Punjab Univ. J. Zool., Vol. 30 (2), pp. 051-055, 2015

ISSN 1016-159 (Print)
ISSN 2313-8556 (online)

Original Article

Pathogenicity of *Aspergillus parasiticus* against *Coptotermes heimi* (Wasmann)

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(**Article history:** Received: November 27, 2015; Revised: December 18, 2015)

Abstract

Termites are economically important insects. They cause damage to furniture, household goods, timber and forest vegetation. Present study was designed to determine the effect of fungus isolates at six different exposure times on *Coptotermes heimi* (Wasmann), which is one of the major destructive termites of Pakistan. Fungal conidia were repellent to *C. heimi*. The workers of *C. heimi* were exposed to fungus for 60, 45, 30, 20, 15, 10 minutes and percentage mortality and consumptions were noted during eight days period. Results showed that workers of *C. heimi* responded effectively to different exposure time. Percentage mortality in 10, 15, 20, 30, 45, 60 minutes exposure was 46.7%, 55.6%, 67.5%, 76%, 85.3%, 100%, respectively. And, control showed 0% mortality. There was marked reduction in consumption by termites subjected to different exposure time. Consumption in all groups was significantly lower than control.

Key words: Termites, consumption, *Aspergillus parasiticus*

To cite this article: AIHETASHAM, A., QAYYUM, F. AND XAACEPH, M., Pathogenicity of *Aspergillus parasiticus* against *Coptotermes heimi* (wasmann). *Punjab Univ. J. Zool.,* **30**(2): 51-55.

INTRODUCTION

Coptotermes heimi (Wasmann) is a destructive species of subterranean termite, distributed in Pakistan , India and Bhutan (Akhtar, 1972 and Roonwal and Chhotani, 1989). It has established in the Arid Arabian peninsula also (Roonwal and Chhotani, 1989). This species usually attacks shisham (Indian rosewood), mulberry and poplar trees in Pakistan. But they can attack up to 35 different species of plants (Roonwal, 1970). *Coptotermes heimi*, which is common in Pakistan and is generally involved in damaging woodwork in the buildings and forest trees.

Due to their severe damage, many types of insecticides which belong to different group have been applied to overcome the termite problem in Pakistan. Fungal conidia and chemicals extracted from plants were also used to suppress the termites but their results highly varied (Ahmed *et al.,* 2005; Ahmed *et al.,* 2008). In Pakistan, scientists are using fungal agents also to control the population of termites. Amongst the fungal agents *Aspergillus parasiticus* is the principal filamentous fungus involved in the Aflatoxin production (Diener *et al.,* 1987; Giorni *et al.,* 2007). Aflatoxins (AFs) are derived secondary metabolites of a polyketide family produced by *Aspergillus parasiticus*. They are considered as potent hepatotoxins causing mortality and reducing the productivity of farm animals (Stark, 1980; Berry, 1988; Magan and Olsen, 2004; Ventura *et al*., 2004; European Commission, 2006; Giorni *et al.,* 2007; Ravindran, 2015). Significant mortality was observed due to Aflatoxin production in lesser cornstalk borer *(Elasmopalpus lignosellus*) Dorner (2003), Mealy bug (*Saccharicoccus sacchari)* Drummond and Pinnock (1990), *Aedes aegypti* (first instar and forth instar) *culex fatigans, Aedes africanus*, *Aedes simpsoni*, *Anopheles gambiae* and *Melanoplus sanguinipes* (Fabricius) nymphs Nnakumusana (1985) by *Aspergillus parasiticus.*

The present study reports impact of pathogenic fungi (*Aspergillus parasiticus) against Coptotermes heimi*. These bioinsecticides can be used against the control of termites and will not pollute the environment, being ecofriendly.

MATERIALS AND METHODS

Termite collection

Subterranean termite *C. heimi* workers were collected from hatchet-split dead standing and fallen wood from Faisal town Lahore, Pakistan in June 2013. Termites were kept in laboratory and maintained at 28°C. Only mature

30-PUJZ-51021172/15/0051-0055

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and healthy workers were used for experimental work.

Media preparation

In a conical flask, SDAY media was prepared by mixing accurate amount of 40g/l Dextrose 10g/l Peptone 10g/l Yeast extract and 20g/l Agar in distilled water and was then autoclaved. Under sterile conditions media was poured into autoclaved petri plates. Then it was allowed to solidified and placed in incubator at 37° C before inoculation.

Isolation of Pathogens

The termites were surface sterilized by 5.25% sodium hypochlorite as described by Bao and Yendol (1971). The dead termites of *Heterotermes indicola* infected by fungus were crushed in sterile distilled water and suspension was made. An inoculum of the suspension was directly streaked on the media. Inoculation of this culture was observed against *C. heimi*.

Identification of Fungi

Smears were prepared by taking fungus from infected dead termites and identified it under microscope. This isolated pure culture was stained by Lactophenol blue solution to examine its morphological characteristics. The small blocks of SDAY medium were also used for slide preparation and detailed characteristics were studied.

Repellency test

Petri plates were oven dried at 100°C for 3 hours. Two semi-circular shaped filter papers were cut and placed in Petriplates leaving behind a narrow space in middle. One was used as control by dipping in distilled water and other tested with conidial suspension. 10 workers were released in the centre of each Petriplate. The setup was covered with black cloth to minimize the effect of light. Temperature was maintained at ±28°C.

Inoculation

Twenty one petri plates filled with growth media were inoculated under sterile conditions with sterile inoculating loop conidia were picked from fungus plate and loop was touched on medium at its center. After that, petri plates were incubated at room temperature (28±2 °C) under total darkness for 3 days. Before each test, conidia were examined under a phase contrast microscope to check for contamination.

Pathogenicity of *A. parasiticus* was observed against workers of *C. heimi* by surface culture method (crawling). Six sets of cultural plates (3 replicates) were maintained. 300 termite workers were exposed and allowed to crawl over the fungal surface for 10, 15, 20, 30, 45 and 60 minutes. Control tests (3 replicates) were also performed by treating with distilled water for each exposure time.

Post exposure mortality

Mortality after exposure was observed by introducing treated termites into 18 sterilized petri plates with wet filter papers (n=300). The plates of treated termites were kept in a dark chamber maintained at an average temperature of 28° C with **±**26° C and an average ambient relative humidity **±**92% RH. Sterile distilled water was sprayed at the inner side of plates to maintain the humidity. 3 replicates of controls were also maintained in a similar manner, by omitting fungal exposure. The termites were checked daily, mortality was recorded for eight days. In the end of experiment the filter paper that served as food source was removed, carefully cleaned all debris, weighed to determine consumption.

RESULTS AND DISCUSSION

Termiticidal activity of *Aspergillus parasiticus* at different exposure times were tested against the workers of *Coptotermes heimi.* The strain of *A. parasiticus* proved to be highly pathogenic against *C. heimi*. All termite workers moved towards filter paper dipped in water showing that *A. parasiticus* was repellent to termite workers so these strains were used further to cheek mortality and feeding test. Crawling method was used for the workers of *C. heimi* significant mortality was observed when they crawled for longer period of time. Termite workers moving on the fungus plate could easily become contaminated with fungus conidia and pass them to other members through body contact and grooming behavior Preston *et al*., (1982). Percentage mortality in 10, 15, 20, 30, 45, 60 minutes exposure was 46.7%, 55.6%, 67.5%, 76%, 85.3%, 100% respectively while control showed 0% mortality (Fig. 1). Percentage mortality of termites increased gradually and continuously from day 1 to day 8 in all fungus treated termite plates showing that fungus worked slowly and effectively against termite's workers. This slow mortality prolife is

generally a great characteristic of exposure to fungi. Fungus treated termites appeared slow moving and sluggish on the first day of termite bioassay congregate on the paper in the manner described by Leong (1966) prior to their death Results are similar to Grace and Zoberi (1992) who work with *bassiana* strain and observered that mortality occur slowly but continuously upto 15 days of exposure. Our results match with Rosengaus *et al*. (1999) who observed that higher spore concentration of fungus (2.2x108) spores ml more lethal to the termite population.

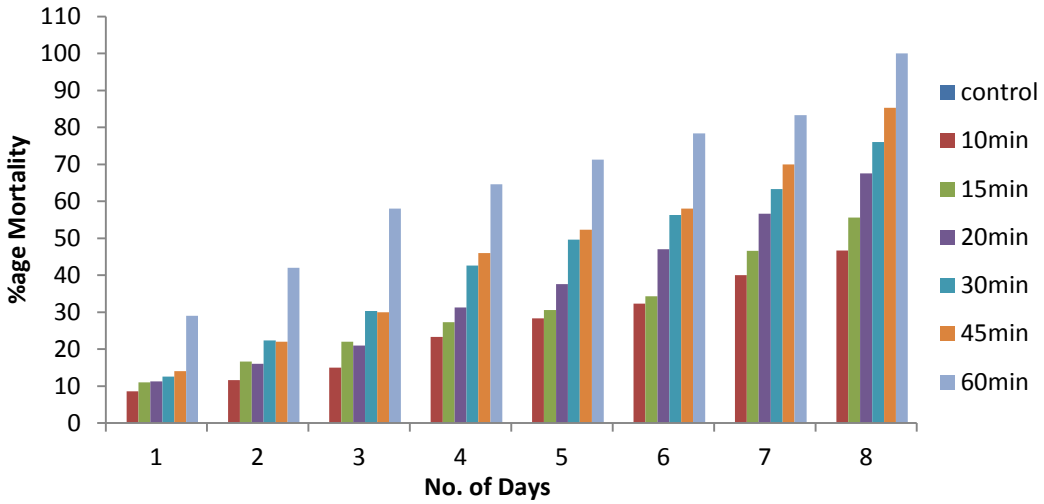

Figure 1. Mortality of termites (*Coptotermes heimi)* after fungal exposure of different time span and number of days.

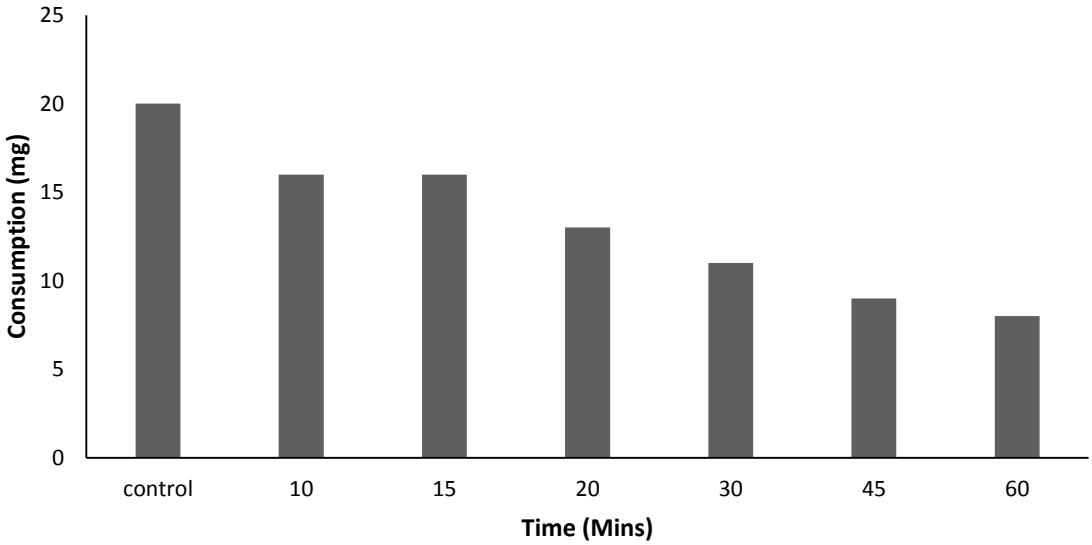

Figure 2. Mean consumption of filter paper by *C. heimi* groups treated with different fungal exposure time after 8 days.

Significant survival in some of the nymphs was observed for longer durations when subjected to lower concentration (106 spores ml). These results are also in confirmation with the work of Rosengaus and Traniello (1997), Liu *et al*. (2002) and Wright *et al*. (2005) who describe that the susceptibility of the termites to fungal infection was usually dose dependent.

Filter paper consumption was significantly reduced in treated group as compared to control group. Treated termites were observed feeding on the filter paper in two days of test initiation. But termite feeding was

greatly reduced in next six days of experiment. Inverse relation was present between exposure time and termite feeding. Significantly less feeding was observed in termites subjected to more exposure time in fungal units than in control (Fig. 2).

The reason why we use filter paper for our experiment was that *Coptotermes heimi* used filter paper as food while many other termites species including *Microtermes obesi* cannot use filter paper as food (Kakde *et al.,* 2005; Hussain, 2006). A possible source of variation in virulence among the strains of the fungus could be difference in environment of different places and ability of strains to survive under different climatic condition. Virulence of pathogen may also depend upon the media on which it is being cultured. Hence strain of *A. parasiticus* used in this study was suitable as biological control agent against *C. heimi*.

REFERENCES

AHMED, S., ASHRAF, M.R., HUSSAIN, M.A. AND RIAZ, M. A. 2008. Pathogenicity of Isolates of *Metarhizium Anisopliae* from Murree (Pakistan) against *Coptotermes heimi* (Wasmann) (Isoptera: Rhinotermitidae) in the laboratory. *Pak. Entomol.,* **30:** 119-125.

AHMED, S., NASEER, A. AND FIAZ, S. 2005. Comparative efficacy of botanicals and insecticides on termites in Sugarcane at Faisalabad*. Pak. Entomol.,* **27:** 23-25.

AKHTAR, M.S. 1972. Studies on the taxonomy and zoogeography of termites of Pakistan. Ph.D. Thesis, University of the Punjab, Lahore.

BAO, LI-LI. AND YENDOL, W.G. 1971. Infection of the Eastern subterranean Termite, *Reticulitermes flavipes* (Kollar) (1) with the fungus *Beauveria bassiana* (Balsamo) Vuill(2) *Entomophaga,* **16**(30): 343-352.

BERRY, C. 1988. The pathology of mycotoxins *J. Pathol.,* **154:** 301–311.

DIENER, U. L., COLE, R.J., SANDERS, T.H., PAYNE, G.A., LEE, L.S. AND KLICH, M. A. 1987. Epidemiology of aflatoxin formation by *Aspergillus flavus*. *Annu Rev Phytopathol.*, **25:** 249-270.

DORNER, J.W., COLE, R.J., CONNICK, W.J., DAIGLE, D.J., MCGUIRE, M.R. AND SHASHA, B.S. 2003. Evaluation of biological control formulations to reduce aflatoxin contamination in peanuts. *Biol. Control*, **26(3):** 318-324.

DRUMMOND, J. AND PINNOCK, D. E. 1990. Aflatoxin production by entomopathogenic isolates of *Aspergillus parasiticus* and *Aspergillus flavus*. *J. Invertebr. Pathol.*, **55(3):** 332-336.

EUROPEAN COMMISSION, 2006. The Rapid Alert System for Food and Feed (RASFF) Annual Report.

GIORNI, P., MAGAN, N., PIETRI, A., BERTUZZI, T. AND BATTILANI, P., 2007. Studies on *Aspergillus* section *Flavi* isolated from maize in northern Italy. *Int. J. Food Microbiol.,* **113:** 330–338.

GRACE, J.K. AND ZOBERI, M.H. 1992. Experimental evidence for transmission of *Beauveria bassiana* by *Reticulitermes flavipes* workers (Isoptera: Rhinotermitidae). *Sociobiol.*, **20:** 23–28.

HUSSAIN, A. 2006. Pathogenicity of exotic strains (*Metarhizium anisopliae*) of entomopathogenic fungi against termites *Odontotermes* spp. And *Microtermes* spp (isopteran: termitidae) in laboratory and field. *Deptt. Agri. Ento., Univ. Agri. FSD*, Pakistan. M.Sc. Thesis. Pp.92.

KAKDE, T.D., WADASKAR, S.P., GAWANDE, S.J., GAWANDE, S.J., GAIKWAD, AND VIDYAPEETH, P.D.K. 2005. Susceptibility evaluation of termites, *Odontotermes obesus* (Rambur) against the fungal entomopathogens*. Resist. Pest Manage. Newsl.*, **14**(2): 26-29.

LEONG, C.Y. 1966. A taxonomic study of the *Malayan Corixidae* (Hemiptera-Heteroptera) with the description of *Micronectam alayana* sp. Novo Bulletin the National Museum, *Singapore*, **33**: 83-90.

LIU, H., SKINNER, M., BRUCE, L., PARKER, AND BROWNBRIDGE, M. 2002. Pathogenicity of *Beauveria bassiana*, *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes) and other Entomopathogenic Fungi against *Lygus lineolaris* (Hemiptera: Miridae). *J. Econ. Entomol.*, **95:** 675–681.

MAGAN, N. AND OLSEN, M. 2004. Mycotoxins in Food: Detection and control. Wood head Publishing Ltd., Cambridge, UK.

NNAKUMUSANA, E.S. 1985. Laboratory infection of mosquito larvae by

entomopathogenic fungi with particular reference to *Aspergillus parasiticus* and its effects on fecundity and longevity of mosquitoes exposed to sporal infections in larval stages. *Curr. Sci.*, **54**(23): 1221.

PRESTON, A.F., ERBISCH, F.H., KRAMM, K R. AND LUND, A.E. 1982. Developments in the use of biological control for wood preservation. *Proc. Amer. Wood-preserv. Assoc.*, **78:** 53-61.

RAVINDRAN, K., DEWEN, Q. AND SIVARAMAKRISHNAN, S. 2015. Sporulation Characteristics and Virulence of *Metarhizium anisopliae* Against Subterranean Termites (*Coptotermes formosanus*). *Int. J. Microbiol. Res.,* **6 (1):** 01-04.

ROONWAL, M.L. 1970. Termites of the Oriental region, pp. 315-391. In: K. Krishna and F. M. Weesner (eds) Biology of Termites, Volume 2. Academic Press, New York and London.

ROONWAL, M.L. AND CHHOTANI, O.B. 1989. The fauna of India and the adjacent countries. Isoptera (Termite). Volume 1. Introduction and Families Termopsidae, Hodotermitidae, Kalotermitidae, Rhinotermitidae, Stylotermitidae and Indotermitidae. *Zoological Survey of India*, Calcutta.

ROSENGAUS, R.B., JORDAN, C., LEFEBVRE, M.L. AND TRANIELLO, J.F.A. 1999. Pathogen alarm behavior in a termite: A new form of communication in social insects. *Naturwissenschaften*, **86 (11):** 544-548.

ROSENGAUS, R.B. AND TRANIELLO, J.F.A. 1997. Pathobiology and disease transmission in dampwood termites [*Zootermopsis angusticollis* (Isoptera: Termopsidae)] infected with the fungus *Metarhizium anisopliae* (Deuteromycotina: Hypomycetes). *Sociobiol.,* **30:** 185–195.

STARK, R.E. 1980. *"Stages of speech development in the first year of life". In Yeni-Komshian, G. H.; J. F. Kavanagh, C. A. Ferguson. Child Phonology. Volume 1: Production. New York, NY:* Academic Press. pp. 73–92.

VENTURA, M., GOMEZ, A., ANAYA, I., DÍAZ, J., BROTO, F., AGUT, M. AND COMELLAS, L. 2004. Determination of aflatoxins B1, G1, B2 and G2 in medicinal herbs by liquid chromatography–tandem mass spectrometry. *J. Chromatography* A, **1048**(1): 25-29.

WRIGHT, M.S., RAINA, A.K. AND LAX, A.R. 2005. A Strain of the Fungus *Metarhizium anisopliae* for Controlling Subterranean Termites. *J. econ. Entomol.*, **98 (5):** 1451-1458.