

Effect of *Metarhizium anisopliae* on Different Group Size of *Coptotermes heimi*

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Coptotermes heimi is a destructive subterranean termite species commonly found on the Indian subcontinent. Environmental concerns of chemical insecticides usage forced scientist to concentrate on alternative methods. *Metarhizium anisopliae* is observed to have pathogenic effects against various arthropods. This study focused on the interaction of *M. anisopliae* with *C. heimi* to check its virulence against the termites. Specific spore concentration of *M. anisopliae* (1.1×10^7 conidia/ml) was applied on different group sizes of termites to check if termite population has any effect on its mortality. Results showed that with the increase of group size of termites, mortality rate decreases.

Keywords: *Coptotermes heimi*; *Metarhizium anisopliae*; Conidia; mortality rate.

1. INTRODUCTION

Termites are an important group of insects that are extensively distributed in temperate, tropical

and subtropical countries of the world [1]. Termites have seven families which then further divided in to more than 2,600 define species [2,3]. Despite having common traits, termites

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have adapted wide range of ecological habitats showing great diversity in cast physiology and morphology, nesting behavior and association with different kinds or microbial community [4]. Their main food is cellulose based material. Most of the termites do not have efficient cellulose digestion system [5] they tend to make symbiotic association with microbial communities that can break cellulose into simpler sugar units [6].

Among these 2,600 species of termites, only small amount (70-80 species) is considered to cause damage to structure [7], forestry and agriculture; both food and cash crops [8]. Subterranean termite, *Coptotermes heimi*, have destructive nature and are heavily found in India, Pakistan [9-12] and Bhutan [13]. Earlier it was known as *Arrhinotermes heimi* [14], but was renamed later as *C. heimi* by Holmgren [15]. The termite specie tends to feed on both living and dead timber and shrub [16]. It is reported that it caused great damage to important tree of Pakistan and India including morus (mulberry), shisham (Indian rosewood). Not only it attacks on forest trees it also feeds on wooden buildings in many part of Pakistan [17].

Economically loss caused by termites gained a lot of attention to invest in the control of termites, especially subterranean termites [18,19]. Insecticides based on organochloride compounds were considered to have great impact on termites' mortality in the past. One insecticide application in or around building have potential to avoid termites for years [20]. Even present days' techniques to control termites are heavily dependent on chemical pesticide [18,21]. Despitess the great advantageous nature of these chemical applications, one cannot deny their effect on environment which begs the question of having alternate, eco-friendly approaches to control termite growth [22-24].

Termites controls using biological methods have gained much attention as an alternate technique to chemical control usages. Biological based techniques were started in 1960's with special focus on fungal pathogens [25-29]. The use of fungi as biological control of termites was built on the view of classical biological technique [30] with the use of fungi virulence that can replicate itself in the nest of termites and can spread from one termite to other by high social interaction, thus cause an epizootic and destroy the whole colony. Most of the researchers focused on the entomopathogenic fungi nature of *Beauveria bassiana* (Balsamo) *Vuillemin* and *Metarhizium*

anisopliae (Metschnikoff) *Sorokin* [23,24,31]. The fungi are broadly spread in soil and broad range of host. Studies showed that these fungal species have potential against termites, however have less successful trial in fields [22,32].

M. anisopliae is a soil fungi generally lived in temperate, arctic, tropical and subtropical parts of world [33]. Even though, the fungi have ability to survive in soil [34,35], it is assumed to be the fungi which has pathogenic nature against wide range of insects such as beetles, moths, flies, cockroaches, locusts and other arthropods [36]. Pathogenic activity of *Metarhizium* strains varies from host to host [37-39]. This study explores the interactions of *C. heimi* with the entomopathogenic fungus *M. anisopliae* to assess the effective candidacy of this pathogenic fungus as biological control.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Termites

C. heimi (Wasmann) were collected from verminous trees of *Populus euramericana* at the of Forman Christian College, Lahore. Termites was identified using morphological traits for soldiers and workers [40].

2.2 Conservation of Termites at Controlled Laboratory Conditions

Laboratory conditions at 25°C and 70% Rh was provided for termite's acclimatization prior to experimentations. Termites were kept on Whatmann filter paper for three days in Petri plates during experiments.

2.3 Culturing of *M. anisopliae* on Selected Media

The culture of *Metarhizium anisopliae* was provided by entomology laboratory at Forman Christian College, Lahore. The fungus was cultured on potato dextrose agar (PDA) media at 27 or 30 C in the dark for 15–20 days.

2.4 *M. anisopliae* Effects on Different Group Size of Termites

An estimate number of spores were made using a hemacytometer. Spores of fungus *M. anisopliae* were distributed on 10 petri dishes having Whatman No. 1 filter paper moistened

with water. All plates contained different group size of termites 1.e 5, 10, 15, 20, 25, 30, 35, 40, 45, 50. The experiment was conducted for 2 weeks and termites' mortality was checked every day. While examining, dead termites were separated in another petri plate from healthy ones. Fungus growth was checked in petri plate having dead termites. Control group in this experiment was plate without the fungus.

3. RESULTS

M. anisopliae at the concentration of 1.1×10^7 conidia/ml gave 100% mortality in the group size of 5, 10, 15, 20, 25 and 30 after 8, 10, 12, 14, 11 and 10 days respectively (Table 1). While 83%, 77.5%, 70%, 67% mortality was

obtained in 35, 40, 45 and 50 group sizes of termites, respectively, after 2 week experiment (Table 1). This data showed that with the increase of group size of termites, mortality rate decreases (Fig. 1).

The impact of standard fungal concentration (1.1×10^7 conidia/ml) on group size of termite was significant ($P=0.05$; $F=91.87$, DF, 1-18 ANOVAs one way) on mean termite % mortality. 100% mortality was achieved after day 8,10,12,14,11 and 10 against termite group size ranged from 5-30 respectively. However, a pronounced effect was recorded where termite mortality decreased with the increase in population size from 83% to 67% and group size ranged 35-50. The root square adjusted was 82.71% (Table 1).

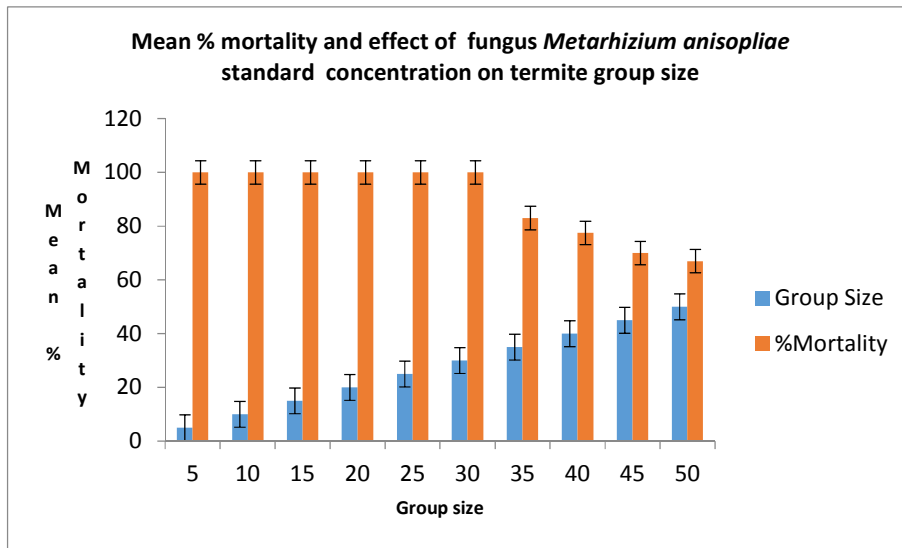


Fig. 1. Mean % mortality and effect of fungus *Metarhizium anisopliae* standard concentration on termite group size after 2 weeks experiment

Table 1. Effect of fungus *Metarhizium anisopliae* standard concentration (1.1×10^7 conidia/ml) on group size mortality of *Coptotermes heimi*

Group size	Days	Mean mortality (%)
05	08	100
10	10	100
15	12	100
20	14	100
25	11	100
30	10	100
35	14	83
40	14	77.5
45	14	70
50	14	67

ANOVA one way (unstacked)

Source	DF	SS	MS	F	P
Factor	1	19375	19375	91.87	0.00
Error	18	3796	211		
Total	19	23171			

$S = 14.52$, $R\text{-Sq} = 83.62\%$, $R\text{-Sq (adj)} = 82.71\%$

Level	N	Mean	Standard deviation
Group size	10	27.50	15.14
Mortality	10	89.75	13.88

4. DISCUSSION AND CONCLUSION

Investigation of pathogenicity of *M. anisopliae* was performed using petri dishes containing only fungi conidia and subjected termites. Researches of efficiency screening [41-47] and condition of infection [41,44,48] of many entomopathogens such as *M. anisopliae* against termites have been performed in only laboratory conditions and without any soil presence, hence opposing microbial community naturally present in soil. Milner and Staples [45] worked on many isolates of *M. anisopliae* against *Nasutitermes exitiosus* and *Coptotermes* spp. and reported 80% mortality of termites. A study showed the comparison virulence of *M. anisopliae* among other fungal strains and reported *M. anisopliae* have highest virulence among all other fungal strains.

With the increase of group size of termites, mortality rate decreases. This can be possible because of the social behavior of termites [49, 50] such as avoiding infected termites. Moreover, they also develop chemical mediated defenses against pathogen which include their ability to sense the toxins released by pathogenic fungi such as *Beauveria* and *Metarhizium* species. Because of this ability, they avoid the contact with fungus spores or any toxin contaminated substrates [51]. Rosengaus et al. [52] describes that termites develop a protective immune system in response to exposure to fungal or bacterial pathogens. These findings demonstrate that termites have developed effective defense systems against pathogens.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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