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EFFECTS OF ENTOMOPATHOGENS ON ALBINO MICE

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The safety of three species of entomogenous fungi *Tolypocladium cylindrosporum* (Gams.), *Verticilium lacanii* (Zimm.) Viegas and *Paecilomyces fumosa-roseus* (Wize.) Brown & Smith was tested on laboratory mice. Acute oral dosages of 4.9 x 10^8 spores/mouse for *T. cylindrosporum*, 5.54 x 10^8 spores/mouse for *V. lacanii* and 4.5 x 10^8 spores/mouse for *P. fumosa-roseus* were administered for two weeks experimental period. No recoveries of the target organisms were recorded from any of the tested animals and there were no significant differences in terms of body weight, food and water consumption and blood counts. No histopathological changes were observed in liver and kidney on microscopic examination.

Key words: Murine safety, Entomogenous fungi, Albino mice.

Introduction

Entomogenous fungi represent a large group of microorganisms that have demonstrated potential as biological control agents against insect pest populations. Mammalian safety evaluations of fungi belonging to the family Entomophthoraceae and other entomogenous fungi are essential prior to their use as candidate biological insecticides in the field. Ignoffo (1975) and Ferron (1978) have reviewed major studies conducted on the microbial control of insects by fungi and interactions with non-target vertebrate hosts. Ignoffo (1973) reviewed *Conidiobolus coronatus* (*Entomophthora coronata*) as the causative agent of mycoses in man and horses, as reported in studies by Emmons and Bridges (1961), Bras *et al* (1965), Andrade *et al* (1967) and Chauhan *et al* (1973). These studies established the possibility of mycoses in vertebrate tissue, thereby making safety evaluations necessary for all species of entomogenous fungi that are potential biocontrol agents.

Keeping the above facts in view, these investigations were performed to assess the mammalian safety of *Tolypocladium cylindrosporum, Verticillium lacanii* and *Paecilomyces fumoso-roseus* on albino mice.

Materials and Methods

Stock cultures of *T. cylindrosporum, Verticillium lacanii* and *P. fumosa-roseus* were obtained from the Department of Biology, Rhode Island College, USA. Sub-cultures were grown on Sabouraud's maltose agar with 1% yeast extract (SMA + Y). Penicillin G 0.5g and 0.5g of Streptomycin sulphate were also added to each litre of medium.

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The mice used in the experiments were standard inbred strains of PCSIR Laboratory animal house, albino mice. The test animals were 4 weeks old and were kept on measured diet in separate cages for an additional 2-weeks period prior to testing. Twelve mice (6♀ + 6♂) served as control, while a similar batch of 12 animals each was used for tests with *T. cylindrosporum, V. lacanii* and *P. fumosa-roseus*. All the animals received the calculated feed. The control animals received sterile distilled water while the test animals received their respective fungal spore suspensions (*ad libitum*) in sterile distilled water with the help of cannula (Griffith and Farris 1942).

The test groups were allowed 15 days experimental period to consume the fungal suspensions and the suspensions were tested for viability prior to and during the test period. Viability test involved streaking on aliquot of the suspensions on sterile plates containing the appropriate medium for each species. Daily records of individual body weight, food and liquid consumption were kept for all the test animals. Faecal samples were collected from all the mice and suspensions were made by mixing 10 mg of faecal materials with 1 ml of distilled water and then plated on sterile media. A second faecal suspension was centrifuged with Tween-80 and washed in order to concentrate the spores and exclude the debris. The isolates were then streaked on agar plates and observed for growth of the target organisms.

The animals were observed for several parameters including abnormal behaviour and changes in appearance. Daily fungal intake was recorded for each mouse and during the experimental period the total number of viable spores consumed were 4.9 x 10^8 for *T. cylindrosporum*, 5.54 x 10^8 for *V. lacanii* 4.5 x 10^8 for *P. fumosa-roseus* for each test animal. At the end of the test

period 4 mice (two females and two males) from the control group and each of the test groups were autopsied. Gross pathological examinations of the animals were made and viscera such as the heart, lung, liver, stomach, intestine and kidney were cut in 2-3 mm pieces and plated on sterile agar. At the same time liver and kidney pieces were fixed in bouin's fluid and processed for histological studies (Askari *et al* 1996). The streaked plates were held for 15 days and observed for recovery of the target organisms. Prior to dissection, blood samples were collected from the mice via cardiac puncture and stained with Wright's stain for cytologic studies. Haematocrit values and differential blood values were recorded and compared between the control and test groups.

Results and Discussion

Recovery of *T. cylindrosprum, V. lacanii* or *P. fumosa-roseus* was not recorded in any of the tissues that were plated during autopsy of the animals. Histological examination of the fixed tissues showed no trace of the three fungal species. Fig 1 and 2 shows the control and treated animal which were autopsied. Fig 3 and 4 are of liver lobe, showing central vien, portal area and radiating hepatic cells. Fig 5 and 6 are of kidney, showing glomerular capsule (Bowman's), medullary rays and arcuate artery. These figures show no significant differences between the tissues of control and treated mice and no histopathological effects observed in the microscopic examination of the tissues. There was no recovery of any of the faecal pellet washings that were plated. These results indicate that the fungal spores were inactivated during the passage through the digestive tract of the host.

All the tissues of both the control and treated mice were free from detectable abnormalities and blood counts did not reveal any significant differences betwe the groups of mice (Table 1). Comparison of body weights showed no significant differences between control and test animals (Table 2). The average feed and liquid consumption was relatively unaffected by the intake of large quantities of spores in both the test groups (Scarborough 1931; Loomis 1978; Clarke 1978; Rowley and Ratcliffe 1988).

The dosage administered to the mice was significantly higher than amounts normally used during control programmes in the field and dosage level when extrapolated (2.6×10^{13} / 70 kg) to man are greater than any possible exposure or contamina-

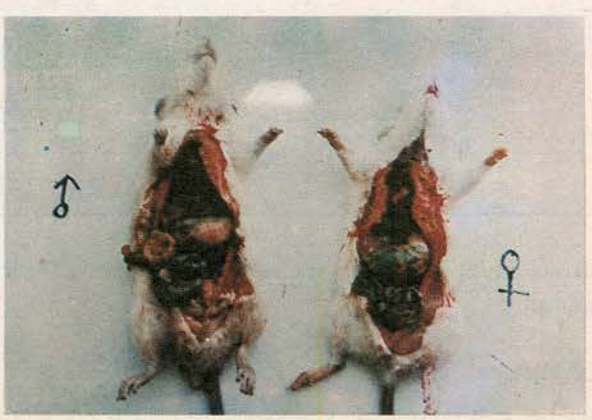

Fig 1. Control albino mice.

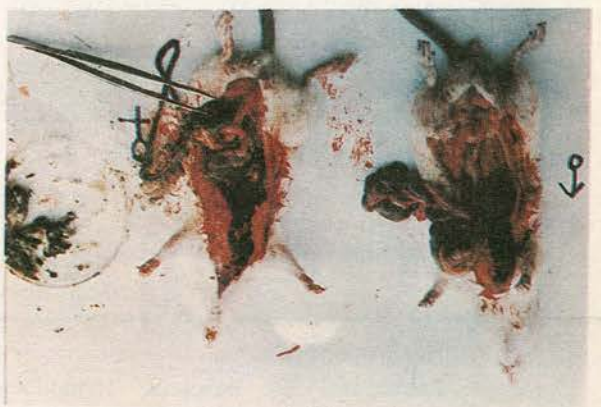

Fig 2. Treated albino mice.

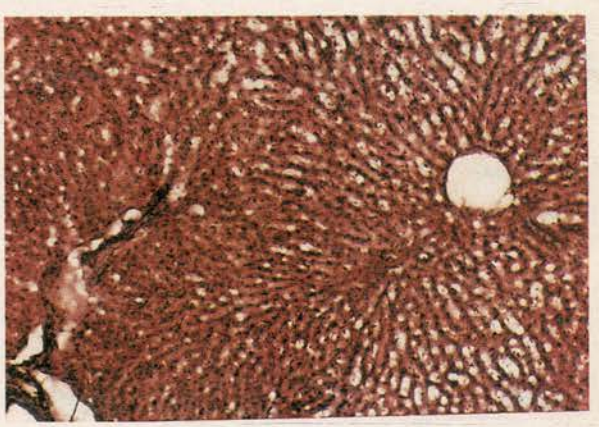

Fig 3. Normal liver tissue of albino mouse 20µ.

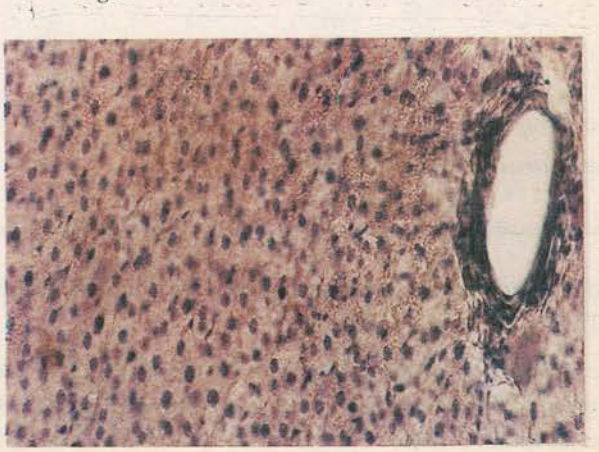

Fig 4. Treated liver tissue of albino mouse 40µ.

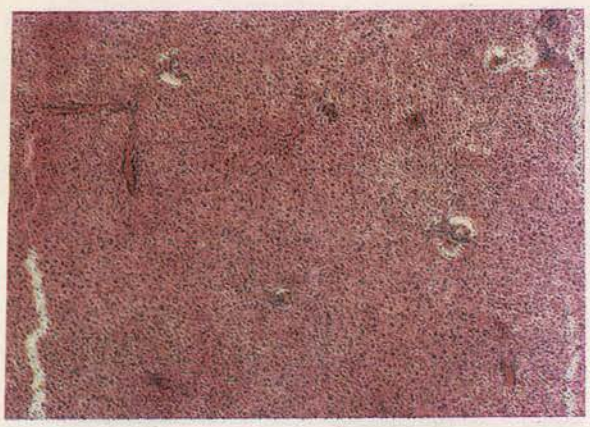

Fig 5. Normal kidney tissue of albino mouse 20µ.

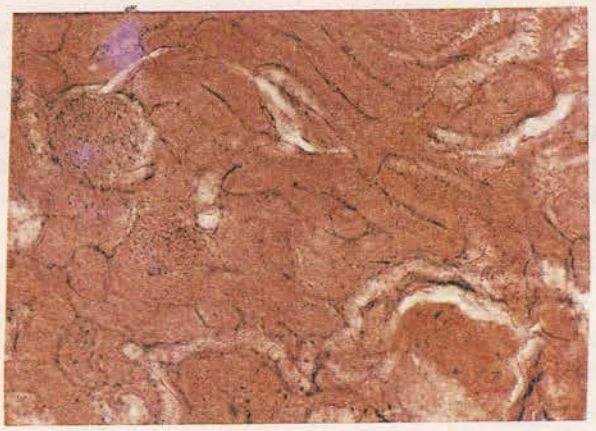

Fig 6. Treated kidney tissue of albino mouse 40µ.

Table 1

Effect of administering acute oral doses of *Tolypocladium cylindrosporum, Verticillium lacanii* and *Paecilomyces fumosa-roseus*

	Haematocrit (%) Packed cell vol		Differential count (%)					Total count	
			Basophil	Lymphocyte	Monocyte	Neutrophil	Eosinophil	W B C	R B C
	♀	46	0	70	2	28	0	6275	7,640,000
	♀	46	0	67	5	26	2	6125	8,230,000
Control	♂	47	0	69	1	30	0	7180	7,300,000
	♂	45	2	68	2	27	1	7000	7,390,000
Tolypocladium	♀	50	0	67	0	31	2	6925	8,040,000
cylindrosporum	♀	40	1	70	1	25	3	6665	7,760,000
	♂	48	0	73	0	26	1	7210	8,800,000
	♂	47	1	68	1	26	4	6030	8,078,000
	♀	40	0	70	0	30	0	6900	7,820,000
Verticillium	♀	45	2	67	5	25	3	6725	7,900,000
lacanii	♂	48	1	68	3	30	4	6100	8,010,000
	♂	47	0	65	4	28	3	7475	8,240,000
	♀	47	0	70	1	27	2	6650	7,780,000
Paecilomyces	♀	45	1	67	0	30	0	6550	7,980,000
fumosa-roseus	♂	48	0	65	4	30	1	7400	8,120,000
	♂	50	1	68	1	26	4	6950	7,810,000

tion during normal field use of entomogenous fungi as biocontrol agents (Wasti *et al* 1980).

It is, therefore, concluded that large oral doses of *T. cylindrosporum, V. lacanii* and *P. fumosa-roseus* are harmless to murine tissue. Further evaluation of these and other entomogenous fungi in non target invertebrates, avian and mammalian tissue is required. Some work is in progress and will determine the environmental impact of these microorganisms.

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Table 2

Average body weight of mice after acute oral doses of *Tolyoocladium cylindrosporum, Verticillium lacanii, Paecilomyces fumosa-roseus*

	No. of animals		Average body weight (g) ± S.E. Initial	Final	Dosage (spores)
Control	♀	6	41.8 ± 1.9057	45.51 ± 1.7755	
	♂	6	38.25 ± 1.2739	43.86 ± 1.6675	0
Tolypocladium	♀	6	34.66 ± 2.1366	34.25 ± 1.7923	
cylindrosporum	♂	6	41.73 ± 0.7837	38.75 ± 0.8139	4.9×10^8
Verticillium	♀	6	25.74 ± 1.1380	27.15 ± 1.7358	
lacanii	♂	6	31.08 ± 1.7196	35.28 ± 2.1770	5.54×10^8
Paecilomyces	♀	6	36.25 ± 2.366	34.15 ± 0.853	
fumosa-roseus	♂	6	32.06 ± 0.279	31.75 ± 1.095	4.5×10^8

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