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Laboratory Evaluation of Entomopathogenic Fungi *Metarhizium anisophilae* and *Beauveria bassiana* Against Termite, *Macrotermes* (Isoptera: Termitidae)

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Abstract: Termites, *Macrotermes* are major agricultural and domestic problem in Ethiopia causing serious damage with loss up to 100%. The use of synthetic termitecids was the most commonly used prevention measure to reduce the termites attack. However, these synthetic termiticides were known to be very harmful to the environment and non-target organisms. Therefore, efficacy of entompathogenic fungi, *Metarhizium anisopliae* (isolates PPRC-2 and MM) and *Beauveria bassiana* (isolates PPRC-56 and 9609) were evaluated against *Macrotermes*. The isolates were obtained from Ambo Plant Protection Research Center, Ethiopia. For each isolate concentrations of 1×10^5 to 1×10^9 conidia mL^{-1} were prepared and used as treatments. Untreated and standard (Diazinon 60% EC) checks were used for comparison. The treatments were laid out in Completely Randomized Design (CRD) and replicated thrice. The fungal isolates were evaluated by direct spraying of spore suspensions on worker *Macrotermes* spp. The result of the study revealed that all fungal isolates used were able to infect and cause mortality at all concentrations. The percent mortality of *Macrotermes* varied from 60 to 100% for *M. anisopliae* isolate MM at 1×10^5 to *M. anisopliae* isolate PPRC-2 at 1×10^9, respectively. Similarly, the percentage mortality of *Macrotermes* varied from 25-95% for *B. bassiana* isolate 9609 at low concentration and isolate PPRC-56 at highest concentration, respectively. The isolates had LT_{50} ranging from 7.74 days in *M. anisopliae* isolate PPRC-2 to 8.80 days in *B. bassiana* isolate 9609. The concentration response with the isolate PPRC-2 showed the lowest LC_{50} of 3.21×10^5 conidia mL^{-1} followed by isolates MM, PPRC-56 and 9609 with LC_{50} of 3.82×10^5, 4.39×10^5 and 5.08×10^5 conidia mL^{-1}, respectively. In conclusions, the present study suggests that the use of entompopathogenic fungi, *M. anisopilae* and *B. bassiana,* at higher concentrations for seven days is an eco-friendly effective mycoinsecticides that causes more than 95% mortality of *Macrotermes*.

Key words: *B. bassiana*, bio-control, entomopathogenic fungi, *M. anisopliae, Macrotermes,* mortality

INTRODUCTION

Termites belong to the insect order Isoptera and have long been recognized as important agricultural and domestic pests (Owusu *et al*., 2008; Samb *et al*., 2011). Among all termites, the largest termite species are the *Macrotermes* spp. known to be fungus growing and mound building. *Macrotermes* builds large epigial nests (mounds) from where they forage outwards to distances up to 50 m in galleries (Osipitan and Oseyemi, 2012). *Macrotermes* are economically important insect pests causing serious damage up to 100% loss to agricultural crops and various domestic products (Abdurahman, 1990; UNFAO, 2000; Sekamatte, 2001; Nyeko *et al*., 2010). Damage by these termite species to agricultural and commercial products also provides entry for secondary infection by pathogen especially *Asperagilous*, which cause indirect yield loss and contamination of products with aflatoxins (Osipitan and Oseyemi, 2012).

Management of termites has largely relied on broad spectrum and persistent organo chlorine insecticides in the world (Logan *et al*., 1990). Nowadays, most of the persistent insecticides were banned or withdrawn from the market for human health and environmental reasons. Also, synthetic insecticides are not affordable by poor farmers of Africa including Ethiopia. Thus, there were serious limitations and increasing legal restrictions associated with the application of persistent and deleterious insecticides; because of which the search for environmentally benign alternative methods of termite management has been intensified by entomologists. Among the diverse potential alternatives available for termite management, the use of entomopathogenic fungi is getting momentum (Michael, 2005).

Entomopathogenic fungi, *Beauveria bassiana* (Basamo) and *Metarhizium anisophilae* (Metchnikoff) are effective in the management of different species of termite (Milner *et al*., 1998; Milner, 2003) which may be used in

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different methods among which direct exposure, soil barrier and in baits system were able to achieve good control in termite colony. The genus *Metarhizium, Beauveria* and *Paecilomyces* are fungal pathogens of insects that have shown great promise in commercial development. *M. anisopliae*, with worldwide distribution has been isolated from more than 200 insect species across seven orders and has shown great potential as bio-control agents. Strains of *Metarhizium* differ in their host range, necessitating selection of the most virulent against a target insect (Zimmermann, 1993).

M. anisopliae has an advantage over *B. bassiana* in microbial management of termite due to their social behavior and high production of fungal biomass (Sun *et al*., 2002). Pik-Kheng *et al.* (2009) reported that isolates of *M. anisopliae* were pathogenic against subterranean termite, *Coptotermes curvignathus,* causing 100% mortality at 1×10^7 conidia mL^{-1} within 3 days post-inoculation. According to David *et al.* (2010), a positive relationship between virulence and repellency effect of different isolates of the fungus *M. anisopliae* on *M. michaelseni* was determined. Further, they compared the volatile profiles of two isolates of *M. anisopliae*, on the same species of termite and found that the volatiles of each isolate act synergistically.

The entomopathogenic fungus, *M. anisophilae*, has more host ranges and is widely used as bio-pesticide agents on several types of insect pests which include onion thrips, storage cowpea, white grub, cattle ticks and different species of termites such as *Reticulitermes* spp., *Rhinotermitidae*, *Coptotermes formosanus* and *Odontotermes formosanus* (Maniania *et al*., 2003; Sun *et al*., 2003; Wang and Powell, 2004; Cherry *et al*., 2005; Samson *et al*., 2005; Bahiense *et al*., 2006; Dong *et al*., 2009). Many strains of *M. ansisopliae* have been isolated from termites and are reported as effective myco-insecticides (Sun *et al*., 2003; Wright *et al*., 2005) for the management of subterranean termites. Entomopathogenic fungi isolate, *Aspergillus terreus*, spore suspension at different concentration was tested on different stage of tick, *Hyalomma anatolicum anatolicum* and has shown promising result as bio-pesticides (Suliman and Mohammed, 2012).

Biological control of different agricultural and domestic pests has been reported as promising option in the current and future state of pest management (Bittencourt, 2000; Chandler *et al*., 2000; Kaaya, 2003; Sharma *et al*., 2011; Jaramillo and Borgemeister, 2006). Entomopathogenic fungi, *M. anisophilae,* pathogenicity has been studied and found to cause mortality on different stage of insect pests such as filth fly parasitoid, *Spalangia Cameroni*, Malaria mosquito, *Anopheles Gambia* (Nielsen *et al*., 2004; Scholte *et al*., 2006). Thus new management approach in *Macrotermes* is deemed of prime necessity in Ethiopia. However, the report on the use of entomopathogenic fungi for the management of termite in Ethiopia is very meager despite the economic importance of *Macrotermes* as insect pest. Therefore, the current study was aimed at determining the efficacy of some available isolates of entomopathogenic fungi (*B. bassiana* and *M. anisophilae*) at different concentrations for the management of *Macroterms.*

MATERIALS AND METHODS

Description of the experimental area: The experiment was conducted in Entomology and Plant Pathology Laboratory, Jimma University, College of Agriculture and Veterinary Medicine (JUCAVM) which is located at 354 km Southwest of Addis Ababa, Ethiopia. JUCAVM is at geographical coordinate of 7°42' N latitude and 36°50' E longitude with an altitude of 1710 masl. The temperature ranges from 11.8°C (minimum) to 26.8°C (Maximum) with maximum relative humidity and mean rainfall of 91% and 1500 mm per annum, respectively (Abera *et al*., 2011).

Termite collections and establishment for test: Termite, *Macrotermes* spp., population were collected from two main sites, namely Saye Kebele of Mana and near Agaro town of Agro Woredas. At these two localities, termite mounds were dug up using spade and soil containing termites were put on plastic sheets. Termites were collected from the plastic sheets using camel hair brush and placed in plastic boxes (polyethylene plastic box) (2217×7 cm^3) as described by Gitonga *et al.* (1995). Wooden plants (termite infested materials) were added to the plastic boxes as feed for the termites. Then the top parts of the plastic boxes were covered with a mesh cloth that allows air ventilation in and out easily but preventing the escape of the termites. Moistened wad of cottons were placed in the plastic boxes to maintain the required moisture level (more than 60%) for the survival of termites. The boxes carrying the termites were transported to JUCAVM, Entomology and Plant Pathology Laboratory and placed in a cool and dark area until needed for the experiments. Periodically, dry wooden materials were provided to the termite's population and the plastic boxes were inspected for maintenance of the required moisture level.

Source of entomopathogenic fungi isolates: Two already cultured species of entomopathogenic fungi (*M. anisopilae* and *B. bassiana*) and two isolates of each species, viz., MM, PPRC-2 (*M. anisopliae)* and 96O9 and

PPRC-56 (*B. bassiana)* used in this experiment were obtained from Ethiopian Institutes of Agricultural Research, Ambo Plant Protection Research Center.

Preparation of spore suspension: To prepare fungal spore suspension, the conidia were harvested by flooding each plate with 10 mL of 0.05% Tween 80 (sigma) as a wetting agent in sterile distilled water. The conidial suspensions were prepared by mixing the solution using a magnetic stirrer for 5 min and the suspensions were filtered through sterile muslin cloth to eliminate the coagulated medium. The spore suspension of 1×10^5, 1×10^6, 1×10^7, 1×10^8 and 1×10^9 per mL of distilled water were prepared for each isolates by using haemocytometer as described by Lacey (1997).

Experimental design and treatments: There were 22 treatments (Table 1) replicated thrice and laid-out in Completely Randomized Design (CRD). In this experiment, there were two controls (the untreated and the standard controls, viz., sterile water and 0.21% a.i. Diazinon, respectively) for comparison. Each isolate was tested at five concentrations (1×10^5, 1×10^6, 1×10^7, 1×10^8 and 1×10^9 conidia mL). The experiments were conducted under laboratory conditions at 25±2°C temperature and 60% RH in dark places.

Median Lethal concentration (LC_{50}) and time (LT_{50}): Median lethal time (LT_{50}) and Median lethal concentration (LC_{50}) were determined for each concentration by taking in to account the time and concentration required at which the inoculums of fungus caused 50% of the mortality on worker termite population. Lethal time (LT_{50}) and lethal concentration (LC_{50}) required to achieve 50% mortality per replicate was obtained from probit analysis.

Table 1: Entomopathogenic fungi isolates investigated in the present study

Fungi and Isolates	Treatments (conidia mL^{-1})	Fungal source
B.bassiana		APPRC
9609	1×10^5	
	1×10^6	
	1×10^7	
	1×10^8	
	1×10^9	
PPRC-56	1×10^5	
	1×10^6	
	1×10^7	
	1×10^8	
	1×10^9	
M. anisopliae		APPRC
MM	1×10^5	
	1×10^6	
	1×10^7	
	1×10^8	
	1×10^9	
PPRC-2	1×10^5	
	1×10^6	
	1×10^7	
	1×10^8	
	1×10^9	
Diazinon	0.21%	
Water	2 mL	

APPRC: Ambo Plant Protection Research Center

Mortality of termite due to fungal isolates: To test the efficacy of each of the fungal isolates on termites, twenty worker termites were placed in a Petri dish on a filter paper and sprayed with the spore solutions as described by Julia and Lina (2010). To determined how many termites were dead without being infested with entomopathogenic fungi, a control group were sprayed with only sterile distilled water as a negative control. The efficacy was evaluated on a daily basis for 15 days, by counting dead termites which were later converted to percentage mortality. Petri dishes containing the treated termites were maintained at 25±2°C in the dark. Mortality data were corrected for the corresponding control mortality by the formula:

$$CM(\%)=\frac{(T(\%)-C(\%))}{(100-C(\%))}\times100$$

where, CM is corrected mortality, T is mortality in treated insects and C is mortality in untreated insects (Abbott, 1925).

Data analysis: The data recorded for different response variables in the study were analyzed statistically by using one-way analysis of variance model in SAS version 9.2 Software packages. Based on significant differences of treatments, mean separation was done using Turkeys' studentized (HSD) test. Mortality rate were corrected using Abbott (1925) formula. United State Environmental Protection Agency (USEPA) probit analysis version 1.5 was used for analyzing median lethal time and median lethal concentrations for each isolates at different concentrations (Finney, 1971). All recorded variables were analyzed according to the following statistical model:

$$Y_{ij} = \mu + T_i + E_{ij}$$

where; Y_{ij} is any response, μ: is the general mean effect, T_i is the ith treatment effect and E_{ij} is the experimental error.

RESULTS

Percentage mortality of worker termites: Percentage mortality of worker termites was calculated for the different concentrations of the four isolates and showed increasing mortality with increasing spore concentration. Cumulative mortality of worker termite over exposure

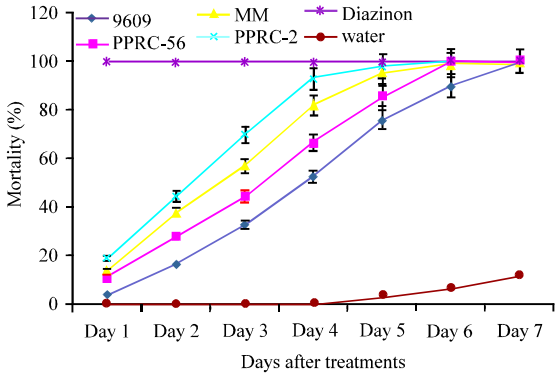

Fig. 1: Cumulative Percent mortality of termite treated with *M. anisopliae* (MM and PPRC-2) and *B. bassiana* (9609 and PPRC-56) isolates

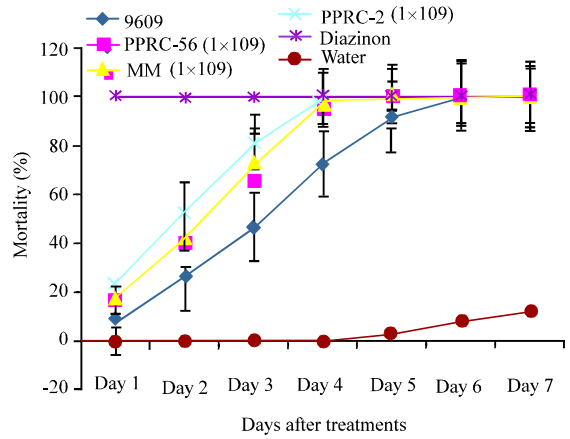

Fig. 2: Percent Mortality of *Macroterms* treated with different fungal isolates at 1×10^9 conidia mL^{-1} (highest concentration)

period (one to seven days) was significantly different ($p<0.05$) for the fungi isolates (Fig. 1). On the first day of exposure, maximum mortality (100%) was registered from standard check, while the untreated control had zero mortality. These were significantly different from all concentration of the isolates. Among the isolates, maximum mortality was observed from PPRC-2 isolates (19 to 100%) followed by MM isolates (14 to 100%) on day one up to day seven after treatment. Minimum mortality was registered from 9609 isolates (4 to 100%) followed by isolate PPRC-56 (11 to 100%) within the same period of exposure. Generally, as time progressed, cumulative mortality for all isolates increased attaining 100% mortality on the seventh day after application of the spore concentrations.

At the highest concentration, 1×10^9 conidial mL^{-1}, all *B. bassiana* and *M. anisopliae* isolates gave complete reduction in population of worker termites resulting to 100% mortality after sixth day of exposure (Fig. 2). The initial data for pathogenicity of all the four isolates indicated that all of them were virulent, even one day after exposure causing significant mortality (up to 20%) when compared with the untreated control (zero mortality). The mortality percentage for all the isolates gradually increased and on sixth day after exposure all of the isolates gave 100% mortality similar to the standard control.

Mean percentage mortality of the worker termites due to the different isolates of the entomopathogenic fungi at different concentrations was significantly different over time (Table 2). After one day of exposure 100% and zero mortality was registered from standard and negative controls, respectively. Among the isolates, maximum and significant mortality (23.33%) was registered from PPRC-2 at highest concentration. However, this concentration was followed by and non-significantly different from PPRC-2 isolates (21.67%) at 1×10^8 conidia mL^{-1}, PPRC-2 isolates (20.00%) at 1×10^7 conidia mL^{-1}, PPRC-2 isolate (16.67%) at 1×10^6 conidia mL^{-1} and MM isolates (18.33%) at 1×10^9 conidia mL^{-1}. Minimum mortality (1.67%) was recorded from 9609 isolates at lowest concentration followed by 9609 isolate (3.33%) at 1×10^6 conidia mL^{-1}, (5%) at 1×10^7 conidia mL-^{1}, (6.67%) at 1×10^8conidia mL^{-1}, (8.33%) at 1×10^9 conidia mL^{-1} and PPRC-56 isolate (5%) at 1×10^5 conidia mL^{-1} and these were on par with each others. This indicates that *M. anisopliae* isolate PPRC-2 at higher concentration is the most pathogenic fungi against termite followed with *M. anisopliae* isolate MM. On the second days after exposure, maximum mortality was also registered from standard check (100%). There was no mortality registered from untreated control (0%). From the isolates, maximum and significant mortality (53.33%) was registered from PPRC-2 at higher concentration followed by PPRC-2 (50%) at 1×10^8conidia mL^{-1}, PPRC-2 (46.64%) at 1×10^7 conidia mL^{-1} and MM (46.67%) at 1×10^9 conidia mL^{-1} which were non-significantly different among each other. Significantly minimum mortality of 3.33% and 8.33% were recorded from 9609 isolates at 1×10^5 and 1×10^6 conidia mL^{-1}, respectively which were also on par with the negative control.

On day three, highest and significant mortality (100%) was registered from standard check (Diazinon). No mortality was registered (0%) from control (Water). Highest and non-significant mortality of 81.67% was recorded from the isolates PPRC-2 at higher concentration followed by PPRC-2 at 1×10^8 conidial mL^{-1} (78.33%), MM isolate at 1×10^9 conidia mL^{-1} (73.33%) and PPRC-2 at 1×10^7 conidial mL^{-1} (71.67%) in that order. However, lower and significant mortality, 13.33 and 21.67%, were obtained from 9609 isolates at 1×10^5 and 1×10^6 conidial mL^{-1}, respectively.

Similarly, on day four, standard check gave significantly higher worker termites' mortality (100%) while no mortality (0%) was obtained from negative

Table 2: Mean percent mortality of termite treated with *B. bassiana* and *M. anisopliae* isolate at different concentrations over time

Fungi and their isolates	Treatments (Conidia mL^{-1})	Percent Mortality of *Macrotermes* — Time after exposure (Days) Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
B.bassiana								
9609	1×10^5	1.67^{kl}	3.33j	13.33^k	26.67i	45.00g	68.33d	100.00a
	1×10^6	3.33^{jkl}	8.33ij	21.67jk	38.33h	60.00f	83.33c	100.00a
	1×10^7	5.00^{ijkl}	21.67^{gh}	40.00ghi	60.00fg	83.33e	100.00	100.00a
	1×10^8	6.67^{hijkl}	23.33^{fgh}	43.33fgh	65.00efg	91.67cd	100.00^a	100.00a
	1×10^9	8.33^{ghijk}	26.00fg	46.67fgh	73.33cde	100.00a	100.00^a	100.00a
PPRC-56	1×10^5	5.00^{ijkl}	16.67hi	30.00ij	43.33h	65.00f	100.00^a	100.00a
	1×10^6	10.00^{fghij}	23.33fgh	36.67hi	55.00g	78.33e	100.00^a	100.00a
	1×10^7	11.67^{fghi}	28.33fg	43.33fgh	66.67def	90.00d	100.00^a	100.00a
	1×10^8	12.67^{efgh}	31.67ef	48.33efg	73.33cde	98.33ab	100.00^a	100.00a
	1×10^9	16.67^{bcdef}	40.00de	65.00cd	95.00a	100.00a	100.00^a	100.00a
M. anisopliae								
MM	1×10^5	10.00^{fghij}	23.33fgh	40.00ghi	60.00fg	81.67e	98.33^a	100.00a
	1×10^6	13.33^{defgh}	31.67ef	50.00efg	73.33cde	96.67abc	100.00^a	100.00a
	1×10^7	15.00^{cdefg}	38.33de	58.33de	83.33bc	100.00a	100.00^a	100.00a
	1×10^8	16.00^{cdef}	41.67cd	65.00cd	93.33ab	100.00a	100.00^a	100.00a
	1×10^9	18.33^{bcde}	46.67bcd	73.33bc	100.00a	100.00a	100.00^a	100.00a
PPRC-2	1×10^5	13.33^{defgh}	31.67ef	53.33ef	76.67cd	93.33bcd	100.00^a	100.00a
	1×10^6	16.67^{bcdef}	41.67cd	65.00cd	93.33ab	98.33ab	100.00^a	100.00a
	1×10^7	20.00^{bcd}	46.67bcd	71.67bc	96.67a	100.00a	100.00^a	100.00a
	1×10^8	21.67^{bc}	50.00bc	78.33b	100.00a	100.00a	100.00^a	100.00a
	1×10^9	23.33^b	53.33b	81.67b	100.00a	100.00a	100.00^a	100.00a
Diazinon		100.00^a	100.00a	100.00a	100.00a	100.00a	100.00^a	100.00a
Water		0^l	0.00j	0.00l	0.00j	3.33^h	6.67^e	11.67b
CV (%)		14.69	9.22	6.70	4.71	2.48	1.48	0.64
HSD		5.42	5.40	5.40	5.40	4.54	5.40	5.40
p-value		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

Means followed by the same letter within a column are not significantly different (Turkey's Studentized range test, p<0.05)

control. Among the isolates, significant and higher mortality (100%) were recorded from the isolate PPRC-2 at 1×10^{9}conidia mL^{-1}, 1×10^{8}conidia mL^{-1} and MM isolates at 1×10^9 conidia mL^{-1} followed by PPRC-2 (96.67%) at 1×10^{7}conidia mL^{-1}, PPRC-56 (95.00%) at 1×10^9 conidia mL^{-1}, PPRC-2 (93.33%) at 1×10^6 conidia mL^{-1} and MM isolate (93.33%) at 1×10^8 conidia mL^{-1}. There were no significant differences among these concentrations of the respective isolates.

After fifth day of exposure, highest and significant mortality, 100%, of workers termite was registered from standard check; isolates PPRC-2 at 1×10^{7}to 1×10^9 conidial mL^{-1}, MM at 1×10^7 to 1×10^9 conidial mL^{-1}, PPRC-56 at 1×10^9 conidial mL^{-1} and 9609 isolate at 1×10^9 conidial mL^{-1}. Minimum significant mortality (3.33%) was recorded from untreated control. The mortality in the negative control treatment might be because of ageing. From the isolates, minimum significant mortality (45.00%) was registered from 9609 isolate at 1×10^5 conidial mL^{-1}.

Six days after exposure, minimum and significantly lower percentage mortality was 6.67% from negative control treatment while the other treatments showed non-significant mortality among each other. From the isolates, 9609 isolates registered 68.33% at 1×10^5 conidial mL^{-1} and 83.33% at 1×10^6 conidial mL^{-1} where as isolate MM scored 98.33% mortality at 1×10^5 conidial mL^{-1}. However, the rest isolates and standard checks, Diazinon were not significant different from each other with 100% mortality.

The trend on percent morality of worker termite gradually increased over exposure period and attained maximum mortality of 100% from sixth day on wards similar to standard check (Diazinon). In addition, it can be noticed that as concentration of the isolates increased, efficacy also increased on specific day after exposure.

Median lethal time (LT_{50}) for fungal isolates: The median lethal time (LT_{50}), time taken for the death of 50% of worker termite due to different isolates at different concentration (1×10^5 to 1×10^{9}conidia mL^{-1}) were found to be different. From the isolates, *M. anisopliae* isolate PPRC-2 has shown the shortest median lethal time (7.74 days) at the highest concentration to observe 50% death of the target insect pests. On the other hand, median lethal time noted for the death of 50% of termite treated with *B. bassiana* isolates 9609 was the longest (8.80 days) at lowest concentration (1×10^5) (Table 3). Thus, the isolates of each entomopathogenic fungi were found to be promising in terms of the time taken as a management option against termite.

Median lethal concentration (LC_{50}) for each fungal isolates: Results of the probit analysis obtained from applying known conidial suspensions of the two fungi

Table 3: Median lethal time (LT_{50}) of *B. bassiana* (9609 and PPRC-56) and *M. anisopliae* (MM and PPRC-2) isolates against termite on day seven after treatment

Fungi and isolates	Treatments (conidia mL^{-1})	LT_{50} (days)	95% CL		Slope[±SE]	P-value[a]
			Lower	Upper		
B. bassiana						
9609	1×10^5	8.80	8.71	8.91	4.58±0.82	2.57
	1×10^6	8.65	8.52	8.77	3.50±0.60	8.72
	1×10^7	8.40	8.26	8.53	3.73±0.61	1.32
	1×10^8	8.31	8.17	8.44	3.91±0.64	1.32
	1×10^9	8.26	8.13	8.40	4.19±0.69	1.92
PPRC-56	1×10^5	8.59	8.46	8.73	3.09±0.53	9.89
	1×10^6	8.46	8.32	8.60	3.31±0.55	2.29
	1×10^7	8.41	8.28	8.55	3.73±0.61	1.36
	1×10^8	8.25	8.09	8.41	3.15±0.52	1.31
	1×10^9	8.11	7.95	8.27	3.58±0.59	1.79
M. anisopliae						
MM	1×10^5	8.54	8.41	8.68	3.41±0.57	3.57
	1×10^6	8.27	8.12	8.42	3.49±0.49	1.18
	1×10^7	8.15	7.99	8.32	3.35±0.55	1.30
	1×10^8	8.10	7.94	8.25	3.80±0.63	2.63
	1×10^9	7.95	7.78	8.13	3.51±0.59	3.28
PPRC-2	1×10^5	8.19	8.01	8.36	2.95±0.48	1.51
	1×10^6	8.07	7.9	8.24	3.25±0.53	1.55
	1×10^7	7.91	7.72	8.16	3.22±0.54	2.75
	1×10^8	7.83	7.65	8.02	3.57±0.60	6.16
	1×10^9	7.74	7.55	7.94	3.35±0.58	8.88

[a]is the single hypothesis one-sided p-value of the association between upper and lower limit (based on Fisher's Exact Test)

Table 4: Median lethal concentration (LC_{50}) of selected fungal isolates against termite, *Macrotermes* treated with aqueous conidial suspensions of different isolates of *B.bassiana* (9609 and PPRC-56) and *M. anisopliae* (MM and PPRC-2) on day seven

Fungi and isolates	Treatments (conidia mL^{-1})	LC_{50} (spores mL^{-1})	95% CL		Slope[±SE]	Fit of probit line	
			Lower	Upper		X^2	Tabular value
B.bassiana							
9609	1×10^5	5.08	4.47	5.54	9.82± 2.30	5.61	11.07
	1×10^6	4.55	3.91	5.02	8.55±1.89	3.29	11.07
	1×10^7	3.63	2.98	4.09	7.83±1.70	3.99	11.07
	1×10^8	3.58	2.95	4.01	8.63±1.93	3.12	11.07
	1×10^9	3.31	2.75	3.67	10.05±2.30	2.48	11.07
PPRC-56	1×10^5	4.40	3.67	4.90	7.54±1.70	3.31	11.07
	1×10^6	4.39	3.70	4.89	7.99±1.80	4.05	11.07
	1×10^7	4.02	3.35	4.49	8.46±1.88	3.32	11.07
	1×10^8	3.57	2.86	4.05	7.27±1.61	2.10	11.07
	1×10^9	3.33	2.64	3.81	6.66±1.41	2.19	11.07
M. anisopliae							
MM	1×10^5	3.82	3.12	4.31	7.38±1.21	2.51	11.07
	1×10^6	3.30	2.60	3.79	6.40±1.34	1.81	11.07
	1×10^7	2.95	2.31	3.40	6.54±1.36	2.02	11.07
	1×10^8	2.71	2.10	3.15	6.34±1.32	1.13	11.07
	1×10^9	2.48	1.93	2.87	7.22±1.62	1.36	11.07
PPRC-2	1×10^5	3.21	2.52	3.72	5.94±1.22	1.11	11.07
	1×10^6	2.90	2.21	3.40	5.45±1.11	1.27	11.07
	1×10^7	2.59	1.94	3.05	5.85±1.24	1.14	11.07
	1×10^8	2.44	1.86	2.84	6.87±1.55	0.90	11.07
	1×10^9	2.35	1.75	2.76	6.56±1.52	0.75	11.07

CL: Confidence limits, X^2-Chi-Square

isolates have shown that the LC_{50} for each isolate was different. Among the two isolates of each *M. anisopliae* and *B. bassiana* tested; *M. anisopliae* isolate PPRC-2 had the least LC_{50} value (2.35×10^9 conidia mL^{-1}) followed by PPRC-2 (2.44×10^8conidia mL^{-1}) and MM (2.48×10^9conidia mL^{-1}). While, the median lethal concentration to killed 50% of termite treated with *B. bassiana* isolates 9609 took the highest lethal concentration (5.08×10^5 conidia mL^{-1}) followed by 9609 isolates (4.55×10^6 conidia mL^{-1}), MM (4.40×10^5 conidia mL^{-1}), MM (4.39×10^6 conidia mL^{-1}) and MM (4.02×10^7conidia mL^{-1}) (Table 4).

DISCUSSION

Spore concentrations of two species of entomopathogenic fungi (*M. anosopliae* (isolates MM and PPRC-2) and *B. bassiana* (9609 and PPRC-56) were

applied on worker termite to determine fungi isolate(s) with high efficacy against *Macrotermes* under laboratory conditions. Both fungi isolates were found to be pathogenic to *Macrotermes*. However, there was a variation in their virulence against *Macrotermes*. The two isolates of *B. bassiana* were significantly less effective when compared with that of *M. anosopliae* isolates in terms of virulence. Among the isolates, PPRC-2, were found more virulent to the *Macrotermes* than the other isolates. Virulence due to *M. anisopliae* isolates on *Macrotermes* was not significantly different as compared to that of standard check after fifth days of the exposure period both causing 100% mortality. This indicates that from all the entomopathogenic fungi, *M. anisopliae* PPRC-2 isolate was the best entomopathogen for the control of *Macrotermes*. The next best entomopathogen was MM, followed by PPRC-56 and 9609 from *B. bassiana* in that order. These findings are in conformity with earlier reports (Singha *et al*., 2006; Ahmed *et al*., 2009; Dong *et al*., 2009; Pik-Kheng *et al*., 2009) who have shown a similar pattern of activity with isolates of these two entomopathogenic fungi against subterranean termite *Coptotermes curvignathus*. Both the fungal species were reported to produce an enzyme exoproteases with insecticidal activity.

The highest concentration (1×10^{9}conidia mL^{-1}) of the fungal isolates *M. anisopliae* (PPRC-2 and MM) had nearly 100% mortality similar to the synthetic chemical (Diazinon) from day four on wards. On average, all the fungal isolates showed a high level of mortality, ranging from 25 to 75% (9609) and 45 to 95% (PPRC-56) for the isolate of *B. bassian* while 60 to 100% (MM) and 75 to 100% (PPRC-2) for *M. anisopliae* isolate when compared to 0.21% Diazinon with 100% mortality over time.

Currently the understanding of how effective the fungal isolates on *Macrotermes* was very limited due to less experiments, but many reports are available showing that the isolates cause a high mortality on other insects (Kannan *et al*., 2008). In this study it has been shown that all the fungal isolates are effective against termite and achieved appreciable mortality. An increase in the concentration of spores generally increased the mortality and generated a faster mortality.

After termites were infested with fungus spores, the mortality rate was lowest on the first two days and increased rapidly on the subsequent days. Percent mortality of termites depends on concentration of conidia and isolates of fungi. Termites that have been treated with conidia of *M. anisopliae* initiate to die within two days after inoculation. After six to seven days white mycelium had developed and green conidia were appeared around the insect cadavers. The reason for this is that fungus spores required the amount of time to thrive and sprout their mycelia into the termites (Krutmuanga and Mekchay, 2005; Brogden *et al*., 2005) opined that mortality increased after the second day of exposure because specific toxin, Destruxin, from the extracts penetrated swiftly into the termites' hemocoel.

In this study, the calculated values of LT_{50} for the isolates suggest that, in general, the *M. anisopliae* isolates PPRC-2 (7.74 to 8.19 days) and MM (7.95 to 8.54 days) elicited mortality quickly than did the *B. bassiana* isolates 9609 (8.26 to 8.80 days) and PPRC-56 (8.11 to 8.59 days). This observation conforms to the earlier reports (Singha *et al*., 2006; Ahmed *et al*., 2009; Dong *et al*., 2009; Pik-Kheng, *et al*., 2009; Cherry *et al*., 2005). The median lethal time taken for fifty percent death of target insect (LT_{50}) was more in the current result may be due to different factors such as difference in physiological combinations, methods of application of spore suspension and the amount used, the age of the insects used plus the condition where the experiments were conducted and other characteristics of entomopathogenic fungi. Also according to Todorova *et al*. (2002) and Alizadeh *et al*. (2007), lethal time (LT_{50}) depends on the spore suspension and with the increase in spore suspension there is a decrease in time taken. Adane *et al*. (1996) reported the lowest times of 2.74 and 5.54 day for *B. bassiana* on bruchid and maize weevil, respectively.

The median lethal concentration (LC_{50}) of different fungal isolates against termite, *Macrotermes* depend upon the fungal species or its isolate. Earlier studies confirmed that conidia of *V. lecanii* were highly pathogenic against aphids (Vu *et al*., 2007). The susceptibility of target insect to fungal infection is concentration dependent (Liu *et al*., 2002; Wright *et al*., 2005). Ansari *et al*. (2004) also reported that mortality of insect pests due entomopathogenic fungi depends on the concentration of conidial suspension, time of exposure and temperature. Entomopathogenic fungi *M. anisopliae* isolates had low LC_{50} values (3.21×10^{5} to 3.82×10^{5}) compared to *B. bassiana* isolates (4.39×10^{5} to 5.08×10^{5}) which is in line with the findings of Singha *et al*. (2011).

CONCLUSION

Based on this finding, we suggest that *M. anisopliae* and *B. bassiana* as a useful option for the management of *Macrotermes* spp. However, further investigations are strongly recommended to be carried out on the possibility of field application as well as finding other isolates of Entomopathogenic fungi that have potential as bio-termiticidal activities against such termite.

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