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**MOSQUITO ENTOMOPATHOGENIC FUNGI - AN OVERVIEW REGARDING A
POSSIBLE INTEGRATION IN *Aedes aegypti* AND *Aedes
albopictus* CONTROL PROGRAMS IN BRAZIL.**

(*FUNGOS ENTOMOPATOGÊNICOS DE MOSQUITOS - UMA ABORDAGEM GERAL CONSIDERANDO-SE UMA
POSSÍVEL INTEGRAÇÃO EM PROGRAMAS DE CONTROLE DE *Aedes aegypti* E *Aedes albopictus* NO
BRASIL*)

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INTRODUCTION

The origin of *Aedes albopictus* is considered to be in southeast Asia, and the recent introduction of the species into the United States and Brazil has been regarded as the most singular medical entomological event of the past decade in Americas^{56,63}. Geographic strains may vary markedly in oral susceptibility to dengue virus, showing therefore different vectorial competencies. One strain from Brazil (State of Espirito Santo) showed to be able to transmit under laboratory conditions, all four-dengue serotypes and a sylvan strain of yellow fever⁵⁷. Furthermore, under laboratory conditions Brazilian strains of *Ae. albopictus*, showed to have a degree of vertical transmission of dengue viruses and have therefore the potential to play a role in the maintenance of dengue viruses in nature¹²¹. Even so, no epidemics has been correlated to this species in Brazil unless when occurring in sympatric with *Ae. aegypti*. Recent

reviews have concerned about the genetics⁵⁹, biology⁶⁰ and importance in Americas⁵⁵ of this species.

In many parts of the world where both species are recorded to be introduced, ecological studies on the interaction between the two species have been done in order to evaluate the possible epidemiological consequences. Competitive displacement can permit that *Ae. aegypti* populations spread throughout the *Ae. albopictus* distribution, eliminating this last species. Otherwise, and as seems to occur in the United States and Hawaii, *Ae. albopictus* could displace *Ae. aegypti* populations⁵⁵. The vectorial competence of each species or introduced strain should be taken in account. Also, inter-specific hybrids mainly between *Ae. albopictus* males and *Ae. aegypti* females can occur in the field⁶¹. The vectorial competence must be in the same way determined to the hybrids.

Dengue epidemics have not evolved where only *Ae. albopictus* populations occur in Brazil and due to this fact authorities have given emphasis and priority, in practice, to *Ae. aegypti* control. Furthermore, the occurrence of *Ae. albopictus* in semi-urbanized and rural environments sometimes in association with its native breeding microhabitats such as tree holes⁵⁸ or bamboo stumps (personal observation), bring an additional difficulty to its control in Brazil. Besides dengue, an additional problem could be raised since epidemics of urban yellow fever could be restarted with *Ae. albopictus* acting as a link to the common sylvatic cycle of the viruses. *Ae. albopictus* population densities much higher than that of *Ae. aegypti* are being quite frequent and even predictable to some regions in the last years. Once the official monitoring programs detect *Ae. aegypti* occurrences, efforts are done until the complete eradication of the foci. In detriment, common high populations densities of *Ae. albopictus* leave out of control and Breteau indexes as great as 25 or 30 are recorded.

Naturally in this situation, a mosquito strain with a supposed

relatively low vectorial competence could cause epidemics, as previously reported to *Ae. aegypti* and sylvatic yellow-fever in Nigéria⁶¹.

As a rule, control campaigns in Brazil have followed the precepts of WHO and PAHO⁸⁹, mainly based in breeding site reduction whether with community participation or not, and treatments with chemical insecticides. In short, this late consists in the use of thermonebulization and residual sprays in resting sites against adults and larvicidal water treatment with pyrethroids and organophosphorus compounds. Besides these practices, a great interest upon alternatives to conventional control can be noted among governmental personnel involved in *Aedes* control campaigns.

Studies and attempts on the biological control of mosquitoes have been extensively revisited in last decades and the reasons why can be summarized as follow:

1. Many examples of agricultural insect pests consistently controlled either by the introduction of natural enemies or by the application of pathogens, lending to the industrialization of microbial insecticides;
2. Frequent epizootic among some mosquito populations permitting the self-maintenance of a natural control that can lower the costs of man-made control;
3. A higher ecological compatibility permitting lower environmental hazard risks due to a greater specificity of the biological control agents when compared with chemical insecticides. In the same way, safer to man and domestic animals. And,
4. A lower risk of resistance development.

The two basic approaches in utilizing fungi as microbial control agents would be either by inoculation of the pathogen in the environment followed by its natural spread (colonization method) or as biological insecticide. The first method should result in a continuous control, but not always total, acting as a density dependent

mortality factor. Some survivors will be always desirable in order to carry and maintain the fungus. The late method results in a non-self maintained control, since the fungus itself is not able to multiply and/or reinfect under natural conditions. This late method is also totally dependable upon man-made controlled applications and could result in the total elimination of the target mosquito population. Due to the gravity of the epidemiological aspects usually related to *Ae. aegypti* and *Ae. albopictus*, it seen that efforts on the second approach should ever have priority. Even so, considering some features pointed out to *Ae. albopictus* in Brazil, colonization could also be taken in account against this species. Any way, compatibility and a possible integration of selected fungi candidates with conventionally used chemical insecticides may concern. General aspects of possible interactions among entomopathogenic fungi and chemical insecticides has been pointed out by many authors^{115,114,134,105}.

In Brazil, there is a lack of laboratory and field studies regarding *Ae. aegypti* and *Ae. albopictus* biological control with *Bacillus thuringiensis* var. *israelensis* (BTI) and *Bacillus sphaericus*. The possibility of integration of BTI and the larvicide temephos was evaluated recently under laboratory conditions, against a field collected *Ae. aegypti* larval population in Campinas (State of São Paulo, Brazil)⁷⁷.

The aim of the present study is to point out the best-known entomopathogenic fungi recorded in *Aedes* species or related culicid in many parts of the world. Some biological features of the cited fungi species or supra specific groups are also presented, as well as aspects regarding to its production, compatibility with chemical insecticides and attempts to utilization in control operations. The present paper intent principally to motivate the search for native species and strains of mosquito entomopathogenic fungi suitable for local use, since there are

very few findings in Brazil. A recent book published by Weizer¹¹³ would be helpful as guideline in fieldwork on collecting and handling pathogens from mosquitoes.

RESULTS

Ecological as well as biological aspects of selected fungi related to *Aedes* species are presented in Tables 1 (**A** to **D**). Aspects of cultivation and large-scale production are presented in Tables 2 (A to D). Some Information on compatibility with chemical insecticides currently used in mosquito control, laboratory or field tests with the fungi will appear in Tables 3 (A to C). In this way, tables **A-** shows Information available to selected Hyphomycetes. Tables **B-** presents aspects to Zygomycetous fungi. Tables **C-** and tables **D-** to Oomycetes and species of the genus *Coelomomyces* respectively.

COMPLEMENTARY REMARKS AND REFERENCES

The records in Tables **A** related to occurrences of the fungi in *Aedes* spp or other mosquito genera were merely compiled from literature, reflecting therefore not only truly natural infections under field conditions but also accidental infection or attempts to infect under laboratory conditions. These late situations, consisting any way in a new host record to the pathogen. Even so, priority was given to records taken under natural conditions, mainly to fungi with worldwide distribution and wide host spectrum.

Geographic regions or countries listed in the present paper, in the same way, can reflect a natural collection site (as for example *Metarhizium anisopliae* occurring in *Ae. crinifer* in Argentina and *Beauveria bassiana* in former USSR) or even a successful attempt to introduction and colonization, as cited to *Coelomomyces stegomyiae* in

Tokelau Islands. Some strains of promising fungi have been traveled with mycologists from one continent to another or even accidentally in infected insects and the original occurrence of such fungi could actually only be speculated.

TABLE 1-A. Occurrences and geographical distribution of some **Hypomycetous** fungi related to *Aedes* spp. or related genus.

[ORDER] <i>Species</i>	OCCURRENCE IN MOSQUITO	RECORDS IN BRAZIL	<i>Aedes</i> AS HOST OR OTHER CULICIDAE (STAGE)*	NON MOSQUITO HOSTS
[MONILIALES]				
<i>Culicinomyces bisporalis</i>	Australia	-	<i>Ae. kochi</i> ()	
<i>Culicinomyces clavisporus</i>	Australia USA	-	<i>Ae. rupestris</i> ()	Other Dipterans ²³
<i>Tolypocladium cilisdrosporu m</i>	New Zealand Czekoslovakia USA	-	<i>Ae. australis</i> <i>Ae. sierriensis</i> (L,A)	<i>Daphnia</i> Copepods <i>Musca</i> ¹⁵
<i>Verticillium lecanii</i>	USA	APHIDS	<i>Ae. triseriatus</i> ()	APHIDS SCALES THRIPS
<i>Beauveria bassiana</i>	USSR	BEETLES CATERPILLARS ANTS, BUGS	<i>Cx. pipiens</i> (L,A)	MANY SPECIES
<i>Metarhizium anisopliae</i>	Argentina USA	BEETLES STINK BUGS CATERPILLARS	<i>Ae. crinifer</i> ¹³⁶ (A) <i>Ae. triseriatus</i> ¹³⁷ (L)	> 300 SPECIES
<i>Paecilomyces farinosus</i>	USA USSR Argentina	BEETLES CATERPILLARS	<i>Ae. sierriensis</i> <i>Ae. excrucians</i> <i>Ae. albifasciatus</i> (L,A)	BEETLES CATERPILLARS

REFERENCES

73,744,75,76

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73,74,75,76

* E= egg, L= larvae, P= pupae, A= adult

TABLE 1-B. Occurrences and geographical distribution of some **Zygomycetous** fungi pathogenic to *Aedes* spp. or related genus.

[ORDER] <i>Species</i>	OCCURRENCE IN MOSQUITO	RECORDS IN BRAZIL	<i>Aedes</i> AS HOST OR OTHER CULICIDAE (STAGE)*	NON MOSQUITO HOSTS
[ENTOMOPHTHORALES]				
<i>Conidiobolus thromboides</i>	Czechoslovakia	-	<i>Cx. pipiens</i> ()	APHIDS
<i>Erynia aquatica</i>	USA	IN APHIDS	<i>Ae. sp</i> <i>Ae. Fitchii</i> <i>Cu. morsitans</i> (L,P,A)	
<i>Eryniae ovispora</i>	Sweden	-
<i>Zoopthora radicans</i>	France	IN LEAFHOPPER CATERPILLAR (A)	9 OTHER INSECT ORDERS
<i>Entomophaga conglomerata</i>	USSR France	-	<i>Ae. comunis</i> <i>Cx. p. pipiens</i> <i>Cx. p. molestus</i> (L,A)	CHIRONOMIDAE TIPULIDAE
<i>Entomophthora culicis</i>	USSR , Tunisia Poland, Spain Switzerland	-	<i>Ae. aegypti</i> ¹⁰ <i>Aedes sp</i> , <i>Culex</i> <i>sp</i> (L,A)	ADULTS SIMULIID ⁸
[HARPELLALES]				
<i>Smitium culicis</i>	USA France	-	<i>Ae. Aegypti</i> , <i>Ae.</i> <i>berlandi</i> <i>Ae. Caspius</i> ; <i>Ae.</i> <i>detritus</i> <i>Ae. Geniculatus</i> ; <i>Ae. melanimon</i> (L)

REFERENCES 73,74,75,76 39 73,74,75,76

* E= egg, L= larvae, P= pupae, A= adult

TABLE 1-C. Occurrences and geographical distribution of some **Oomycetous** fungi pathogenic to *Aedes* spp. or related genus.

[ORDER] <i>Species</i>	OCCURRENCE IN MOSQUITO	RECORDS IN BRAZIL	<i>Aedes</i> AS HOST OR OTHER CULICIDAE (STAGE)*	NON MOSQUITO HOSTS
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[LAGENIDIALES]

<i>Lagenidium giganteum</i>	USA, India Africa, UK Antarctica		<i>Ae. aegypti</i> ⁴⁶ <i>Ae. melanimon</i> <i>Cx. quinquefasciatus</i> <i>Psorophora</i> sp. (L)	<i>Daphnia</i> COPEPODS ³⁵ CERATOPO- GONIDAE ⁸⁵
<i>Crypticola clavulifera</i>	Australia	-	<i>Ae. kochi</i> <i>Forcipomyia marksae</i> (L)	-

[PERONOSPORALES]

<i>Pythium</i> sp. (near <i>P. aderens</i>)	USA	-	<i>Ae. sierriensis</i> <i>Cu. inornata</i> <i>Cu. Insidens</i> <i>An. freeborni</i> (L)	-
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[SAPROLEGNIALES]

<i>Leptolegnia chapmanii</i>	USA	-	<i>Ae. triseriatus</i> <i>Cx. Quinquefasciatus</i> <i>Culex</i> sp. <i>Mansonia</i> sp. (L)	-
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73,74,75,76 39 73,74,75,76 /

* E= egg, L= larvae, P= pupae, A= adult

TABLE 1-D. Occurrences and geographical distribution of some **Chytridiomycetous** fungi pathogenic to *Aedes* spp. or related genus.

[ORDER] <i>Species</i>	OCCURRENCE IN MOSQUITO	RECORDS IN BRAZIL	<i>Aedes</i> AS HOST OR OTHER CULICIDAE (STAGE)*	OBLIGATE ALTERNATIVE HOST
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[BLASTOCLADIALES]

<i>Coelomomyces stegomyiae</i> var. <i>stegomyiae</i>	Malaya, Japan New Guinea Tokelau Solomon Isl. Philippines Sri Lanka		<i>Ae. aegypti</i> <i>Ae. albopictus</i> <i>Ae. Polynesiensis</i> <i>Ae. Multiformis</i> <i>Ae. Quadrimaculatus</i> <i>Ae. flavopictus</i> (L,A)	<i>Phyllognathopus viguieri</i> ¹⁰⁰
<i>Coelomomyces stegomyiae</i> var. <i>chapmani</i>	Taiwan China		<i>Ae. albopictus</i> <i>Ae. subalbatus</i> <i>Ae. yanbarensis</i> (L)	NOT KNOWN
<i>Coelomomyces dentialatus</i>	Madagascar		<i>Ae. aegypti</i> <i>Ae. albopictus</i> (L)	NOT KNOWN
<i>Coelomomyces dentialatus</i>	Burma, Fiji China, USA Thailand		<i>Ae. aegypti</i> <i>Ae. albopictus</i> <i>Ae. polynesiensis</i> <i>Ae. alcasidi</i> (L) <i>Toxorhynchites rutilus</i> <i>septentrionalis</i> ⁹⁵	NOT KNOWN
<i>Coelomomyces indicus</i>	India, Egypti Nigeria, Zambia Thailand, Kenia		<i>Ae. aegypti</i> ¹⁰¹ beyond 20 to 24 species in other genera ^{97,99} (L)	<i>Cyclops</i> sp ¹⁰¹
<i>Coelomomyces psorophorae</i>	USA Canada		<i>Ae. aegypti</i> <i>Aedes</i> spp (L,A) <i>Cx. quinquefasciatus</i> <i>Cu. inornata</i> <i>Psorophora howardii</i>	<i>Cyclops vernalis</i>

REFERENCES

73,74,75,76

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73,74,75,76

* E= egg, L= larvae, P= pupae, A= adult

TABLE 2-A. Aspects of cultivation and large-scale production of some **Hyphomycetous** fungi related to *Aedes* species.

[ORDER] <i>Species</i>	CULTURE	PRODUCTION	MASS STORAGE
[MONILIALES]			
<i>Culicinomyces bisporalis</i> <i>Culicinomyces clavisporus</i>	Different media according to the strain ⁷	Possible	Dry mycelium ^{25,40} . Few months at -20°C ⁶⁹
<i>Tolypocladium cilindrosporum</i>	Easily done Submerged or surface	Possible	Some months ^{40,44,70}
<i>Verticillium lecanii</i>	Already done. Granular or submerged aerated ¹¹⁸	Conidia or blastospores ¹¹⁸	
<i>Beauveria bassiana</i>	Easily done Submerged or surface	Already done ^{27,107,108}	Dry mycelium ⁷¹
<i>Metarhizium anisopliae</i>	Easily done Submerged or surface	Already done in Brazil ^{39b,108,108}	Depend upon the substrate of growth ⁷² .
<i>Paecilomyces farinosus</i>	Already done ¹²⁴		

TABLE 2-B. Aspects of cultivation and large scale production of some **Zygomycetous** fungi related to *Aedes* species.

[ORDER] <i>Species</i>	CULTURE	MASS PRODUCTION	STORAGE
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[ENTOMOPHTHORALES]

<i>Conidiobolus thromboides</i>	Easily done ⁹ Forms resting spores ^{37,38}	Resting spores in liquid media ^{10,37,38}	
<i>Erynia aquatica</i> <i>Erynia ovispora</i>	Very difficult Need cold water		
<i>Zoophthora radicans</i>	Easily done in suitable media ^{11,21,80}	Dry mycelium method. Patented ¹²	
<i>Entomophthora conglomerata</i>	Probably not easy	Regarding the genus ¹²⁵	
<i>Entomophthora culicis</i>		Regarding the genus ¹²⁵	

[HARPELLALES]

<i>Smithium culicis</i>	Some species can be cultured		
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TABLE 2-C. Aspects of cultivation and large scale production of some **Oomycetous** fungi related to *Aedes* species.

[ORDER] <i>Species</i>	CULTURE	MASS PRODUCTION	STORAGE
[LAGENIDIALES]			
<i>Lagenidium giganteum</i>	Easily done. Zoosporogenesis stimulated ¹⁶	Already done ³⁰	Encapsulation in alginates ³⁸
<i>Crypticola clavulifera</i>	Easily done		
[PERONOSPORALES]			
<i>Pythium</i> sp. (near <i>P. adhaerens</i>)	Easily done		
[SAPROLEGNIALES]			
<i>Leptolegnia chapmanii</i>	Easily done		Not done

TABLE 2-D. Aspects of cultivation and large scale production of some **Chytridiomycetous** fungi related to *Aedes* species.

SPECIES	CULTURE	MASS PRODUCTION	STORAGE
<i>C. stegomyiae</i> var. <i>stegomyiae</i>	-	-	-
<i>C. stegomyiae</i> var. <i>chapmani</i>	-	-	-
<i>C. dentialatus</i>	-	-	-
<i>C. macleayae</i>	-	-	-
<i>C. indicus</i>	In vivo ¹⁰²	-	-
<i>Coelomomyces psorophorae</i>	In vitro or In vivo ^{131,94}	Difficult	Sporangia in millipore filter at 5 ⁰ C ²⁶

TABLE 3-A. Compatibility with chemical insecticides and evaluations against *Aedes aegypti* and *Ae. albopictus* of some **Hyphomycetous** fungi.

[ORDER] <i>Species</i>	COMPATIBILITY WITH CHEMICAL INSECTICIDES	LABORATORY STUDIES AND EVALUATIONS AGAINST <i>Ae. aegypti</i> AND/OR <i>Ae. albopictus</i>
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[MONILIALES]

<i>Culicinomyces bisporalis</i>		LC ₅₀ =10 ³ -10 ⁴ conidia/ml against 3 rd instar <i>Ae. aegypti</i> larvae ⁷
<i>Culicinomyces clavisporus</i>		10 ⁵ -10 ⁷ conidia/ml resulted in 20-48% mortality in 2 nd instar <i>Ae. albopictus</i> larvae ⁵⁴ .
<i>Tolypocladium cilidrosporium</i>		Pathogenesis in <i>Ae. aegypti</i> ⁵³ .
<i>Verticillium lecanii</i>	42 insecticides evaluated. Diflubenzuron showed to be toxic ⁵	Higher mortality at 10 ⁸ blastospores or conidia/ml against <i>Ae. aegypti</i> larvae ² ;
<i>Beauveria bassiana</i>	Same as <i>P. farinosus</i> . Can be damage by Malathion ^{39,88,106} <i>B. brongniartii</i> was also evaluated ⁶ .	total control in 5 days against adults ⁴⁹ .
<i>Metarhizium anisopliae</i>	Same as <i>P. farinosus</i> . Can be inhibited by Malathion ⁸⁸ . Temephos, Leptophos and Malathion highly toxic to sporulation ⁴ . Affected by diflubenzuron ⁷ .	Toxaemia or septicemia as mode of action in <i>Ae. albopictus</i> . Efficient at 3x10 ⁵ conidia/ml against 4 th instar larvae ^{1,110} .
<i>Paecilomyces farinosus</i>	Growth and sporulation not affected by Diazinon, Pirimicarb and Cypermethrin ⁴³ .	Two Russian strains showed at least 50% efficiency against larvae of some mosquitoes ⁵¹ .

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106, 34

TABLE 3-B. Compatibility with chemical insecticides and evaluations against *Aedes aegypti* and *Ae. albopictus* of some **Zygomycetous** fungi.

[ORDER] <i>Species</i>	COMPATIBILITY WITH CHEMICAL INSECTICIDES	LABORATORY STUDIES AND EVALUATIONS AGAINST <i>Ae.aegypti</i> AND/OR <i>Ae.albopictus</i>
[ENTOMOPHTHORALES]		
<i>Conidiobolus</i> <i>thromboides</i>	Malathion: incompatible ^{39a} , conidia germination ⁸⁶ and resting spores inhibited ⁸⁷	
<i>Erynia</i> <i>aquatica</i>		10.7% adult mortality in <i>Ae. aegypti</i> when inoculated in pupae ³
<i>Erynia</i> <i>ovispora</i>		
<i>Zoophthora</i> <i>radicans</i>		CL ₅₀ =350-600 conidia/mm ² against <i>Ae. aegypti</i> adults ²²
<i>Entomophthora</i> <i>conglomerata</i>	Regarding the genus ¹¹⁹	
<i>Entomophthora</i> <i>culicis</i>	Not affected by Diflubenzuron.	
<i>Smithium</i> <i>culicis</i>	81	
REFERENCES	106, 34	

TABLE 3-C. Compatibility with chemical insecticides and evaluations against *Aedes aegypti* and *Ae. albopictus* of some **Oomycetous** fungi.

[ORDER]	COMPATIBILITY WITH CHEMICAL INSECTICIDES	LABORATORY STUDIES AND EVALUATIONS AGAINST
<i>Species</i>	<i>Ae. aegypti</i> AND/OR <i>Ae. albopictus</i>	
[LAGENIDIALES]		
<i>Lagenidium</i> <i>giganteum</i>	BHC, DDT, Toxaphene, Chlorpyrifos and Fenthion are toxic.	General ^{36,30} . Recycle in <i>Ae. aegypti</i> and persisted for 10 weeks ¹¹² .
	Methoprene, Malathion and Temephos are probably compatible at recommended rates ³²	
<i>Crypticula</i> <i>clavulifera</i>		
<i>Pythium</i> sp. (near <i>P. adhaerens</i>)		78
<i>Leptolegnia</i> <i>chapmanii</i>		??
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