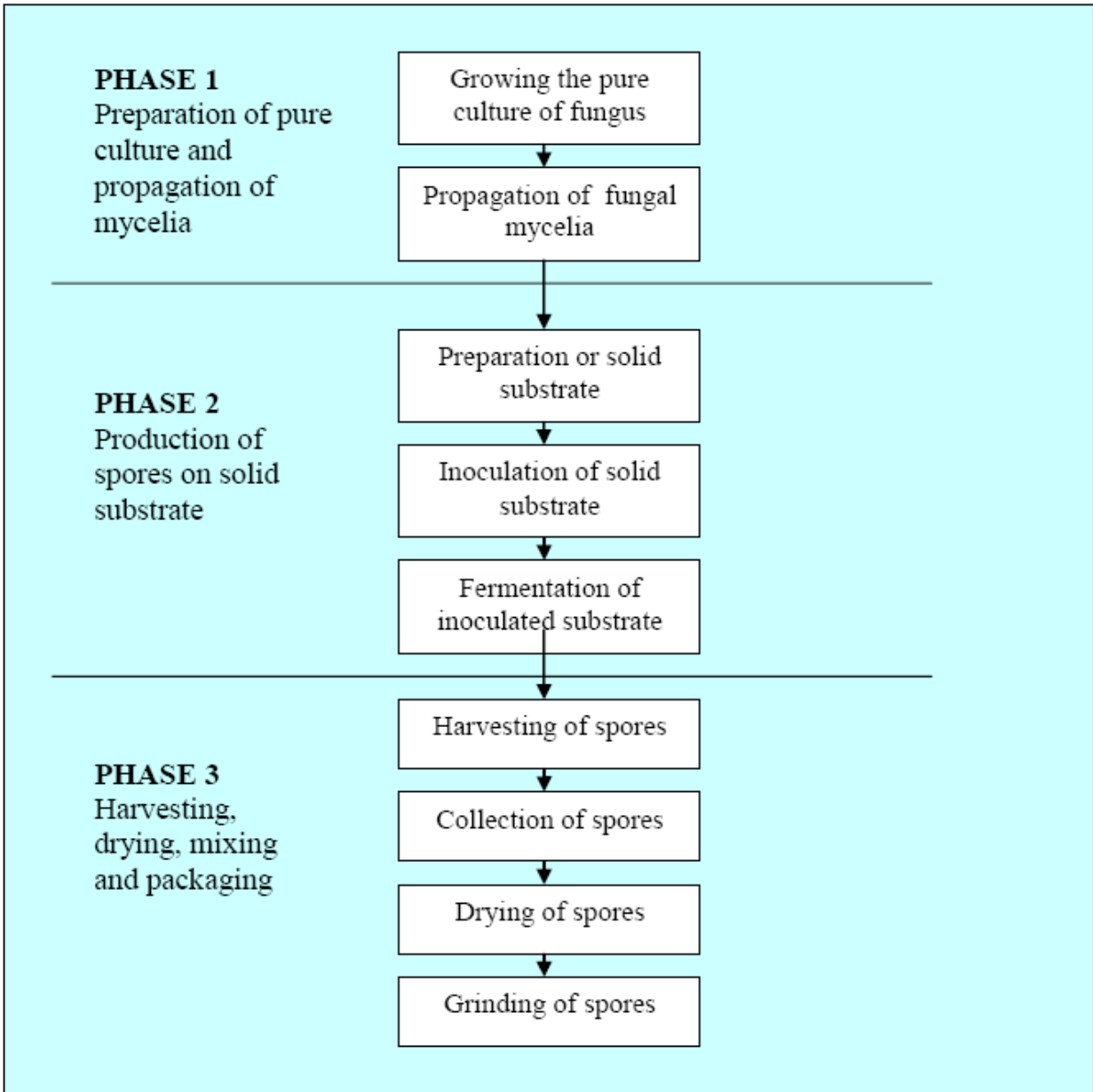
**Nematode:** Another group of microorganisms that can control pests is the entomopathogenic nematodes, which control weevils, gnats, white grubs and various species. These fascinating organisms suppress insects in cryptic habitats (such as soil-borne pests and stem borers). Commonly used nematodes in pest management belong to the genera *Steinernema* and *Heterorhabditis*, which attack the hosts as infective juveniles (IJs). Entomopathogenic nematodes (EPN) can be mass-produced *in vivo* and *in vitro* in solid media or liquid fermentation.

**Mode of action:** IJs are free-living organisms, which enter the hosts through mouth, anus, spiracles or cuticle (Figure 2). They are able to release their bacterial symbionts in to the haemocoel of hosts, killing the host within 24–48 h. The nematodes can complete up to three generations within the host, after which the IJs leave the cadaver to find the new hosts.

### **Production and Development:**

Some microbial biopesticides are easy to produce and develop and can be manufactured using simple and in-expensive technologies. The BVs and EPN can be produced *in vivo* in insects and entomopathogenic fungi, such as *Hirsutella*, *Beauveria* and *Metarhizium*, are produced on grains. Such simple technologies are useful for developing countries where a substantial demand exists for local production and distribution at the farmers’ level.

**Conclusion:** Entomopathogens can be important tools in IPM strategies in both organic and conventional production systems. Depending on the crop, pest, and environmental conditions, entomopathogens can be used alone or in combination with chemical, botanical pesticides or other entomopathogens.



**Figure 1:** Chart showing the flow of the process involved to produce the spores of fungi