PATHOGENICITY OF *ASPERGILLUS NIGER* AGAINST VARIOUS SPECIES OF TERMITES

KHALID IDREES KHAN, RAFAT H. JAFRI AND MUZAFFAR AHMAD

Department of Pharmacy, (KIK) and Department of Zoology, (RHJ, MA) University of the Punjab, Lahore-54900, Pakistan

Abstract: The workers of *Microtermes unicolor*, *Microcerotermes championi* and *Heterotermes indicola* were infected with a surface culture of *Aspergillus niger* for a period of 10, 20, and 30 minutes and the mortality pattern was described. Their 100% death occured quickly when the insects were crawled over a conidial culture of *A. niger* for a longer period of time. Out of 3 termite species *M. championi* was found to be more susceptible to *A. niger* than *M. unicolor* and *H. indicola*.

Key words: *Aspergillus niger*, pathogenicity, termites.

INTRODUCTION

Several ectoparasitic genera, *Ectomyces*, *Termiteria* (Tate, 1927, 1928) and *Antennopsis* (Buchli, 1951) are recorded to cause a mycosis in termites. Krejzova (1976) isolated a strain of *Paecilomyces fumoso-roseus* from *Zootermes formosanus* and *Reticulitermes lucifugus*. A few parasitic fungi on termites were also reported and described by several investigators (Bao and Yenndol, 1971; Kimbrough *et al.*, 1972; Yendol and Rosario, 1972; Rossi, 1974; Rossi and Rossi, 1977 a,b; Blackwell and Kimbrough, 1976, 1978.)

The mucoralian fungus, *Absidia coerulea* (Bainer) has also been found to cause a mycosis in *R. virginicus* (Lund and Engelhard, 1962). Comparative pathogenicity studies of *Metarrhizium anisopliae* (Metch) and *Beauveria bassiana* (Balsamo) against workers and soldiers of the formosan termite *Coptotermes formosanus* (Shiraki) have been made by Leong (1966). These two entomogenous fungi showed similar symptoms with *B. bassiana* being more pathogenic.

In laboratory test Beal and Kias (1962) exerimentally infected two subterranean species *Reticulitermes virginicus* (Banks) and *R. flavipes* (Kollar) with *Aspergillus flavus*. Sannasi (1968) observed dead queen and king of *Odontotermes obesus* and found *A. flavus* as the causative agent. *Aspergillus flavus* was also isolated from *Bifiditermes beesoni* collected from stem of *Pyrus communis* and it was fairly pathogenic to *M. championi*, *Heterotermes indicola* and *B. beesoni* (Khan, 1981).

According to Lenz (1968) a strain of *Aspergillus niger* was known to produce at least two toxins and one of them was found to be toxic to the termite *H. indicola*. But infection by *A. niger* on termites was not thoroughly described. Therefore, the present work was undertaken to test the pathogenicity of *A. niger* against the various species of termites.

MATERIALS AND METHODS

In order to test pathogenicity of *A. niger* against various species of termites in Pakistan, a pure culture of *A. niger* was obtained from the laboratory of Professor G. Becker (Bundesanstalt fur Malerialprufung, Berlin, West Germany). Its pathogenicity was tested against *Microtermes unicolor* Snyder, *Microtermes championi* Snyder and *H. indicola* (Wassman).

The inoculum was prepared by growing *A. niger* on Sabouraud's dextrose agar medium with yeast extract (SDA+Y) for 7 days as described by Yendol and Rosario (1972). The culture was homogenized in 100ml sterile distilled water. One ml aliquot of this homogenized suspension was then added to each petri dish (100x15mm) containing SDA+Y medium. These cultures were incubated at 25 ± 1 ^{O}C for 72 hours.

Microtermes unicolor, *Microcerotermes championi* and *H. indicola* workers were divided into four groups. Each had 25 termites. Group No. 1 of each species of termite was kept as control. While workers of group Nos. 2,3 and 4 of each species of termite were directly placed on 72 hours sporulating culture of *A. niger* and allowed to crawl over the fugal surface for 10, 20 and 30 minutes respectively. The control groups were treated in the same fashion, except that the fungal inoculation was omitted. When the inoculating period terminated, termites of each group were transferred to other petri dishes containing a double layered filter paper as a bed, slightly dampened with sterile water and their mortality pattern was noted after every 24 hours and the dead termites were carefully examined.

RESULTS AND DISCUSSION

In order to demonstrate the etiological agents, 72 hours infected dead specimens were removed from the holding Petri dishes, surface sterilized with 5.25% sodium hypochlorite and then rinsed several time in sterile water. The specimens were then placed on SDA+Y medium and incubated for 3 days at 25 ± 1 °C. Within this period, mycelium and a few spores of *A. niger* grew out of the infected termite onto the medium. The fungus was reisolated in pure culture, identified and Koch's postulates were fulfilled as described by Bao and Yendol (1971).

Observations after every 24 hour indicated that termites became gradually inactive and tend to appear in groups leaving behind the dead individuals. Their percent mortality was calculated daily and presented in Table 1-3. However, the data of LT_{50} and LT_{100} is shown in Table-4 for comparison. The termite workers (25 individuals) were divided into four groups as mentioned earlier. Group 1 of each species of termite was kept as control while group 2,3 and 4 were allowed to crawl over 72 hours conidial culture of *A. niger* for 10, 20 and 30 minites respectively.

At 48 hours following infection of *Microtermes unicolor* by *A. niger*, there was 52%, 60% and 48% mortality in group Nos. 2,3 and 4 respectively. There was 100% motality at 144 hours, 144 hours and 120 hours, in group Nos. 2,3 and 4 respectively (Table-1).

Table I. Percentage mortality of workers of *Microtermes unicolor* infected by 72 hour old culture of *Aspergillus niger*

Hours after infection	Group-1 (control)	Group-2 (crawling) 10 min.	Group-3 (crawling) 20 min.	Group-4 (crawling) 30 min
24	4	24	28	32
48	4	52	60	48
72	4	68	80	72
96	4	80	88	84
120	4	88	92	100
144	4	100	100	-

The LT_{50} was calculated as 46.8 hours hours, 41.8 hours and 50.4 hours, in group Nos. 2,3 and 4, respectively (Table-IV)

While at 48 hours following infection of *Microcerotermes championi* by *A. niger*, there was 76%, 50% and 80% mortality in group Nos. 2,3 and 4 respectively. However, their 100% mortality occurred at 96 hours, 96 hours and 72 hours following infection respectively (Table-II)

Table II. Percentage mortality of *Microcerototermes championi* infected by 72 hours old culture of *Aspergillus niger*

Hours after infection	Group-1 (control)	Group-2 (crawling) 10 min.	Group-3 (crawling) 20 min.	Group-4 (crawling) 30 min.
24	4	40	28	32
48	4	76	50	80
72	4	92	76	100
96	4	100	100	-

LT_{50} was calculated as 30 hours, 48 hours and 32.4 hours in group Nos. 2,3 and 4 respectively.

At 48 hours following infection of *H. indicola* by *Aspergillus niger*, there was 28%, 32% and 24% mortality in group Nos. 2,3 and 4, respectively; and their 100% mortality occurred at 264 hours, 216 hours respectively (Table-III).

Table III. Percentage mortality of *Heterotermes indicola* infected by 72 hours old culture of *Aspergillus niger*

Hours after infection	Group-1 (control)	Group-2 (crawling) 10 min.	Group-3 (crawling) 20 min.	Group-4 (crawling) 30 min.
24	0	8	16	16
48	0	28	32	24
72	4	32	44	44
96	4	44	64	60
120	4	52	72	76
144	4	72	80	84
168	4	76	88	92
192	4	80	96	96
216	4	88	100	100
240	4	92	-	-
264	4	100	-	-

LT_{50} was calculated as 115.2 hours, 79.2 hours and 81.4 hours in group Nos. 2,3 and 4, respectively.

An analysis of the results showed that all the species of termites were susceptible to *A. niger* infection. The comparative data on 100% mortality pattern shows that when the termites were allowed to crawl for a longer duration of time *i.e.*, up to 30 minutes, their mortality occured more quickly. Similarly, in case of *H. indicola*, the data of LT_{50} also showed that less time was required to kill 50% of the termites when these were allowed to crawl over a fungal growth for longer period *i.e.* 20 or 30 minutes. However, in case of *Microterms. unicolor* and *Microcerotermes. championi*, the mortality pattern based on LT_{50} did not show any significant difference in causing 50% death of termites when crawled for 10 or 30 minutes.

The comparative data on the mortality pattern of all three species of termites shows that *Microcerotermes championi* died in a shorter time than the other species of termites (Table-IV)

Table IV. LT_{50}/LT_{100} (in hour) of *Microtermes unicolor* infected by 72 hours old culture of *Aspergillus niger*

Termite species	LT_{50}/LT_{100} (crawling) 10min.	LT_{50}/LT_{100} (crawling) 20min.	LT_{50}/LT_{100} (crawling) 30min.
Microtermes unicolor	46.8/144	41.8/144	50.4/120
Microcerotermes championi	30/96	48/96	32.4/72
Heterotermes indicola	115.2/264	79.2/216	81.4/216

These findings indicated that the workers of *Microcerotermes championi* were more susceptible to *Aspergillus niger* infection as compared to *Microtermes unicolor* and *Heterotermes indicola* workers.

These findings support the view of Becker (1965) that differences exist in the action of a strain of fungus to various species of termites. The time interval from fungal invasion to death of the insect host varies considerably among different species of fungi and even between host as found in the present studies and others (Becker and Kerner-Gang, 1964; Leong, 1966; Bao and Yendol, 1971).

Aspergillus niger appears to be a potential pathogen for the control of termites in laboratory conditiions. Further studies are needed for testing the pathogenicity of this fungus against termites in the field conditions.

Acknowledgement

This work was supported by a P. L. 480 Grant FG.Pa-201 (Project A17. FS 21)

REFERENCES

BAO, LI-LI AND YENDOL, W.G., 1971. Infection of eastern subterranean termite, *Reticulitermes flavipes* (Kollar) (1) with the fungus *Beauveria bassiana* (Balsamo) Vuill (2). *Entomophaga*, **16**: 343-352.

BEAL, R. H. AND KAIS, A.G., 1962. Apparent infection of subterranean termites by *Aspergillus flavus* (Link). *J. Insect. Pathol.*, **4**: 448-489.

BECKER, G., 1965. Versuche uber den Einflub von Braun faulepilzen auf wahl and Ausnutzung der Holznahrug durch Termiten. *Material u Organismen*, I: 95-156.

BECKER, V.G. AND KERNER-GANG, W., 1964. Schadigung und Forderung von Termiten durch Schimmelpilze. *Z. Ang. Ent.*, **53**: 429-448.

BLACKWELL, M. AND KIMBROUGH, J.W., 1976. Ultrastructure of the Termite-associated Fungus *Loboulbeniopsis termitarius*. *Mycologia*, **6**: 541-550.

BLACKWELL, M. AND KIMBROUGH, J.W., 1978. *Hormisciodeus filamentous* Gen. Et. Sp. Nov., A termite infecting fungus from Brazil. *Mycologia*, **70**: 1274-1280.

BUCHLI, R.R., 1951. *Entennopsis gallica*, a new parasite on termites. *Trans. 9th Int. Cong. Ent. Amsterdum*, **1**: 519-524.

KHAN, K.I., 1981. Studies of pathogens of termites of Pakistan, Ph. D. thesis, Punjab University, Lahore, Pakistan.

KIMBROUGH, J.W., MORALES, M.I. AND GOUGER, R.J., 1972. A new species of *Dimeromyces* (Laboulbeniales) on subterranean termites. *Mycologia*, **64**: 388-393.

KREJZOVA, R., 1976b. Pathogenicity of a strain of *Paecilomyces fumoso-roseus* isolated from Zootermopsis Sp. (*Isoptera*). *Vest. Cs. Spol. Zool.*, **40**: 286-288.

LENZ, M., 1968. Zur wirkung von Schimmelpilzen auf verschiedene Tieraten. *Z. Angew. Zool.*, **55**: 385-425.

LEONG, L.H.K., 1966. Infection of the Formosan subterranean termite *Captotermes formosanus* Shiraki by the fugus, *Metarrhizium anisopliae* (Metch.) Serokin. Master Degree Thesis. Univ.

Hawaii. Deptt. Entomol.

LUND, A.E. AND ENGELHARD, N.T., 1962. Subterranean termites *Absidia coerulea* Bainer (Mucorales). *J. Insect. Pathol.*, **4**:131-132.

ROSSI, W., 1974. Una nuova specie di *Loboulbenia* (Ascomycets, Loboulbeniales) parasita di termite. *Doriana*, **5**: 1-5.

ROSSI, W., AND CESARI ROSSI, M.G., 1977a. Sui fungli parassiti di termiti, Italiane (Parasitic fungi on Italian termites). *G. Bot. Ital.*, **111**: 165-170.

ROSSI, W., AND CESARI ROSSI, M.G., 1977b. Due Nuove specie Di Dimeromyces (Loboulbeniales). Rivista Di *Parassitologia.*, **38**: 109-113.

SANNASI, A., 1968. Apparent infection of queen and drones of the mound-building termite *Odontotermes obesus* by *Aspergillus flavus*. *J. Invert. Pathol.*, **10**: 434-435.

TATE, P.C., 1927. On *Ectomyces calotermes*, n.g., n.sp. and *Ascomycetes* parasitic on *Calotermes samonus* Holngren. *Isoptera. Protermitidae. Parasitol.*, **19**: 54-60

TATE, P.C., 1928. Note on the *Ectomyces* and *Termitaria* fungi parasitic on termites. *Parasitol.*, **20**: 77-78.

YENDOL, W.G. AND ROSARIO, S.B., 1972. Labotatory evaluation of methods for inoculating termite, with *Ectomophthraceous* fungi. *J. Econ. Ent.* **65**: 1027-1029.

Received: November 18, 1993