Original Article

Pathogenicity of Aspergillus parasiticus against Coptotermes heimi (Wasmann)

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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

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) Donner (2003), Mealy bug (Saccharicoccus sacchari) Drummond and Pinnock (1990), Aedes aegypti (first instar and forth instar) culex fatigans, Aedes africanus, Aedes simponi, 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
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 ±2°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
Termicitidal 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 prose 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 observed that mortality occur slowly but continuously up to 15 days of exposure. Our results match with Rosengaus et al. (1999) who observed that higher spore concentration of fungus (2.2x10^8) spores ml more lethal to the termite population.

![Percentage Mortality vs Days](image1.png)

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

![Time vs Consumption](image2.png)

**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 termite 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.

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