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REVIEW ARTICLE

Characterization and importance of fungal bio-control agents

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Abstract

Various methods are adopted to manage the plant diseases. Fungicide being used to manage the disease is in practice which is hazardous for human and animals causing serious health disorder. Now-a-days much emphasis is being given on biological control of phytopathogens responsible for causation of plant diseases. Much more information is available on mode of action and application of bio-agents exploited for biological control but the microorganisms utilized in this respect are little known as regards to their morphological features and taxonomy. This communication particularly deals with the bio-agents which are entomogenous, nematophagous, predaceous in nature and mycoparasites. Ephasis has been given on their characters and classification and use as bio-control agents.

Key words -Bio-control, Bio-agents, Insectivorous, Nematophagous, Mycoparasites, Kalisena.

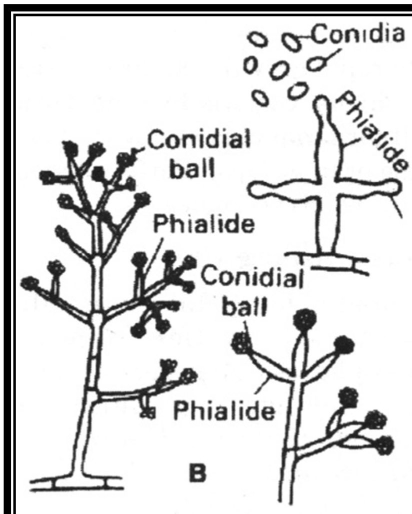
Introduction

The term biological control was first used in scientific literature in 1914. Baker and Cook (1974) described biological control as "reduction of inoculums density or disease producing activities of a pathogen or parasite in its active or dormant state, by one or more organisms, accomplished naturally or thoroughly manipulation of environment, host, or antagonists or by mass introduction of one or more antagonists." Biological control is coming up as an alternative strategy for disease management which is also ecology conscious and environment friendly. The bio-control agents are eco-friendly and cost effective also. Some of the bio-agents increase the plant emergence, growth of seedlings and bio-agents of pathogens act as their enemies (parasitoids). Certain genera of fungi have greater potential for being effective bio-control agents eg. *Trichoderma, Gliocladium, Chaetomium;* entomogenus fungi as *Entomophthora, Beauveria, Laboulbenia; n*ematophagous *viz.*, *Catenaria, Zoopage, Paecilomyces* and predaceaus fungi like *Arthrobotrys, Dactylella, Dactylaria* and mycoparasites in nature as *Syncephalis, Piptocephalis, Piptocephalus* and *Dimargaris*.

Bio-agents

(1)*Trichoderma* - *Trichoderma* (Teliomorph *Hypocrea*) is a fungal genus found in many eco-systems. Some strains have the ability to reduce the severity of plant diseases by inhibiting plant pathogens, mainly in the soil or on plant roots, through their high antagonistic and mycoparasitic potential (Viterbo and Horwitz, 2010). The presence of fungal prey and the availability of root derived nutrients have been major attractors for ancestors of *Trichoderma* to establish them in the rhizosphere and to facilitate the evolution of positive interaction with plants (Druzhinina *et al.,* 2011 and Kumar and Prabhu, 2008; Mahalik and Sahu, 2017). Among the fungal antagonists, *Trichoderma* has gained the maximum popularity as bio-control agent (Barron, 1977). *T. viride, T. harzianum* and *T. virens* have been found effective against many phytopathogens and are considered as generalized bio-control agents.

Morphology of *Trichoderma* spp. is a key identification characteristics such as colony morphology, colony edge, reverse colour, alongwith, colour, shape, size of conidia and their formation and phialides. Credit goes to Rifai (1969), who laid down the foundation of the taxonomy of this interesting group of fungi.

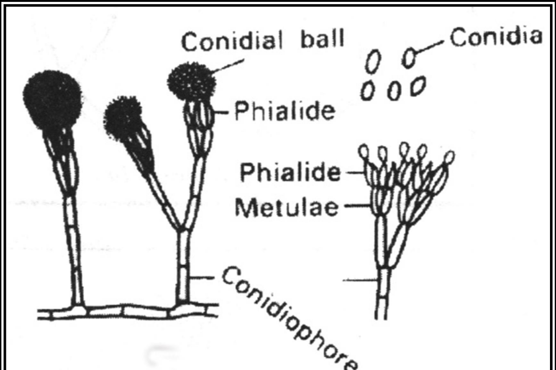


Trichoderma viride

The colour of mycelium remains yellow, green to dark green after 2-3 days. No characteristic odour. Conidial production uniform with green conidia distributed throughout. Reverse of the Petri-plates lemon yellow in colour with no concentric ring. Phialides long, swollen in middle, slender and horn shaped, frequently bend towards the apex of the bearer. Phialospores (conidia) are usually globose or obovoid often, perfectly smooth walled (Rafai, 1969).

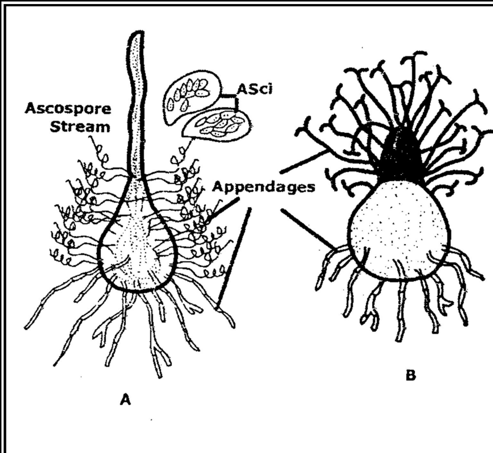
Various *Trichoderma* spp. have been widely used as antagonistic fungal agents against several pests as well as plant growth enhancers.

Prospects of biological control of soil-borne plant pathogens using the genus *Trichoderma*, as one of the promising bio-control agents, has been described. These are free-living, saprophytic fungi and are highly interactive in root, soil and foliar environments. They can also compete with other microorganisms; for example, they compete for key exudates from seeds that stimulate the germination of propagules of plant pathogenic fungi in soil and, more generally, compete with soil microorganisms for nutrients and/ or space. The genus *Trichoderma* has been described for successful reductions of Fusarium wilt in many crops with application of different species. Faster metabolic rates, anti-microbial metabolites, and physiological conformation are key factors which chiefly contribute to antagonism of these fungi. Mycoparasitism, spatial and nutrient competition, antibiosis by enzymes and secondary metabolites, and induction of plant defence system are the typical biocontrol actions of these fungi.



Gliocladium deliquescens

(2) *Gliocladium virens-* *Gliocladium* is an asexual fungal genus in the Hypocreaeae. Certain other species including *Gliocladium virens* were recently transferred to the genus *Trichoderma* and *G. roseum* became *Clonostachys rosea* f.sp. *rosa* in the Bionecteriaceae. *Gliocladium* is a mitosporic, filamentous fungus. Species of *Gliocladium* rarely produce a sexual state. Species of *Gliocladium* are considered to have pathogenic potential against phytopathogens although they are not commonly thought of as a disease causing agent in humans and animals. Gliotoxin is a metabolite of *G. deliquescens* (now *Trichoderma*). The significance of gliotoxin has not yet been determined.
Microscopically, *Gliocladium* species produces hyphae, conidiophores, and conidia borne from hyaline phialides. The conidiophores are erect, dense, and have a brush-like structure which produce tapering, slimy phialides.



***Chaetomium*:**
***C. globosum* (A), *C. thermophile* (B)**

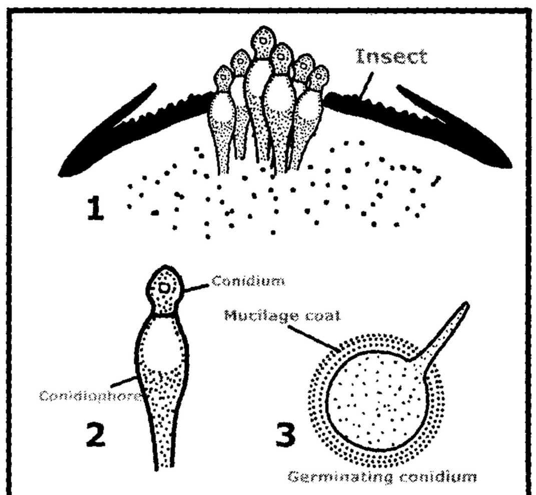
(3) *Chaetomium* - It is a well-known genus of the family Chaetomiaceae with several species. It includes the species, which are found on straw, cloths, waste products etc. Sometimes they cause severe damage to them. Perithecia are superficial, ostiolate, provided with greatly elongated beak like structures. Loose, tightly coiled hairs like appendages appear on the body and on beak. Perithecial wall thin and paraphyses lacking. The ascal wall disappears at maturity and asci have thin, gelatinous wall, which may dissolve before the ascospore matures. The ascospores at maturity are embedded in a jelly and often come out in cirrhus, which is ribbon-like slender of spores held together by mucous and coming out through ostioles. Asci are club-shaped and thin walled. Ascospores are mostly lemon shaped, dark-coloured and one-celled. The genus is named on the character (chaite = hair).

Chaetomium is one of the extremely familiar genera of Pyrenomycetes (Ascomycotina) encountered on various agricultural commodities. The genus contains more than 200 species, all characterized by dark coloured perithecia with short necks which are clothed with irregularly coiled or tightly spiraled hairs. It is a common colonizer of soil and cellulose containing substates. Globosurn has been reported effective in reducing damage caused by seed rot and damping off, several seed borne and soil borne plant pathogens like *Pythium ultimum, Alternaira raphani, A. brassicicola, Fusarium* spp. and its antifungal metabolites have also been reported (Biswas *et al.,* 2011a) and resistance to wheat spot blotch induced by its crude extract (Biswas *et al.,* 2011b). Seed coatings with selected isolates of *C. globosum* protect corn, oat and barley from seedling blight caused by *Fusarium* spp. and *Drechslera sorokiniana*. Increased field stands of maize hybrids were noticed when the seeds were coated with *C. globosum* ascospores.

Seed treatment with ascospores of *C. globosum* reduces damping off of sugarbeet caused by seed borne *Phoma betae* and soil borne *Pythium ultimum* or *Rhizoctonia solani.*

Entomongenous fungi

(1) *Entomophthora*-*Entomophthora* includes fungi which are chiefly parasitic on insects. The most familiar species is *Entomophthora muscae*, commonly called the fly fungus, which is often found on the bodies of houseflies. Innumerable conidia come out which have been shot off the conidiophores growing out of the body of the fly. This forcible ejection of the conidia is a prominent characteristics of *Entomophthora.*



Entomophthora muscae: 1-3, Asexual stage

The mycelium has a definite tendency to form septa and then to fragment into portions which we call hyphal bodies. Such bodies multiply by division or budding, and each eventually produces a conidiophore bearing a conidium at its tip. In some species, such as *Entomophthora fumosa* and *E. fresenii,* hyphal bodies copulate and develop zygospores. Some of the saprobic species have a well-developed mycelium which persists as such. Few of the Entomophthoras have been grown in culture.

Entomophthora reproduces asexually by means of sporangiola, functioning as conidia, which are borne on simple or branched conidiophores. The conidia are forcibly discharged from the conidiophores, and, in most species, germinate by producing germ tubes.

The conidia of the *Entomophthora* are covered by a mucilaginous substance which adheres to any object against which the conidium is catapulted. In *Entomophthora muscae* and other species, if the conidium lands on a substratum suitable for its growth, it germinates and forms mycelium; otherwise it produces a secondary conidium. Zygospores are formed in several species, but their further development is unknown. It is probable that they germinate by means of germ tubes. In principle, zygospore formation in most Entomophthorales investigated is similar to that in the Mucorales, but the details are somewhat different in characteristics.

Entomophthora spp. parasitic on insects are -

(i) *E. aphidis* on *Apphis* sp. of *Pisum sativum* (Butler and Bisby, 1931).

(ii) *E. coranata* on *Myzus persicae* (Ramaseshaiah, 1967).

(iii)*E. fumosa* on rice brown plant hoppers (*Nilaparvata lugens*) (Sawal *et al.,* 1978 and Shrivastava and Nayak, 1978).

(iv)*E. muscae* on housefly (*Musca domestica*) (Kapoor and Chowdhry, 1996).

(v) *Entomophthora* sp. on Bihar hairy caterpillar (Thontadarya *et al.,* 1973).

(2) *Beauveria bassiana* - The species is named after the Italian entomologist, Agostino Bassi, who discovered it in 1815 as the cause of the muscardine disease which then led to carriers transmitting it by airborne means. It was formerly also known as *Tritirachium shiotae*. The name, *B. bassiana* has long been used to describe a species complex of morphologically similar and closely related isolates. Rehner and Buckley have shown that *B. bassiana* consists of many distinct lineages that should be recognized as distinct phylogenetic species and the genus *Beauveria* was redescribed with a proposed type for *B. bassiana* in 2011. In light of this work and the known existence of cryptic species, it is important to characterise isolates used to develop biological insecticides.

Beauveria bassiana can be used as a biological insecticide to control a number of pests such as termites, whiteflies, and many other insects. As an insecticide, the spores are sprayed on affected crops as an emulsified suspension or wettable powder or applied to mosquito nets as a mosquito control agent. As a species, *Beauveria bassiana* parasitizes a very wide range of arthropod hosts. However, different strains vary in their host ranges, some having rather narrow ranges, like strain Bba 5653 that is very virulent to the larvae of the diamondback moth and kills only few other types of caterpillars. Some strains do have a wide host range and should therefore be considered nonselective biological insecticides. These should not be applied to flowers visited by pollinating insects.

B. bassiana is parasitic on adult beetles of

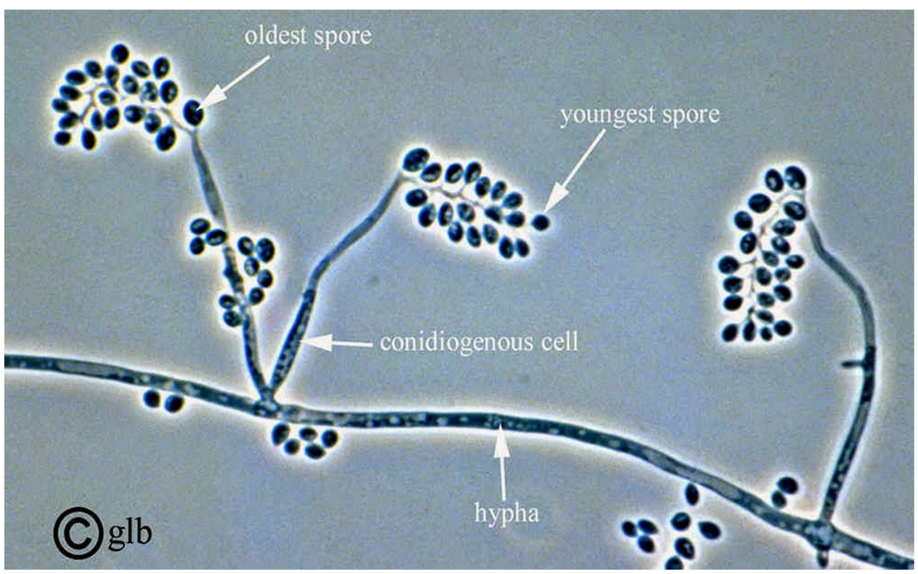
(i) *Lachnosterma consagunea* (Rao and Vijaylakshmi, 1959).

(ii)Caterpillars of *Plusia* sp. (Rama Raje Urs *et al.,* 1965).

(iii)Banana leaf beetle (*Nodostromoa subconstatum*) (Roy and Pujari, 1979).

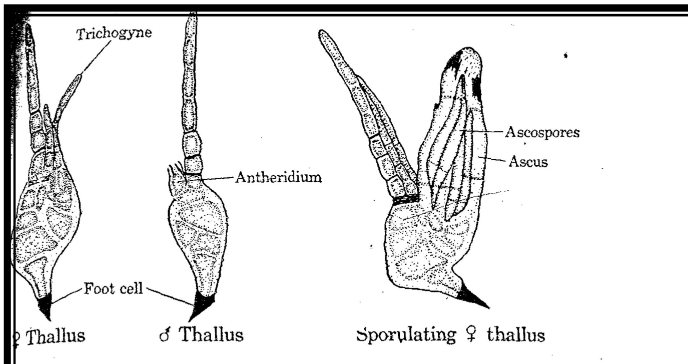
(iv)*B. tenella* on caterpillar (Rahalkar and Thirumalachar, 1974).

(v)*B. bassiana* strain *globulifera* on *Monoscrosporium ellipsosporium,* (Choudhry *et al.,* 1979).



Morphology of *B. bassiana*

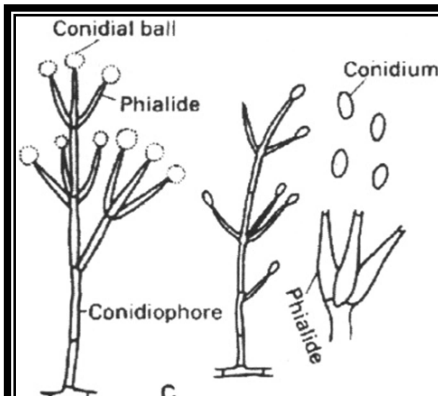
(3) *Laboulbenia* - This is a rather large group of highly specialized parasites of insects and arachnids which occur mostly superficially on their hosts (Thaxter, 1902).



Laboulbenia formicarum

The soma consists of a receptacle and its appendages. Male and female reproductive structures may be borne on the same individual or, in dioecious species, on different individuals. The receptacle is anchored to the host cuticle by a dark basal cell called the foot cell. In some species, a rhizoid (haustorium) grows out of the basal cell and penetrates more deeply into the host. The fungus obtains all its nourishment from the host.

In some *Laboulbenia* specialization becomes even more extreme. Not only are most forms specialized on a particular insect species, but also some of them attack only individuals of one sex, and these only on a particular spot of their body.



Verticillium albo-atrum

The ascocarps of the *Laboulbenia* are minute, stalked perithecia without paraphyses or periphyses, containing four to eight-spored asci. Sexual reproduction is by means of spermatization; non-motile sperm cells (spermatia) are released from male gametangia (antheridia) and spermatize the trichogynes of the ascogonia.

Some other species of *Laboulbenia* parasitic on insects are : *L. coarchata, L. fissa, L. maculata, L. microsoma* and *L. podontiae* (Thaxter, 1914). It has been observed that *L. acanthophora* on *Pericallus* sp. (Coleoptera), *L. assamensis* on *Cathscopus* sp. (Coleoplera) are the known parasites of insects.

(4) ***Verticillium*** - Conidiophore branches are fertile, verticillate, a few in whorls. *V. albo-atrum,* has the perfect stage which is placed in order Hypocreales. Some species have been reported pathogenic on aphis (Nagaich, 1973).

Nematophagous fungi

The microorganisms that parasitize or prey on nematodes, fungi hold an important position and some of them have shown great potential as bio-control agents (Duddington, 1957; Duddington and Wyborn, 1972). Many species of fungi have been found associated with nematodes but only few of them are successful bio control agents (Barron, 1977 and Bandopadhyay *et al.,* 2001). The fungi involved in the biological control of nematodes are grouped into : Endoparasitic fungi, Predacious fungi and Opportunistic fungi.

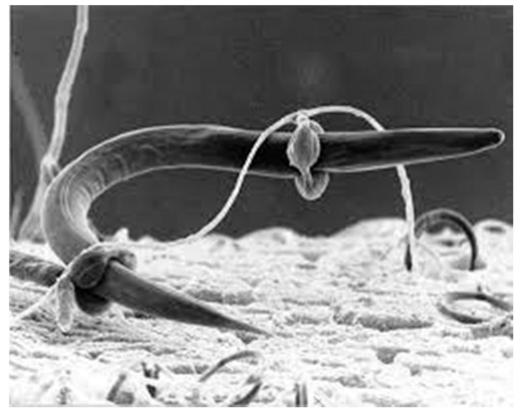
(1) Endoparasitic fungi:- They do not produce extensive mycelium but exist in the environment as conidia, which infect nematodes by either adhering to the surface of the nematodes or being ingested. The spores rapidly germinate and invade the entire nematode, with assimilate hyphae absorbing all the body contents. Two zoosporic fungi, *Catenaria auxiliaris* and *Nematophthora gynophila,* have a role in regulating population of *Heterodera* spp. Some other endoparasites which have some role in bio-control are *Acrostaiagmus*, and *Nematoctonus* etc. (Singh and Gupta, 1986; Singh *et al.,* 1993; Kumar and Singh, 2006).

(2) Predacious fungi:- There are certain species of predacious fungi which capture and kill nematodes in soils. With age, these fungi exhibit reduction in nematode-trapping efficiency. Predatory fungi produce extensive hyphal network in the environment and produce trapping devices at intervals along with each hypha. The traps capture nematodes either mechanically or by adhesion, allowing fungus to penetrate the nematode rapidly and digest the body contents. Predatory fungi form different types of traps, such as unmodified adhesive hyphae (*Stylopage* spp.), adhesive nets (*Arthrobotrys* spp.), adhesive knobs (*Dactylella* spp.), constricting rings. (*A. dactyloides* and *Dactylaria brochopaga*) and non-constricting rings (*Dactylaria candida*) to capture the nematodes. *Arthrobotrys* isolate gives good protection against root-knot nematodes on tomato. These results have better hope for their use in bio-control of nematodes.

(3) Opportunistic fungi:- Nematodes belonging to Heteroderid group (*Heterodera* and *Meloidogyne*) and at the sedentary stages of their life-cycle are vulnerable to attack by these fungi either within the roots or when exposed on the root surface or in the soil. Once in contact with cysts and egg masses, these fungi grow rapidly and eventually parasitize all eggs that are in early embryonic developmental stages. Wide range of opportunistic fungi parasitize eggs and cysts of nematodes.

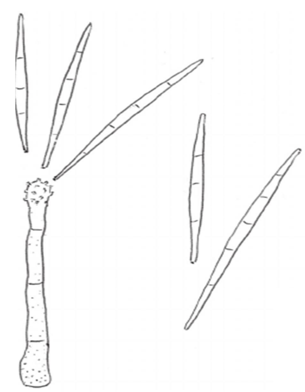
Trichoderma harzianum, Trichoderma virens, Aspergillus niger, Paecilomytes lilacinus, Verticillium chlamydosporium are found promising bio-control agents.

Catenaria



Trapping of nematode

Dactylella



Dactylaria

Some fungi killing and capturing the nematodes

(i) *Arthrobotrys conoides* and *A. oligospora* on *Acroboles* sp. *Cephalobus* sp. and *Plectus* sp. (Sachidananda and Ramakrishnan, 1971).

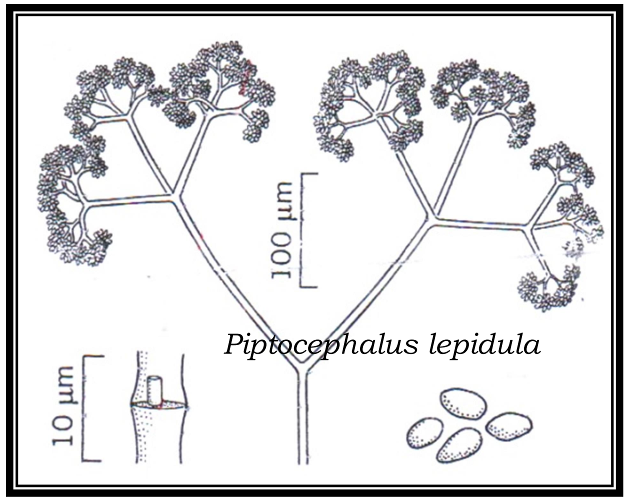
(ii) *Catenaria vermicola* parasitic on larvae and eggs of *Heterodera avenae* (Chowdhry and Dhawan, 1984).

(iii) *Dactylaria brochopaga* on *Diplogaster* sp., *Cephalobus* sp. and *Rhabdilis* sp. and *D. psychrophilis*: (Sachidananda and Swarup, 1966, 1967).

(iv) *Dactylella asthenophaga* on some parasitic nematodes (Sachidananda and Ramakrishnan, 1971).

Paecilomyces lilacinus

The fungus has well developed septate and profusely branched hyaline mycelium. The septa have a central pore through which the cell contents migrate freely from one cell to another cell. The most important means of reproduction is formation of conidia and terminal chlamydospores. The conidiophores ramify in grouped branches or irregularly. The one celled conidia are separated from the phialides in the form of chains. Most of the conidia are prismatic and some are oval or short elliptical. The cell wall is made of chitin. The conidiospore (conidium) is single celled and transparent. This fungus has been reported to parasitize eggs of many sedentary endoparasitic nematodes (Dube and Smart, 1974; Cannayane and Sivakumar, 2001). The infection process begins with growth of the fungal hyphae in the gelatinous matrix; eventually the eggs of *Meloidogyne* or *Tylenchulus* species are engulfed by the mycelial network, which becomes prostate and spirals over the smooth egg surface. The proliferated hyphal branches penetrate the eggs. Once the hyphae are in contact with egg, a series of ultrastructural changes occur in the egg. *Pecilomyces lilacinus* infects *Meloidogyne arenaria* aggs and juveniles. When an egg became occupied by fungal hyphae, changes in egg shell structure occur. It has been observed that *P. lilacinus* was effective egg parasite of *M. incognita acrita* and *Globodera pallida* (Jatala *et al.,* 1979). This fungus has the greatest potential for application as a bio-control agent. Sharma and Trivedi (1989) reported that *P. lilacinus* penetrated the eggs of *M. incognita* nematode and fed upon the internal contents heaving empty shells.

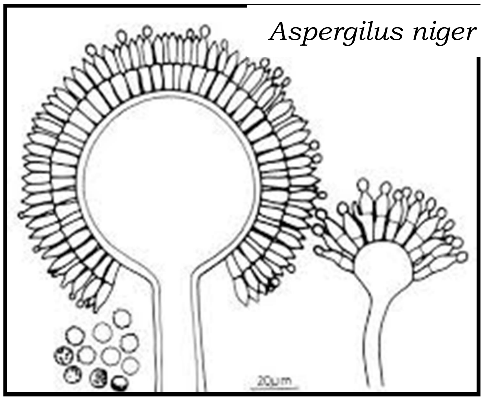


Mycoparasites

This group of fungi are also called as mycophagous fungi which include *Piptocephalis, Piptocephalus, Syncephalis* and *Dimargis*. In *Piptocephalis and Piptocephalus,* the sporangiophore is beautifully dichotomously branched. The merosporangia are cylindrical and simple and may or may not disarticulate; they often are borne on a deciduous head cell. Interestingly, species of *Piptocephalus* apparently have evolved two methods of spore dispersal with some having wet clusters of merosporangia while others remain dry, apparently wind dispersed. Spore dispersal of wet clusters could be by arthropods. Zygospores with an ornamentd zygosporangium wall and apposed suspensors that may be entwined and may bear protuberances; the zygospores are formed in the substrate. The host-parasite relationship of *Piptocephalis unispora*, a species with ovoid single spored merosporangia, has been studied at the ultrastructural level. When the germ tube of this fungus contacts the hypha of a suitable hosts, an appressorium is formed from which a small infection peg develops. This peg penterates the cell wall of the host and gives rise to a branched hustorium. Likewise, the host-parasite relationship between *Piptocephalis virginiana* and *Choanephora cucurbitarum* has been studied.

Baker and her colleagues (1977) tested 50 genera of fungi representing as potential hosts for *Syncephalis sphaerica*. The fungus infacted all species of Mucorales tested as well as some non-mucoraceous species. It also possesses the ability to grow and to sporulate on certain synthetic media. *Piptocephalis debaryana* parasitizes *Mucor* spp. (Methrotra, 1960) and *Syncephalis* like *S. nodosa, S. sphaerica, S. trispora* have been reported as the mycoparasites of *Mucor* spp. (Mehrotra and Prasad, 1965, 1967).

***Aspergilus niger* (Kalisena)-** It is commonly called as "Black mould" and is a common contaminant in laboratories. The mycelium is scanty, hyaline to white or light yellow. Conidiophores arise from the "foot cell" and terminate in an inflated apex upon which radiating phialides are formed. Conidial heads appear globose. They are black, globose, or radiate. Conidia are produced in chains on the sterigmata. They are single-celled, pale to dark brown, more or less globose, with low to prominent ridges and echinulations that are with rough or echinulate surfaces (Thom and Raper, 1945). *A. niger* has been utilized to form 'Kalisena' by Vineeta Sen (1999).



Aspergilus niger

Biofungicide (Kalisena) Larger Image-

Kalisena (*Aspergillus niger* AN27)

An effective biofungicide as well as biofertilizer, based on the technology developed by IARI, New Delhi.

Advantage of use -

1. A biofungicide controls *Fusarium* spp., *Pythium* spp., *Rhizoctonia* spp., *Macrophomina* spp, and *Sclerotinia* spp.

2. Can work under diverse abiotic conditions (temperature ranging from 0-45^{0}C, no specific humidity and pH range from 3-10).
3. Excellent biofertilizer cum-growth promoter.
4. Enhance seed germination percentage, speed up initial plant growth.

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