BIODIVERSITY OF MOSQUITOCIDAL FUNGI AND ACTINOMYCETES

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ABSTRACT

Fungi belonging to several genera are most frequently associated with insect diseases including mosquitoes. The genus Coelomomyces is composed of a large group (ca.70 species) of obligately parasitic aquatic fungi, the overwhelming majority of which have been reported from mosquito larvae. All of them are obligate parasites and require a mosquito host and a copepod host to complete their life cycle. There are several isolates of the fungus Lagenidium giganteum, which are pathogenic for mosquito larvae. They are facultative parasites and not tolerant to organically polluted water or brackish water. Significant numbers of adults of Culex pipiens are infected with Entomophthora spp. Entomophthora aquatica infecting Aedes larvae was also reported. Investigation of the potential of some isolates of Beauveria bassiana for larval mosquito control was not promising. Another species, Beauveria brongniartii (=tenella) was found to be virulent against a wide variety of mosquito larvae. Culicinomyces clavoporous were originally found as contaminants in laboratory populations of mosquitoes. They are facultative parasites and unlike other Fungi Imperfecti, conidia are produced on submerged mosquito cadavers in water. Some isolates of Metarrhizium anisopliae were found to be pathogenic to mosquito larvae. The fungus is a facultative parasite and all mosquito species tested are susceptible to its conidia.

The secondary metabolites of Aspergillus, Penicillium, Fusarium, Paecilomyces, Rhizopus, Amanita, Syncephalastrum, Monilia and Tolypocladium species have been reported to be toxic to mosquitoes. The secondary metabolites of actinomycetes, tetranectin, avermectins, faerifungin and macrotetrolides and flavonoids produced were found to be toxic to mosquitoes and the species genera involved are Micromonospora, Actinomadura, Actinoplanes, Micropolyspora, Nocardiopsis, Oerskonia, Thermomonospora, Sreptoverticillium and Chainia

INTRODUCTION

The emphasis since the early 1960s on the development of integrated mosquito control programmes for vector and non-vector mosquitoes resulted in an intensification of research on a variety of biological control agents considered potentially useful in such programmes. This research produced a wealth of new information on the biology and mosquito control potential of organisms as diverse as predatory insects and fish, parasitic nematodes and a wide variety of pathogens including viruses, bacteria, protozoa and fungi.

FUNGI AS PARASITES

The fungal genera most frequently associated with insect disease are listed in Table 1. The host range of some species are limited to a few species of insects, while wide host ranges are the rule with certain species. There are many strains among the various species and some may vary extremely in their host range.

Table 1: Taxonomic distribution of entomogenous Fungi

Mastigomycotina	Ascomycotina	Deuteromycotina
Coelomomyces	*Ascosphaera*	*Acrostalagmus*
Myiophagus	*Calonectria*	*Aegerita*
Blastulidium	*Cordyceps*	Aschersonia
Aphanomyces	*Hypocrella*	Aspergillus
Leptolegnia	*Laboulbeniomyces*	*Beauveria*
Lagenidium	*Myriangium*	*Cephalosporium*
	Nectria	*Gibellula*
Zygomycotina	*Ophiocordyceps*	*Hirsutella*
	Podonectria	*Hymenostilbe*
Culicola	*Torrubiella*	*Isaria*
Entomophaga	*Sphaerostilbe*	*Metarrhizium*
Entomophthora		*Microcera*
Massospora	Basidiomycotina	*Paecilomyces*
Strongwellsea		*Penicillium*
Tarichium	*Septobasidium*	*Nomuraea*
Triplosporium	*Uredinella*	*Syngliocladium*
Zoophthora		*Synnematium*
Zygnaenobia		*Tetracroin*
		Verticillium

Conidium invasion through the respiratory or alimentary tract is reported but these are relatively rare invasion sites. Conidia usually germinate on the cuticle or from appressoria and then penetrates. Enzymes and mechanical forces are involved. In most cases, yeast-like fragments of mycelium called hyphal bodies are produced which usually float free and apparently multiply in the haemocoel. Some strains produce toxins in this stage to cause death; when organ invasion does occur, the fat body is almost invariably the preferred site. After death, or even before, in those strains, which are weak toxin producers, the mycelium ramifies the internal organs until the insect is virtually filled with fungus. Conidiophores are those produced which erupt through the cuticle and produce conidia on the exterior of the insect. In the genus *Coelomomyces*, the infection is induced in the definitive host (mosquito larva) by free-swimming zygotes from an intermediate host (copepods).

Insect pathogenic fungi produce toxins of two overlapping categories, i.e., those toxic *per os* and those toxic on injection. The former may have potential as insecticides while the latter are useful in elucidating the modes of action of pathogens. The first toxin to be studied was “aflatoxins” from *Aspergillus flavus* against honey bees (Burnside, 1930). Many antibiotics proved lethal to phytophagous mites. Materials toxic on injection are produced *in vitro* by *Metarrhizium anisopliae* (destraxins A & B) (Tamura & Takahash, 1970), *Beauveria bassiana* (beauvericin) and *Cordyceps militaris* (Cordycepin).

FUNGI PARASITIC TO MOSQUITOES

So far, 13 fungal genera are reported to be pathogenic to mosquito, viz., *Leptolegnia, Pythium, Lagenidium, Crypticola, Coelomomyces, Conidiobolus, Entomophthora, Erynia, Smittium, Culicinomyces, Beauveria, Metarrhizium* and *Tolypocladium*. They are distributed among five taxonomic classes, viz., Chytridiomyctes, Oomycetes, Zygomycetes, Trichomycetes and Deuteromycetes (Hyphomycetes). Some of the fungi are listed in Table 2.

COELOMOMYCES

The genus *Coelomomyces* is composed of a large group (ca.70 species) of obligately parasitic aquatic fungi, the overwhelming majority of which have been reported from mosquito larvae. They are among the few pathogens recorded as causing epizootics that result in high levels of mortality in larval populations. Keilin first described the genus *Coelomomyces* in 1921 and allied it with Chytridiales because sporangia production in *Coelomomyces* was similar to that in *Physoderma* and *Catenaria*, which were classified as Chytridiales. Strong interest in *Coelomomyces* was generated when parasitized mosquito larvae were collected by inspectors of the Georgia State Malaria Control in War areas. Whisler (Whisler *et al*., 1975)discovered that infection of larvae of *Culiseta inornata by Coelomomyces psorophorae* was only obtained when the copepod *Cyclops vernalis* was present. On close inspection, it was found that the copepods were full of motile cells that fused in pairs either in the body of the copepod or immediately after their emergence. These zygotes then went on to infect the mosquito. When other species of *Coelomomyces* were examined, it was also found that copepods (Pillai and Smith, 1968; Federici and Roberts, 1975) served as alternate hosts.

Table 2: Selected entomogenous fungi active against mosquitoes

Fungus	**Mosquito stage**	**Mosquito genus**	**Habitat**
Coelomomyces spp.	Larvae	All major genera	Aquatic, including brackish water (with some species of *Coelomomyces*)
Lagenidium giganteum		*Culex*	Aquatic, not brackish or high

Fungus	**Mosquito stage**	**Mosquito genus**	**Habitat**
	Larvae		organic pollution
Entomophthora aquatica	Larvae, pupae	*Aedes, Culiseta*	Aquatic
Entomophthora spp. & E.conglomerata	Adults	Culex	Water surface & vegetation surrounding water works and sewage systems
E. destruens	Adults	Culex	Over wintering & dark, moist resting places
Beauveria brongniartii (*tenella*)	Larvae	All major genera	Aquatic, including tree holes
B. bassiana	Adults	All major genera	Resting sites of adults
Metarrhizium anisopliae	Larvae	All major genera	Aquatic, including brackish & organically polluted water
Culicinomyces sp. & *C. clavosporus*	Larvae	All major genera	Aquatic
Fusarium oxysporum	Larvae	*Culex* and *Anopheles*	Soil
Tolypocladium cylindrospermum & T. inflatum	Larvae	Aedes	Soil & detritus

Table 3: *Coelomomyces* and their crustacean and mosquito hosts.

Coelomomyces	**Crustacean Host**	**Mosquito Host**
C. psorophorae	*Cyclops vernalis*	*Culiseta inornata*
C. punctatus & C. dodgei	*Cyclops vernalis*	*Anopheles spp.*
C. opifexi	*Trigriopus* sp.	*Aedes australis*
Coelomomyces sp.	*Elaphoidella taroi*	*Aedes spp.*
C. liensis	Acanthocyclops	*Culex spp.*

C. indicus	*Microcyclops varicans*	*Anopheles spp.*
C. stegomyiae	*Phyllognathopus viguerii*	*Aedes spp.*

LAGENIDIUM GIGANTEUM

There are now several isolates of this fungus, which are pathogenic for mosquito larvae (Umphlett & Huang, 1972; Couch & Romney, 1973; Galagali *et al.*, 1984). The zoospores of this fungus usually invade host larval mosquitoes through the buccal cavity or penetrate the cuticle of the head capsule. Mycelium develops in the hemolymph and eventually the insect is filled with resting spores or sporangia. If sporangia are present, tubes are forced through the cuticle into the water. The sporangia contents migrate to the tips of the exit tubes. The zoospores formed from this material directly infect other mosquitoes. *L. giganteum* isolates have limited infectivity for anopheline mosquitoes, however, they have a wide host range among culicines (Galagali *et al.*, 1984). They are not tolerant to organically polluted water or brackish water.

ENTOMOPHTHORA

Significant numbers of adult *Culex pipiens* are infected with *Entomophthora* spp. and *E. conglomerata* in summer months in Moscow. Mortalities of up to 100% from *E. destruens* in populations of *C. pipiens* in cool and damp resting sites have been recorded in many areas in Europe.

Entomophthora aquatica infecting *Aedes larvae* was found in Connecticut (Anderson & Ringo, 1969). The organism was difficult to culture and mosquito infection was not obtained in the laboratory. There have been virtually no studies to develop *Entomophthora* spp. as mosquito control agents.

BEAUVERIA

Beauveria bassiana is widely used in insect control studies, but investigation of the potential of some isolates for larval mosquito control was not promising (Clark *et al.,* 1968; Geetha and Balaraman, 1999). Another species, *B. brongniartii* (=*tenella*) was found to be virulent against larvae of a wide variety of mosquito larvae (Pinnock *et al.,* 1973).

CULICINOMYCES

Culicinomyces clavoporous was found in 1972 in Australia and North Carolina where they appeared as contaminants in laboratory populations of mosquitoes (Couch *et al.*, 1974). They are readily cultured on simple laboratory media and infect the host through the alimentary tract after ingestion of conidia. Unlike other Fungi Imperfecti, conidia are produced on submerged mosquito cadavers in water. The host range is restricted to nematocerous flies, particularly Culicidae and Chironomidae.

METARRHIZIUM

Metarrhizium anisopliae was used for insect control in the latter part of the 19th century in Eastern Europe. Later, some isolates were found to be pathogenic to mosquito larvae also (Roberts, 1974; Balaraman *et al.,* 1979). The fungus is easily produced in simple laboratory media, and all mosquito species tested are susceptible to the conidia of *M. anosopliae*. The principal mode of action in most cases is obstruction of air passage through the two tracheal trunks of the larvae. In mosquito larvae all air enters the insect through two spiracles at the tip of the siphon. These spiracles are surrounded by perispiracular valves regulated by muscles. *Metarrhizium* conidia adhere to these valves, germinate and penetrate through the cuticle into the hemocoel. The spiracles become obstructed in the process, and the lack of air and perhaps toxins produced by the fungus in the hemolymph, causes death of the host.

SECONDARY METABOLITES OF MICROORGANISMS

Microbial cells that stop dividing produce synthetases that catalyse transformation of unused substances into molecules called secondary metabolites and this process of secondary metabolism occurs also in plants. Secondary metabolites consist of natural products that (a) are restricted in taxonomic distribution, (b) are synthesized for a finite period by cells that have stopped dividing, and (c) most probably function as convenient disposal packages of excess primary substances. A minority of secondary substances also can affect growth, health or behavior of non-producer cells. Included in this category of allelochemic substances are hormones, pheromones, toxins, and antibiotics. Substrates of secondary metabolism consist of unused pools of such primary metabolites as acetate, pyruvate, malonate, mevalonate, shikimate, prephenate, amino acids and purines.

MOSQUITO ACTIVE SECONDARY METABOLITES

Among the microbes, fungi and actinomycetes are best known for their ability to produce a great variety of secondary metabolites (Rose, 1979; Vining, 1990). They have also proved themselves to be a source of powerful agents, which could be used in the control of pests and parasites. The biological properties of the secondary metabolites of fungi range from antibiotics to mycotoxins (Rose, 1979; Cole & Cox, 1981). These have been reported to cause retardation of growth, low level of fecundity, loss of fertility, mortality, repellency, etc.

The secondary metabolites of *Aspergillus*, *Penicillium*, *Fusarium*, *Paecilomyces*, *Rhizopus*, *Amanita, Syncephalastrum*, *Monilia* and *Tolypocladium* species have been reported to be toxic to mosquitoes (Roberts, 1981; Mishra *et al*., 1987; Weiser & Matha, 1988).

The secondary metabolites of actinomycetes, tetranectin (Ando, 1983), avermectins (Pampiglione *et a*l, 1985), faerifungin (Anon, 1990) and macrotetrolides (Zizka *et al.,* 1989) and flavonoids (Rao *et al.,* 1990) produced, respectively, by *Streptomyces aureus, Streptomyces avermitilis*, *Streptosporangium albidum*, *Streptomyces griseus* and *Streptomyces* sp., were reported to be toxic to mosquitoes. It has also bean reported that the secondary metabolites of *Micromonospora*, *Actinomadura*, *Actinoplanes*, *Micropolyspora*, *Nocardiopsis*, *Oerskonia*, *Thermomonospora*, *Sreptoverticillium* and *Chainia* are toxic to mosquito larvae.

Vijayan and Balaraman (1991) studied the ovicidal, larvicidal and adulticidal activities of the metabolites of fungi and actinomycetes against *Culex quinquefasciatus*, *Anopheles stephensi* and *Aedes aegypti*. The metabolites of 34 fungi and 3 actinomycetes, 133 fungi and 35 actinomycetes, and 17 fungi were found to kill the eggs, larvae and adults, respectively. The species of fungi and actinomycetes, which exhibited mosquitocidal activity are listed in the Table 4 and 5.

Table 4 : Source of fungal metabolites active against eggs/larvae/adults of mosquitoes

Alternaria	*Aspergillus*
Botrytis	*Cladosporium*
Chaetomium	*Fusarium*
Geotrichum	*Gliocladium*
Heterosporium	*Paecilomyces*
Penicillium	*Sporotrichum*
Sporothrix	*Trichothecium*
Trichoderma	*Thielavia &Trichosporium*

Table 5 : Source of actinomycetes metabolites active against eggs/larvae/adults of mosquitoes

Actinomadura	*Actinoplanes*
Amorphosporangium	*Ampullariella*
Dactylosporangium	*Elytrosporangium*
Micromormospora	*Microbiospora*

Nocardia	*Planomonospora & Streptomyces*

CONCLUSION

Fungi are a very complex group with great morphological diversity and they affect/kill mosquitoes due to debilitation. Yet another group of fungi (including the well known parasitic form *Metarrhizium* and *Beauveria*) have been observed to secrete certain secondary metabolites, which are known to kill mosquitoes. Apart from fungi, a few genera of actinomycetes have also been noted to elaborate certain secondary metabolites, which have been observed to kill mosquitoes.

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