INVESTIGATION ON ENTOMOGENOUS FUNGI IN THE CERRADO REGION AND THEIR UTILIZATION FOR MICROBIAL CONTROL OF PESTS

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1. Objectives

The development of biological control methods is important for the control of insect pests on soybean, rice, bean, pastures, etc. which are cultivated in the Cerrado region. Although microbial control is one of the promising strategies among the biological control methods, information on the distribution or the biological characteristics of the pathogens in this region which is essential for microbial control is limited. Therefore, in order to develop a base for microbial control, entomogenous fungi prevailing in this region were collected, isolated, bioassayed, and preserved.

2. Materials and Methods

Collection of entomopathogens: Insect cadavers infected with fungi which were collected from various locations, along with those obtained by rearing of healthy insects from the field, were used as sources of isolation.

Identification of fungi: The fungi were identified by microscopical observation using cadavers or slide cultures.

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Isolation of fungi: Sabouraud's dextrose agar medium containing 1% of yeast extract (SDY) was used for the isolation of the fungi. Fungi were isolated from conidia on the surface of cadavers, and when conidia were not formed on the cadavers, the fungi were kept in humid chambers at 25°C to allow sporulation. Conidia of Deuteromycetes were streaked on SDY plate, kept at 25°C for several days, and the isolated colonies were transferred to pure cultures. Conidia of Entomophthorales discharged from cadavers buried in plain agar were collected on a SDY plate, and pure cultures were obtained. *Metarhizium anisopliae* in soil samples were directly isolated by using Yaginuma's selective medium (Table 1). Two gram of each soil sample collected from various sites was suspended in 200 ml of sterilized distilled water, and applied on the surface of Yaginuma's medium directly or after 10 or 100 time dilution, and kept at 25°C to obtain colonies of *M. anisopliae*. The isolated fungi were cultured on SDY slant media for subculturing, preservation and as inocula.

TABLE 1 - Composition of Yaginuma's medium.

Oatmeal	30 g
75% PCNB W.P.[a]	0.67 g
58% Basic CuSO4 W.P.[a]	0.86 g
Chloramphenicol[a]	0.3 g
Streptomycine sulphate[a]	0.3 g
Agar	20 g
D.W.	1000 ml
pH	6.0

[a] Added after autoclaving.

Comparison of virulence of fungal isolates: Virulence of isolates of *Beauveria bassiana* against *Nezara viridula* was compared. The isolates used in this experiment consisted of #7 isolated from a larva of *Diatraea*

saccharalis collected from DF which had died during rearing in the laboratory, #11 isolated from an adult of Pentatomidae collected from DF, and F-287 isolated from *Aphrophora rugosa* collected from Japan. These isolates were cultured on SDY slants and the conidia were suspended in distilled water with 100 ppm of Tween 20 and diluted to obtain a conidial concentration of 1 x 10^7 to x 1 x 10^3/ml. The experimental insects consisted of 2nd instar nymphs of *Nezara viridula* which had been reared from eggs obtained from adults collected in the field. The insects were dipped into each suspension and reared after the inoculation. Each batch of 10 insects was reared in a Petri dish 6 cm in diameter with peanuts and water at 25°C under long day conditons (14L-10D). Cadavers obtained during the rearing were placed in humid chambers to allow outer fungal growth, and examined under a microscope to determine whether they were killed by the inoculated fungi.

Miscelaneous: Brazilian and Japanese methods of isolation of fungi from soil samples with healthy insects, culture of fungi, preservation, etc. were compared and suggestions were made if necessary. EMPA was visited and the facilities for microbial control experiments were inspected.

3. Results and Discussion

Collection and identification of fungi: The fungi isolated from cadavers of insects that were collected are indicated in Table 2 and Figures 1 to 10. As the specimens from each study site were collected from various areas, although the numbers of cadavers at each site were not directly proportional to the infection rate of the host populations, they reffected the density of cadavers. As soybean fields were investigated frequently, *B. bassiana* on Chrisomelidae and *N. rileyi* on Lepidoptera were frequently detected. These fungal species are also commom species in Japan. Although *B. brongniartii* and *Paecilomyces farinosus* are rather commom species in Japan, they were not found in our studies, but *P. farinosus* was reported on *Spaethiella tristis* in oil palm plantation in Amazon State (Garcia et al. 1988).

TABLE 2 - Materials collected.

Locality	Host	Pathogen	Number of specimens collected
A	LEPIDOPTERA sp. AD.	*Beauveria bassiana*	1
	Lagria villosa LA.	*Paecilomyces* sp.	3
B	*Anticarsia gemmatalis* LA.	*Nomuraea rileyi*	6
	LEPIDOPTERA sp. LA.	*Beauveria bassiana*	1
	Lagria villosa AD.	*Conidiobolus apiculatus*	8
C	*Anticarsia gemmatalis* LA.	*Nomuraea rileyi*	1
	Cerotoma sp. AD. (reared)	*Beauveria bassiana*	1
	Lagria villosa LA.	*Paecilomyces* sp.	1
	COLEOPTERA sp. AD.	*Beauveria bassiana*	3
	COLEOPTERA sp. AD.	*Metarhizium anisopliae*	2
	CURCULIONIDAE sp. AD.	*Beauveria bassiana*	2
	COLEOPTERA sp. AD.	*Beauveria bassiana*	1
	PENTATOMIDAE sp. AD.	*Beauveria bassiana*	1
D	COLEOPTERA sp. AD.	*Beauveria bassiana*	1
E	*Anticarsia gemmatalis* LA.	*Nomurae rileyi*	1
	Diabrotica speciosa AD.	*Beauveria bassiana*	4
	Maecolaspis sp. AD.	*Beauveria bassiana*	6
	CHRYSOMELIDAE sp. AD.	*Beauveria bassiana*	1
	DERMAPTERA spp.(2spp.) AD.	*Beauveria bassiana*	3
	PENTATOMIDAE sp. AD.	*Beauveria bassiana*	1
F	LEPIDOPTERA sp. LA.	*Nomuraea riyeli*	9
G	*Hortensia* sp. NI, AD.	*Conidiobolus apiculatus*	44
	ORTHOPTERA sp. ?	*Hymenostilbe sp.*	1
H	*Anticarsia gemmatalis* LA.	*Nomuraea rileyi*	6
	Diatraea saccharalis AD.	*Akanthomyces* gracilis	7
	LEPIDOPTERA sp. AD.	*Akanthomyces* sp.	2
	Cerotoma sp. AD.	*Beauveria bassiana*	6
	Diabrotica speciosa AD.	*Beauveria bassiana*	29
I	*Cerotoma* sp. AD.	*Beauveria bassiana*	1
	Diabrotica speciosa AD.	*Beauveria bassiana*	1
	Maecolaspis sp. AD	*Beauveria bassiana*	1
	Neobaridia amplitaris AD.	*Beauveria bassiana*	1
	Lagria villosa AD.	*Metarhizium anisopliae*	1
J	*Leptopharsa heveae* LA, AD.	*Hirsutella* sp.	8
K	*Diatraea saccharalis* LA.	*Beauveria bassiana*	2
	Nezara viridula NI	*Serratia marcescens*	1

A: Soybean field, CPAC Campus
B: Soybean field in DF
C: Soybean field & weeds, Sítio Asano, Rio Preto PAD DF
D: Pastures DF
E: Soybean field, Fazenda Tanani, DF
F: Cowpea field, CNPAF Campus, GO
G: Rice paddy, CNPAF, GO
H: Soybean field, Fazenda Itamarati, MT
I: Experimental field of rice & soybean, Alcohol Factory, Fazenda Itamarati, MT
J: Rubber plantation, Fazenda Itamarati, MT
K: Insects reared in laboratory
AD: Adult
LA: Larvae
NI: Nymph

FIG. 1 - Infection of *Diabrotica speciosa* with *Beauveria bassiana*.

FIG. 2 - Infection of *Anticarsia gemmatalis* with *Nomuraea rileyi*.

FIG. 3 - Infection of *Lagria villosa* with *Conidiobolus apiculatus*.

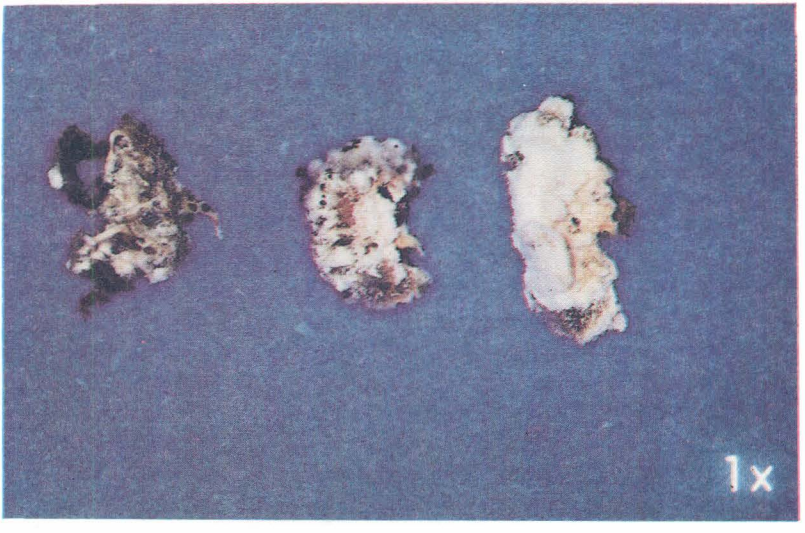

FIG. 4 - Infection of *Lagria villosa* with *Paecilomyces* sp.

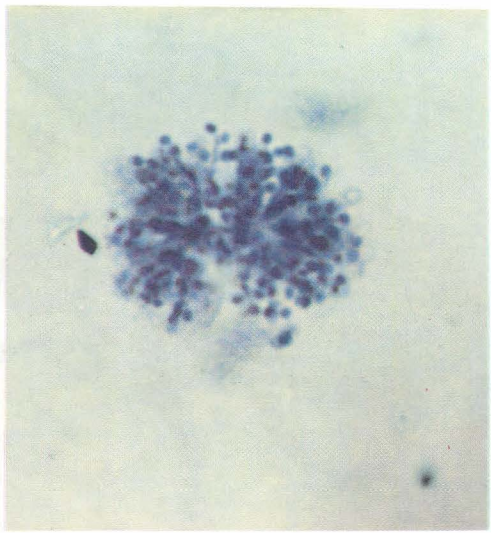

FIG. 5 - Structure of conidia of *Beauveria bassiana*.

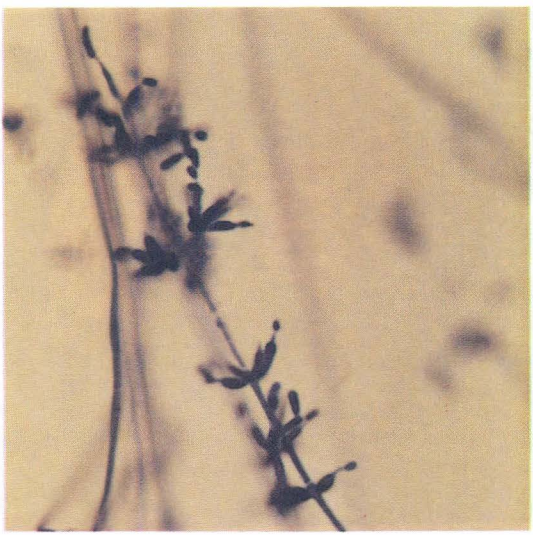

FIG. 6 - Structure of conidia of *Paecilomyces* sp.

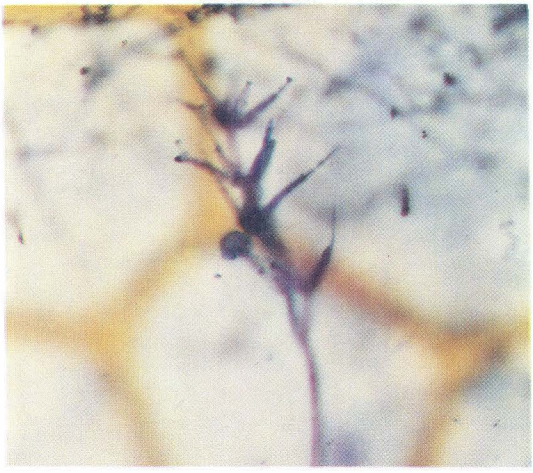

FIG. 7 - Structure of conidia of *Hirsutella* sp.

FIG. 8 - Synnema of *Akanthomyces gracilis*.

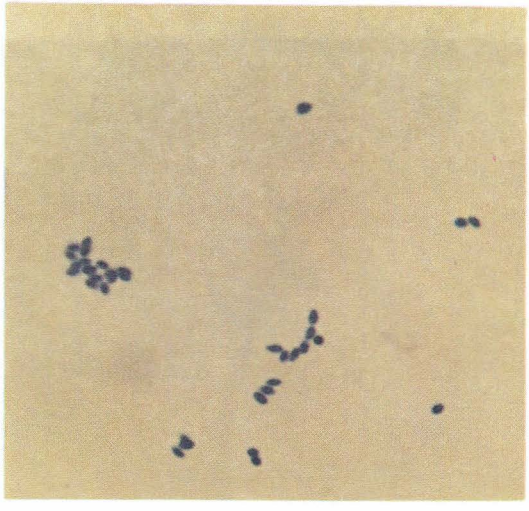

FIG. 9 - Conidia of *Nomuraea rileyi*.

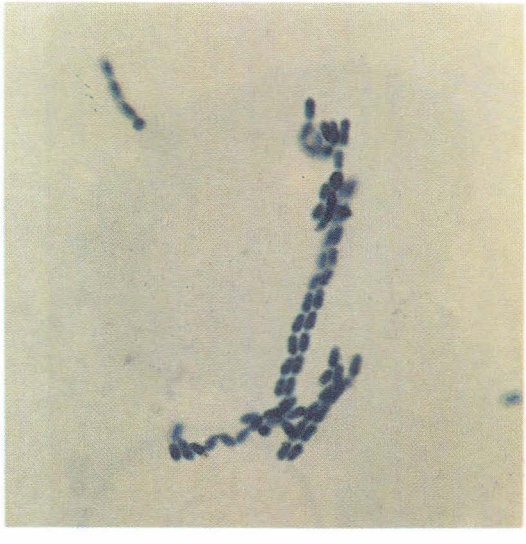

FIG. 10 - Conidia of *Metarhizium anisopliae*.

Sporothrix insectorum which has been reported as parasite on *Leptopharsa heveae* in Brazil, one of the important pests of rubber trees, was not found on *L. heveae* in the Fazenda Itamarati-MT. However, an other fungus, *Hirsutella* sp. was detected on it in MT. *Sporothrix insectorum* is extensively used for biological control of *Leptopharsa heveae* on rubber plantation in Mato Grosso State. *Hirsutella verticillioides* is also utilized as biological control agent, but it is endemic on *L. heveae* population in Para and Mato Grosso State (Charles, 1937; Junqueira et al. 1987). The other entomogenous fungi have been reported by Alves, R.T. 1986 and Alves, S.B. 1985). A specie of *Paecilomyces* on *Lagria villosa* was rather often found in DF. This specie may be a new species of *Paecilomyces*, and it will be necessary to investigate it in detail. These entomogenic fungi have also been reported by several investigators (Alves, R.T. 1986; Alves, S.B. 1985; Charles, 1937; Junqueira et al. 1987).

Isolation of fungi: The isolates obtained from the cadavers are listed in Table 3. Different isolates were prepared if the collection sites were different even when the fungal species and the host species were the same. Among the isolates, *B. bassiana* was the most common fungus. *Hirsutella* sp. on *L. heveae*, and *Akanthomyces* spp. on Lepidoptera adults were too old to be isolated. The isolates are preserved at CPAC, and can be used when needed.

TABLE 3 - Fungi isolated from the cadavers.

Species of fungus		Number of isolates
Bacteria		
Serratia marcensces		1
Fungi		
Zygomycetes:	Entomophthorales	
	Conidiobolus apiculatus	3
Deuteromycetes:	Moniliales	
	Beauveria bassiana	20
	Metarhizium anisopliae	3
	Nomuraea rileyi	2
	Paecilomyces sp.	4

Comparison of virulence: The mortality rate of *N. viridula* dipped in the fungal suspensions is listed in Table 4. Mortality by *B. bassiana* was observed only in the plot of 1 x 10^6 and 1 x 10^7/ ml of isolate #7. Mortality caused by other isolates was not detected. Insects inoculated with fungi

easily develop Septicemia and the mortality rate by other causes often increases. However in this experiment, there was no correlation between the inoculum size and mortality or the number of days after inoculation until death, and the effect of the inoculation was only confirmed by the mortality of 2 individuals. Also, the LC_{50} values could not be calculated due to the low mortality of the fungi. This phenomenon can be ascribed to the low virulence of the fungi, or the moulty of the insects before the invasion of the fungi in their hemocoels. This assumption is based on the fact that 28% of the insects moulted within 1 day and 72% whithin two days after the inoculation. Therefore, it is preferable to use old instar nymphs which have a longer instar period or adults for infection experiments with *N. viridula*.

TABLE 4 - Mortality of *Nezara viridula* inoculated with isolates of *Beauveria bassiana*[a] (mortality with inoculated fungus is indicated []).

Isolate number	Concentration (conidia/ml)	Mortality (%)	Days to death	
			mean	s.e.
control	0	55	17.5	10.1
#7	10^3	50	19.8	20.3
	10^4	56	23.4	19.0
	10^5	58	11.6	6.7
	10^6	45[5]	16.4[7]	10.3
	10^7	63[5]	19.3[5]	9.7
#11	10^3	58	15.0	18.9
	10^4	60	10.1	5.6
	10^5	65	20.8	28.6
	10^6	65	26.2	18.2
	10^7	58	27.8	16.1
F-287	10^3	65	15.6	16.6
	10^4	56	15.5	20.2
	10^5	55	17.8	13.0
	10^6	46	12.8	8.8

[a] Ten individuals x 2, who died accidentally during the experiments were excluded from the number.

Miscelaneous: As techniques of isolation of pathogens from soil samples using living insects had not been applied at CPAC, this method was introduced and demonstrated. However, suitable insects were not reared at CPAC, and the technique was not practiced. It is suggested that in the entomology laboratory of EMPA provision should be made for the construction of rooms for mass production of fungi and mass-rearing of insects.

4. Problems

(1) The minimum equipment required for studies on microorganisms has been provided and it is possible to carry to research. However, in order to handle microbes efficiently, gas burners are necessary and gas pipes should be supplied. Also, laboratories for asseptic work should have ceilings.

(2) Some enterprises in Brazil have been producing *M. anisopliae* several years ago, and the utilization is more developed than in Japan.

However, when the conidial suspension is inoculated directly to autoclaved rice, fungal growth is slow and contamination may occur. If shaking cultures are used, far more inocula could be readily obtained and growth could become faster. Therefore, it is preferable to use a shaker incubator.

(3) Fungi should be preserved in liquid nitrogen or in deep freezers at 100°C. Fungi are preserved in sterilized water at CPAC, and this method is considered to be superior to subculturing on media.

(4) Microbial control cannot be successfully achieved without using appropriate pathogens, conditions, and application methods. These conditions depend of the target insect, pathogens used, and area for application. To obtain appropriate pathogens, it is necessary to collect them from various areas, and to conduct bioassays. For bioassays, the biology and ecology of target insects should be studied and these insects need to be reared through out the year. In the present experiment, the number and species of experimental insects were not sufficient, and proper infection experiments could not be conducted. It is thus necessary to set up a system to rear experimental insects for routine work at CPAC.

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