ISOLATION AND USE OF ENTOMOGENOUS FUNGI IN THE CERRADOS FOR THE CONTROL OF INSECT PESTS

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Introduction

In the Cerrado region, soybean, bean and rice are cultivated in addition to grassland agriculture. A large number of insecticides has been used for the control of insect pests attacking these crops. Among other methods, there are a few studies on microbial control using entomopathogenic fungi. Entomogenous fungi were collected, isolated from soil and bioassayed in order to study their distribution in soil at biological characteristics.

Materials and Methods

1. Collection of entomopathogens

1.1 Isolation from cadavers of insects killed by fungi

Insect cadavers were collected from various fields. Sabouraud's dextrose agar mediums supplemented with 0.5% yeast extract (SDY) containing 100 ppm of Chloramphenicol was used for the isolation of the fungi. Fungi were isolated from conidia on cadavers. When no conidium was observed, the cadavers were kept in humid chambers for conidium formation after sterilization of the surface with a solution of chlorinated lime. Conidia were streaked on a plate, incubated at 27°C, and isolated colonies were transferred to pure culture.

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1.2 Isolation of entomogenous fungi from soil

Soil samples were collected from various locations. Three selective media were Yaginuma's medium, Veen's medium and Baath's medium (Yaginuma, 1986), used for the isolation of entomogenous fungi, *Metarhizium, Beauveria* and *Paecilomyces* (Table 1, Figure 1 and Figure 2). One gram of each soil sample was suspended in 9 ml of sterilized distilled water containing 0.04% Tween 40, and shaken during 15 minutes. Two ml of the soil-suspension was mixed with 8 ml of sterilized distilled water, and 0.5 ml was inoculated on each selective medium. The solution was incubated at 27°C to obtain colonies of entomogenous fungi. Isolated colonies were transferred to plates of SDY, and sporulated. Densities of *M. anisopliae* in the soil samples were estimated by counting the colonies on medium.

TABLE 1 - Composition of three selective media.

Yaginuma's medium	
(Selective medium for isolation of *Metarhizium*)	
Oatmeal	30 g
distilled water	1000 ml
Agar	20 g
pH	6.0
(After autoclaving)	
75% PCNB W.P.	0.67 g
58% Basic copper sulfate W.P.	0.86 g
Chloramphenicol	0.3 g
Streptomycin sulfate	0.3 g
Veen's medium	
(Selective medium for isolation of *Beauveria* and *Metarhizium*)	
Dextrose	10 g
Peptone	10 g
Ox gall powder	15 g
Rose bengal	66 mg
Agar	30 g
Distilled water	1000 ml
(After autoclaving)	
Chloramphenicol	500 mg
Cycloheximide	250 mg
Baath's medium	
(Selective medium for isolation of *Paecilomyces* and *Metarhizium*)	
Malt extract	20 g
Agar	15 g
Distilled water	1000 ml
(After autoclaving)	
$CuSO_4$	400 mg

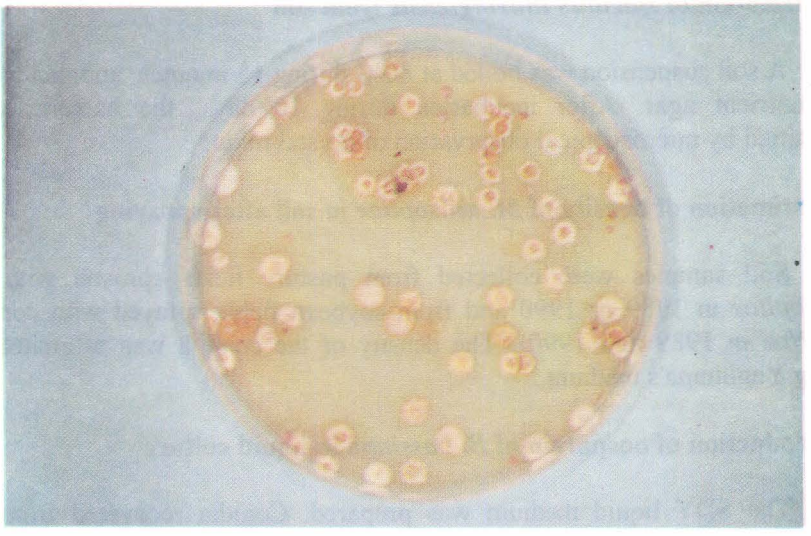

FIG. 1 - Yaginuma's medium for isolation of *Metarhizium anisopliae.*

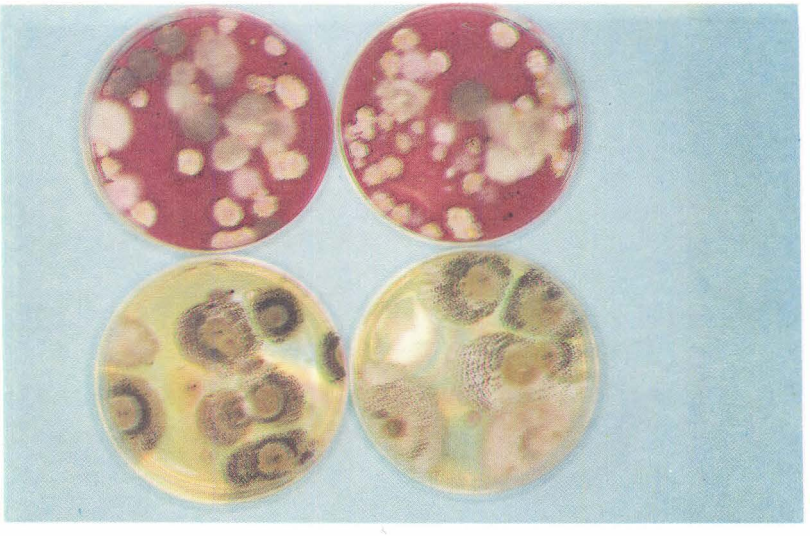

FIG. 2 - Colonies of *Metarhizium anisopliae* on Veen's medium (upper) and Baath's medium.

1.3 Isolation of *Bacillus thuringiensis* from soil

A soil suspension was boiled at 80°C during 15 minutes, and inoculated on nutrient agar. After incubation during 10 days, the bacteria were identified by microscopical observation of crystal toxin.

2. Estimation of density of *M. anisopliae* in soil after spraying

Soil samples were collected from pasture fields sprayed with *M. anisopliae* in 1989 or 1990 and from soybean fields sprayed with conidia (10^{12}/ha in 1989 and 1990). The density of the conidia was estimated by using Yaginuma's medium.

3. Production of oosporein of *B. bassiana* in liquid culture

The SDY liquid medium was prepared. Conidia recovered from 32 isolates from insect cadavers were inoculated in the medium, and shaken at room temperature during 7 days. Secretion of oosporein into the medium was detected by a change of color to red (Figure 3).

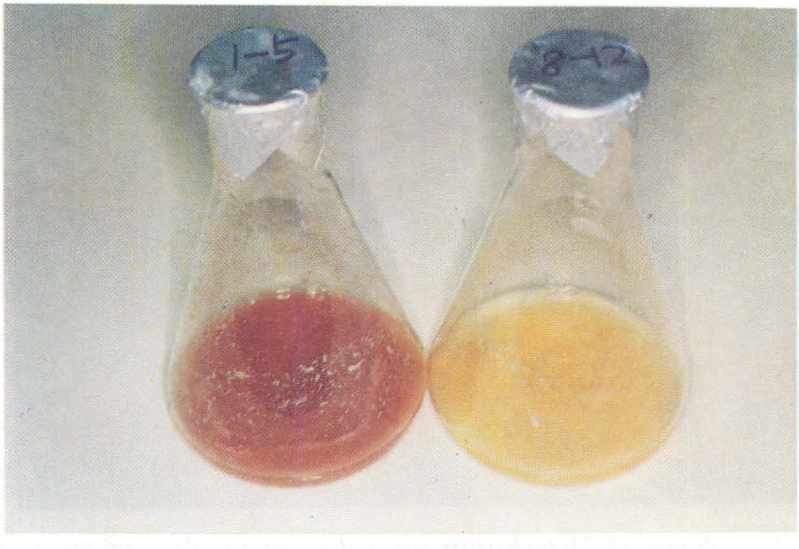

FIG. 3 - Liquid medium turning red by secretion of oosporein.

4. Survival of conidia of *B. bassiana* on leaf in soybean field

The isolates used in this experiment consisted of #1-4 isolated from *Cerotoma arcuata*. The isolates were cultured on rice (Figure 4), and the conidia were suspended in distilled water with 0.1% of Tween 40 and a suspension of 4.2 x 10^8/ml was sprayed on soybean. The soybean leaves were collected immediately after spraying, after 3 and 7 days. Five leaves collected were suspended in water with 0.1% Tween 40. The suspension was inoculated on plates of Veen's medium.

FIG. 4 - Isolates of *Beauveria bassiana* cultured on rice.

5. Comparison of virulence of isolates

Virulence of isolates of *B. bassiana* against the lace bug, *Vatiga illudens* was compared. Isolates #1-1, #5-1 and #7-1 from *Cerotoma arcuata*, FRB49 and FRB101 from sting bug and FRB97 from spittle bug were tested. These isolates were cultured on rice (Figure 4). Ten grains of rice were suspended in 10 ml of water with 0.1% Tween 40, and diluted with

water 10 to 1000 times. Nymphs and adults of the insects were collected from a cassava field. Leaves of cassava were dipped into each suspension. Approximately 8 or 10 insects were reared with a leaf dipped in a Petri dish at room temperature.

Results and Discussion

1. Collection of entomopathogens

1.1 Isolation from cadavers of insects killed by fungi

The cadavers of the insects killed by the fungi collected are shown in Table 2. Many cadavers of Coleoptera insects infected with *B. bassiana* were collected from soybean fields. Some cadavers of sting bugs and ants infected with *B. bassiana* were observed in soybean fields.

TABLE 2 - Insect cadavers collected.

Locality	Field	Host	Pathogen	Number of cadavers collected
DF	Soybean	*Cerotoma arcuata*	*Beauveria bassiana*	6
		Diabrotica speciosa	*Beauveria bassiana*	2
		Nezara viridula	*Beauveria bassiana*	1
		ant	*Beauveria bassiana*	1
		LEPIDOPTERA sp.	*Nomurae rileyi*	3
DF	Soybean	*Cerotoma arcuata*	*Beauveria bassiana*	6
		Diabrotica speciosa	*Beauveria bassiana*	1
DF	Soybean	*Cerotoma arcuata*	*Beauveria bassiana*	2
DF	Soybean	*Cerotoma arcuata*	*Beauveria bassiana*	2
		Diabrotica speciosa	*Beauveria bassiana*	1
DF	Soybean	*Cerotoma arcuata*	*Beauveria bassiana*	11
		Diabrotica speciosa	*Beauveria bassiana*	2
		COLEOPTERA sp.	*Beauveria bassiana*	1
		LEPIDOPTERA sp.	*Beauveria bassiana*	1
Itamarati MT	Soybean	*Cerotoma arcuata*	*Beauveria bassiana*	11
		LEPIDOPTERA sp.	*Nomuraea rileyi*	3
Itamarati MT	Cotton	LEPIDOPTERA sp.	*Nomuraea rileyi*	1
Itamarati MT	Soybean	*Euschistus heros*	*Beauveria bassiana*	5
		COLEOPTERA sp.	*Metarhizium anisopliae*	1
Itamarati MT	Soybean	*Cerotoma arcuata*	*Beauveria bassiana*	4
Manaus	Forest		*Beauveria bassina*	1
			Aschersonia sp.	1

1.2 Isolation of entomogenous fungi from soil

The results of detection of fungi from soil by using three kinds of selective media are shown in Table 3. *M. anisopliae* was detected in 42 of the 45 soil samples collected. As the soil samples were collected mainly from soybean fields, the detection rate was about 93%, a value higher than that recorded in Japan. *B. bassiana* was detected only from 4 soil samples in spite of the large number of insect cadavers with the fungus found in fileds.

TABLE 3 - Number of soil samples from which entomogenous fungi were detected by using three kinds of selective media.

		N° of soil samples where the fungi were detected		
Locality	N° of soil samples collected	*Metarhizium anisopliae*	*Beauveria bassiana*	*Paecilomyces* sp.
DF	20	20	4	0
Itamarati	18	17	0	0
Foz de Iguaçu	4	2	0	0
Manaus	3	3	0	1
Total	45	42	4	1

The population of *M. anisopliae* in the soil samples which is shown in Table 4, ranged from 5.2 x 10 to 1.9 x 10^4 colony-forming unit(CFU)/g dried soil. These results were similar to those obtained in Japan. Yaginuma's medium was particulary useful for the estimation of the density of the fungus composed with other media.

TABLE 4 - Population of *Metarhizium anisopliae* in soil.

Sample nº	Locality	Field	Population of *Metarhizium anisopliae* (CFU/g dried soil		
			Yaginuma's	Veen's	Baath's
1	DF	soybean	2.0×10^4	0	2.6×10^2
2	DF	soybean	1.7×10^4	0	0
3	DF	soybean	1.8×10^3	0	0
4	DF	soybean	1.9×10^4	0	0
5	DF	soybean	3.8×10^3	0	0
6	DF	soybean	2.8×10^3	0	6.7×10^2
7	DF	soybean	2.0×10^3	0	0
8	DF	soybean	5.2×10^3	0	0
9	DF	soybean	2.6×10^3	6.2×10^2	0
10	DF	soybean	2.2×10^3	0	1.3×10^2
11	DF	soybean	8.4×10^3	0	0
12	DF	soybean	4.9×10^2	0	4.9×10^2
13	DF	soybean	1.2×10^4	8.4×10^3	1.3×10^2
14	DF	soybean	2.8×10^3	3.4×10^3	0
15	DF	soybean	7.2×10^2	0	0
16	DF	soybean	1.8×10^4	0	0
17	DF	soybean	6.4×10^3	0	0
18	DF	pasture	1.0×10^3	0	4.5×10^2
19	DF	pasture	4.2×10^2	0	2.8×10^2
20	DF	pasture	2.4×10^3	0	3.1×10^3
21	Itamarati	paddy	2.6×10^3	0	0
22	Itamarati	cotton	1.2×10^4	3.8×10^2	0
23	Itamarati	soybean	1.6×10^4	2.6×10^2	2.6×10^2
24	Itamarati	bean	6.2×10^3	0	0
25	Itamarati	soybean	7.8×10^3	2.7×10^2	0
26	Itamarati	soybean	2.8×10^3	7.7×10^2	0
27	Itamarati	bean	1.1×10^4	3.8×10^3	0
28	Itamarati	soybean	1.4×10^4	3.0×10^3	7.8×10^2
29	Itamarati	pasture	0	0	0
30	Itamarati	pasture	0	1.0×10^2	0
31	Itamarati	soybean	7.1×10^3	0	0
32	Itamarati	soybean	9.6×10^2	0	0
33	Itamarati	cotton	1.1×10^4	1.8×10^3	0
34	Itamarati	soybean	4.5×10^3	1.3×10^3	1.3×10^2
35	Itamarati	uncultivated	0	1.1×10^2	0
36	Itamarati	uncultivated	5.2×10	0	4.1×10^2
37	Itamarati	rubber tree	3.1×10^3	0	0
38	Itamarati	rubber tree	5.4×10	0	0
39	Foz de Iguaçu	forest	0	0	0
40	Foz de Iguaçu	forest	0	0	0
41	Foz de Iguaçu	forest	4.3×10^2	0	0
42	Foz de Iguaçu	forest	3.1×10^2	0	0
43	Manaus	forest	1.4×10^2	0	0
44	Manaus	forest	2.2×10^2	0	1.1×10^2
45	Manaus	forest	8.0×10^2	4.0×10^2	6.7×10^2

1.3 Isolation of *Bacillus thuringiensis* from soil

Bacillus thuringiensis was not detected from the soil samples. As isolates of *B. thuringiensis* pathogenic to Coleoptera have been discovered recently, it will be necessary to continue this investigation.

2. Estimation of population of *M. anisopliae* in soil after spraying

As shown in Table 5, the population of *M. anisopliae* in the samples of soils from a grassland after spraying in 1990 were higher than those in 1989 and the control. These results indicate that the population of *M. anisopliae* in soil decreased with time. In the soybean fields, the density of the fungus in the soil samples after spraying were not different from that of the control (Table 5).

TABLE 5- Population of *Metarhizium anisopliae* in pasture field and soybean field after spraying.

Treatment	Sampling	Pasture field	Soybean field
M. anisopliae	1	2.1×10^3	1.7×10^3
was sprayed in 1989	2	9.3×10^3	3.8×10^3
	3	1.2×10^3	4.3×10^3
M. anisopliae	1	1.2×10^4	
was sprayed in 1990	2	1.3×10^4	
	3	1.4×10^4	
	1	1.2×10^2	1.4×10^3
CK	2	1.3×10^2	2.0×10^3
	3	1.4×10^2	1.4×10^4

3. Production of oosporein of *B. bassiana* in liquid culture

Five isolates of *B. bassiana* did not produce oosporein in liquid culture. It has been reported that the substance is also produced by other fungi, and is harmful to birds. Therefore, the isolates which did not produce oosporein could be used for the microbiological control of insect pests.

4. Survival of conidia of *B. bassiana* on leaf in soybean field

The number of conidia on 5 leaves of soybean collected immediately after spraying, and after 3 and 7 days was estimated to be 3.0×10^7, 6.2×10^5 and 4.8×10^5, respectively. After 3 days, the number decreased by about one fiftieth, but after 7 days, if did not decrease appreciably, presumably due to the lack of rain during the experiment. If it had rained, the number may have decreased more conspicuously.

5. Comparison of virulence of isolates

Mortality of *Vatiga illudens* reared on leaves dipped in a conidial suspension is shown in Table 6. The nymphs of the insect were more susceptible to all the isolates than the adults. After 3 days, cadavers of the insects killed by the fungus were already found. Most of the conidia were produced from ten grains of rice in the case of the isolates #5-1 and #7-1, and the highest mortality rate was also observed for the same isolates. As the concentration of the suspension of each isolate differed, the virulence of the isolates could not be compared directly to that of other isolates. The LC50 values were not calculated due to the high mortality rate of the differents isolates of the fungi. It was considered that the isolates #5-1 and #7-1 could be used for the control of the insect in this experiment.

TABLE 6 - Mortality of adult and nymph of lace bug, *Vatiga illudens* inoculated with isolates of *Beauveria bassiana*. (After 4 days).

	FRB49		FRB97		FBB101		#1-1		#5-1		#7-1	
	A*	N**	A	N	A	N	A	N	A	N	A	N
undiluted	100***	100	87.5	100	90	100	100	100	100	100	100	100
1/10	37.5	100	62.5	100	100	100	70	80	70	100	100	100
1/100	37.5	37.5	62.5	87.5	60	100	50	90	60	50	70	60
1/1000	12.5	12.5	0	12.5	30	0	50	80	50	50	80	80

Concentration of undiluted solution of FRB49, FRB97, FBB101, #1-1, #5-1 and #7-1 were $8x10^7$, 7.2×10^7, 3.0×10^8, 1.8×10^8, 4.5×10^8 and 4.2×10^8/ml, respectively.
Mortality of control was 0%.
* A: adult
** N: nymph
*** percentage

Reference Bibliografic

YAGINUMA, K. and TAKAGI, K. Improvement of a selective medium for isolation of *Metarhizium anisopliae* (Metschnikoff) Sorokin. **Japan Journal of Applied Entomology and Zoology**, v. 30, p.300-301, 1986.