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Pathogenicity of Beauveria bassiana (Balsamo) Vuillemin (Deuteromycotina : Hyphomycetes) against the Odontotermes obesus (Rambur) workers (Isoptera : Termitidae) under laboratory conditions

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ABSTRACT

Odontotermes obesus (Rambur) (Isoptera: Termitidae) is a common termite pest found in almost all crop plants in South East part of Rajasthan (India).It causes considerably economic losses in tropical and subtropical areas and occurrence of termites in the crop field has become a major threat to farmers. Presently, the control methods mostly swing towards chemical that has led to high cost, persistence and adverse effects to the environment. Presently, emphasis has been given on safer bio control agents like fungi, protozoans, bacteria and virus etc. that are environmental friendly. The present investigation is an attempt to control Odontotermes obesus (R) workers with pathogenic fungus Beauveria bassiana . The pathogenicity of Beauveria bassiana (Balsamo) was tested against the worker termite at four different concentrations (4.5×10^8, 4.5×10^7, 4.5×10^6 and 4.5×10^5 conidia/ml.). Complete population check was observed after 8 days of post inoculation at 4.5×10^8 conidia / ml with LC_{50} value of 8.67×10^7 conidia / ml. under laboratory conditions. ANOVA further supported the significance of the result ($F= 89.61$; df=3 and 8, $P < 0.05$). LT_{50} and LT_{90} for highest dose (4.5×10^8 conidia / ml) were 2.55 days and 5.15 days respectively with inverse correlation with concentrations. Various developmental changes such as abnormal appendages, fragile body, shrunken body, body covered with fungal mycelium were also indicative of chitin inhibitory nature of fungus thus revealing two fold effect on the treated insects. Thus the result shows that mycoinsecticide Beauveria bassiana has a great potential to control the termite and reduce the burden of chemical insecticides on the environment.

Keywords: Beauveria bassiana, Odontotermes obesus, mortality, ANOVA, Entomopathogenic fungus, mycoinsectisides,

I. INTRODUCTION

Termites are important pests which can cause a tremendous amount of damage to agriculture crop field as well as other structure of wood. Apart from wooden structures theses also cause extensive damage to agriculture crops like wheat, maize, sugarcane, cotton, groundnut, pulses etc, vegetables, horticulture plants and forest trees. The annual loss has been accounted to millions of rupees, where about 10-25 percent loss is shared by most agriculture fields and forest crops. Severe losses due to termites have been recorded on wheat and sugarcane in north-eastern parts of India (Varshney, 2004). According to Wood et al. (2010) report annual crop loss was upto 31 percent in pepper, okra and tomatoes, whereas 5 to 50 percent crop loss was reported in maize and wheat.

Pardeshi et al. (2010) observed widest niche breadth of Odontotermes obesus (R.) in crop fields that increases the possibility of attack to the crops at the time of foraging. At germination stage, the termite losses up to 90-100 percent have been recorded in sugarcane at Pakistan (Sattar and Salihah, 2001). Further, prevalence of termites is more in dry crop fields due to lack of irrigation facilities resulting in heavy crop loss.

Prevention of termite damage has been a challenge because of their large populations and cryptic behavior. In India around 200 species of termites are found, amongst which Odontotermes, Microtermes, Coptotermes, Heterotermes, Cryptotermes and Microcerotermes are considered to be the major pests of different economically important crops (Roonwal and Chottani, 1989).

Various methods in termite control were explored in the past including physical, cultural, chemical and biological methods (Pearce, 1997). At present, termites are controlled mainly through synthetic chemicals (Chloropyriphos, Methyl-parathion, Phorate etc.). The worldwide awareness about the environment contamination, insecticides resistance, associated resurgence in insects, accumulation of pesticides residues in food chain, high cost etc. give necessity to search for alternative forms of pest control. The time has come to restructure the future termite control measures using the concept of Integrated Pest Management. Many microbial organisms have strong association with termites and some of them are parasites. Some species are used as commercial biological control of pest with purpose of suppression as well as management of pest. (Quasim, 2015).

Entomopathogenic fungi constitute the largest group with more than 700 species causing mycosis in insects' pest. Various strains of entomopathogenic fungi such as Beauveria bassiana (Quesada et al., 2006), Lecanicillium sp. (previous name, Verticillium) (Jung et al., 2006) and Metarzium (Quesada et al., 2007) have been used to control aphids, lepidopteran larva, termite and other insect pests.

India is bestowed with a rich biodiversity of entomopathogens and exploitation of these natural and renewable resources are essential in a successful biocontrol programmed. Entomopathogenic fungi differ from other insect pathogens because they are able to infect through the host's integument, so ingestion is not required hence and infection is not limited to chewing insects (Fuxa, 1987).

Entomopathogens in soil are not destroyed by solar radiation, as humidity is relatively high and stable in termite nest. They are, in addition, more effective because the termites are social insects which live in nests where the temperature and humidity diverge slightly and are suitable for entomopathogenic fungi (Alves et al., 1995) and with considerable social interaction (Delante et al., 1995; Creffield, 1996). This makes termites a good candidate for control with microbial pathogens which live in the nest. The complexity of the nest soil environment is also regarded significant when developing strategies for biological control of termites. Entomopathogens have proved their efficacy as they are basically abundant in soil. Apart from the soil insect entomopathogens are also useful for many crops of economic importance.

In the present investigation we have taken Beauveria bassiana (Balsamo) Vuillemin for their pathogenicity effect against termite (Odontotermes obesus) workers in the laboratory. It will help in exploring the effectivity of fungus against termites and make a decision for their use in the field conditions for control of the termites.

II. MATERIALS AND METHODS

Termite collection and maintenance

The colonies of the termite species Odontotermes obesus (R) were collected from different crop fields. They were reared in the laboratory in plastic containers with soil, dried wooden sticks and saw dust. The temperature was maintained at 25±5^{0}C and RH was 75± 5%. The worker termites were acclimatized for a period of seven days for before starting bioassay experiments.

Fungal isolate

A pure culture of Beauveria.bassiana (Balsamo) Vuillemin was obtained from Maharana Pratap University of Agriculture and Technology, Udaipur, Rajasthan, India and cultured in laboratory. B. bassiana was grown on Potato Dextrose Agar (PDA). Plates were incubated at 26±2 ^{0}C for 10–14 days and aerial conidia were harvested by flooding the plate with sterile H_2O with 0.05% Tween 80. Conidial suspensions were filtered through a single layer of muslin cloths and final spore concentrations were determined by direct count using a haemocytometer. Conidial concentrations were serially diluted with sterile distilled water to achieve 4.5 x 10^8, 4.5 x 10^7, 4.5 x 10^6 and 4.5 x 10^5 conidia/ml.

Bioassay test

Laboratory reared termite workers were treated with different concentrations (4.5 x 10^8, 4.5 x 10^7, 4.5 x 10^6 and 4.5 x 10^5 conidia/ml) of B.bassiana on the filter paper disc (8.2cm diameter) in the sterile petri dishes (100mm x15mm). 1.0 ml of conidial suspension of different concentrations was applied to the filter paper by a pipette. The conidial suspensions were applied in 10-15 drops evenly distributed on the filter paper. Thirty termite workers were added to each petri dish. Each concentration was applied to three replicates. 1.0 ml 0.05% Tween-80 solution was added on the filter paper in Petri dishes as the control for each treatment. Petri plates were kept under laboratory conditions of 27^0±2^0C and 70±5 RH%. The bioassay was carried out to verify the worker termites mortality at every 24 hrs. up to 8 days after exposure. Mortality assessment was then made by counting dead and live worker insects and Abbott's percent of mortality was calculated. Morphological abnormalities were also observed daily.

Statistical analysis

All data were subjected to analysis of variance; Two-way analysis of variance (ANOVA) test was conducted to demonstrate among conidial concentrations and check treatment. Percent mortality was compared by Duncan multiple range test at 0.05 percent probability level (Duncan, 1955). Data represented the means of three replicates. Further, obtained data were corrected using Abbott's formula. The average worker termite mortality data were subjected to probit analysis, for calculating LC_{50} ,LT_{50} calculated by using the Finney(1971). Results with $P < 0.05$ were considered to be statistically significant.

III. RESULTS

Efficacy of Beauveria bassiana (Balsamo) under laboratory conditions (Table 1-3, Graph -1)

Observations revealed that Beauveria bassiana caused a gradual positive lethal effect on the termite worker of O. obesus. Essential linear relationship was between concentrations of conidia and mortality. As the concentration and time were increased the percent mortality also increased. The maximum percentage of mortality was achieved in the highest conidial concentration (4.5X10^8 conidia/ml) i.e. 52.47% after 72 hrs. of treatment and 100% after 196 hrs. while the minimum percentage mortality was recorded in the lowest conidial concentration (4.5X10^5 conidia/ml) with an average of 18.30% after 72 hrs. of treatment and 77.78% after 196 hrs. (Table -1). After 10-14 days of inoculation, conidia sporulated on the cadavers of termites which confirmed the death due to fungal toxicity. ANOVA revealed that F value was statistically significant for concentrations (F=89.61; df=3, 8; P< 0.05) when termite workers were directly exposed with different conidial concentrations in laboratory (Table-2). When Chi square test for goodness of fit was conducted, the test revealed that it was non-significant ($\chi 2$ =3.61, 2.26, 1.11 and 3.12 at df =6) which means that there was no significant differences between the observed and predicted proportion of mortality.

The termite workers were susceptible to B. bassiana (B) Vuill. in a dose dependent manner. Results of the probit-transformed mortality data obtained from applying known conidial suspensions have been used to determine the LC_{50}, LC_{90}, LT_{50} and LT_{90} values (Graph 1). The values of median lethal concentration (LC_{50}) was 8.67X10^7 conidia/ ml and LC_{90} was 4.52 X 10^{12} conidia/ ml of fungal culture. Comparative data on time to death was analysed as estimates of lethal Time (LT_{50}), which is number of days to achieve 50 percent mortality. With decreasing conidial concentrations LT_{50} values increased to be longer for termite workers (Table -3). At highest dose 4.5 x 10^8 conidia/ml, the LT_{50} value was 2.55 days whereas lowest concentration 4.5 x 10^5 showed 4.53 days. LT_{90} values vary from 5.15, 6.11,6.78 and 7.59 days for 4.5 x 10^8, 4.5 x 10^7, 4.5 x 10^6 and 4.5 x 10^5 conidia/ml respectively which were also statistically significant.

Morphological and Behaviour abnormalities

As the fungus brought characteristic abnormalities in insect that indicates clearly its chitin inhibitory nature. During research, the treated worker depicted different morphological abnormalities. Observations after inoculation till death indicated that infected worker termites became inactive and assembled in groups. Infected termites also depicted signs of unsteady motion of their antennae and appendages after 24 hrs. of inoculation. Infected termite workers with the fungus B.bassiana also led to unusual behaviour like sluggishness, non co-ordinate activity, decreased feeding, shrinkage of the body surface (flaccid), abnormal appendages (Fig:1 B,C), reduced activity greatly and ultimately die. After death termites worker distorted from a healthy creamy colour to light brown due to pigmentation in the region of abdomen (Fig:1 B,C), ventral cuticle of abdomen was totally indistinguishable. The dead termites became shrunken (Fig:1 B,C), and crumpled. Fungal conidia attack insects by penetrating cuticle and producing toxins and draining nutrients from the insects body and sooner or later killing the insect. Mouth parts and appendages became short and stumpy which inactivate the movement of worker.

In the ambient temperature the fungal mycelium grew outwardly through the cuticle, and wrapping the dead termites (Fig:2). The head, thorax and other appendages of worker were found to be covered with white mycelial growth due to the infection of B. bassiana on the surface of the worker.

IV. DISCUSSION

Realization of entomopathogenic fungi used for biological control of insects has increased now a day and some of them are also available commercially too (Shah & Pell, 2001). Fungal conidia are responsible for infection by contact to the host through the cuticle and it involves complex biochemical interactions between the host and the fungus (Lacey et al., 2008). According to Lacey et al. (2008) the successful use of entomopathogenic fungi as insect control agents ultimately depends on the use of the right propagate, formulated in best possible manner and applied at proper dosage and time.

When the termite workers were treated with different suspension of B. bassiana for 8 days, percent mortality was observed 100%, 97.38%, 88.10% and 77.59% at 4.5 x 10^8, 4.5 x 10^7, 4.5 x 10^6 and 4.5 x 10^5 conidia/ml doses respectively. Ahmed et al. (2009) observed that higher spore concentration of M. anisopliae (2.2x10^{10}) spores/ml was more lethal to the termite population. When the hyphae invaded the body of the termite, hyphae grew, reproduced and produced large number of conidia, the whole inner structure of termite was destroyed by the hyphae of B.bassiana. These observations confirmed to the earlier reports (Ahmed et al. 2009, Dong et al. 2009 and Pik-Kheng et al. 2009

) showing a similar pattern of activity with entomopathogenic fungus. The fungus B. bassiana was reported to produce exoproteases with insecticidal activity.

Karthikeyan and Jacob(2010) also reported that B.bassiana fungus had a 56.67 to 80.00 percent mortality at 10^8 to 10^9 spores/ml in adult Rice Blue Beetle, Leptispa pygamea Baly (Coleoptera : Chrysomelidae) and LC_{50} values was 2.26 X 10^4 spores/ml under laboratory condition. Mc Coy et al. (2000) reported that the conidia of B.bassiana have suppressed root weevil larval populations when applied at high inoculums rates. The results are also in confirmation with the work of Rosengaus and Traniello (1997) , Liu et al. (2002) and Wright et al. (2004), who reported that the susceptibility of the termites to fungal infection was often concentration dependent. Shimazu (2004) noted that adhesion of dry conidia of to the pine borer Monochamus alternates from contact provided effective control of the insect.

Time was another important factor affecting the percent mortality of treated insects, the higher the concentration, the less the time required to reach the LT_{50}. For a fungal pathogen B.bassiana against termites, it causes mortality after a lag period to allow the maximum number of contacts with the infected carrier before death (Carruthers and Soper, 1987). The workers that picked up fungal conidia must travel through the colony and encounter other individuals before dying. Quesada Moraga et al. (2006) elucidated that the efficacy of the entomopathogenic fungus began clearly after 48 hrs. after inoculation and the hyphae penetrated the integument inside the trachea, epithelial and epidermal cells, after 72 hrs. the fat tissues were damaged and lethality may increase to100 percent after 96 hrs. Changjin (2009) reported that Conidia from the M. anisopliae were highly virulent for O. formosanus causing approximately 100% mortality 3 days post inoculation in the concentration of 3X10^8 conidia/ml. Hyphae of fungus seriously destroyed hemolymph, various tissues, pipelines and produced large number of conidia in the body of termite.

The time interval from fungal invasion to death of the insect host varies among different species of entomopathogenic fungi, and between hosts as found in our study. The reasons that manage the length of this period are the dose of inoculums, virulence of the fungal strain, production of mycotoxins and condition of the host (Chouvenc et al., 2011). Singha et al. (2010) observed the morphology and behavior changes in the treated termite and suggested that fungal growth can cause serious damage to the pest disturbing its major physiological activities resulting in its death.

Development of various malformations in treated workers further supported the fact that fungus is acting on the fact that fungus is acting on the cuticular structure of the insect. Since a cuticle is the governing factor for insects survival, the attack of fungus brings a complete check on the population. As the success of insects in resisting desiccation, breathing and adapting to adverse environmental condition is directly related to the proper functioning of the body cuticle. The abnormal structural features of the cuticle of the treated O. obesus suggest that the fungal growth can cause serious damage to the insect, disturbing its major physiological functions ultimately leading to death. This was supported by our observations on the behavioural response of the insect to the exposed fungus species Beauveria bassiana. The insect cuticle is an extra cellular matrix, secreted and maintained by epidermis (Smyth, 1968). The cuticle covering of the body of termite falls under the soft cuticle category. Hence, it is easily susceptible to fungal growth resulting in chemical as well as morphological abnormalities as reflected in the micrographs. The death may also be due to secretions of various metabolites like proteases, hydrocynic acid and also due to suppression of immune system.

V. CONCLUSION

To conclude, the present study revealed that the entomopathogenic fungus B.bassiana is highly virulent against O. obesus worker, a serious pest of different crops. Biological control with pathogenic fungi is a promising alternative to chemical control against the termites (Grace,1997 and Milner et al., 1998). Biological control with pathogenic fungi might provide long lasting insect control strategies without damage to the environment or non-target organisms (Khetan,2001). Also, social interactions within a termite colony and its dark, damp habitat would seem to favor survival and distribution of pathogenic fungi (Grace, 1997).

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Table 1. Efficacy of different concentration of *Beauveria bassiana** against worker termite (*Odontotermes obesus*)

Concentration	Corrected % Mortality							
	24 hrs	**48 hrs**	**72 hrs**	**96 hrs**	**120 hrs**	**144 hrs**	**168 hrs**	**192 hrs**
4.5 x 10^8	19.48 ± 3.35^{cd}	42.86 ± 3.57^{cd}	52.47 ± 2.83^{bcd}	63.41 ± 3.78^{cd}	85.19 ± 3.71^{bcd}	98.67 ± 2.31^{bcd}	100.00 ± 0.00^{d}	-
4.5 x 10^7	14.92 ± 1.54^{d}	30.95 ± 2.06^{d}	42.68 ± 1.86^{acd}	53.71 ± 3.21^{d}	71.60 ± 2.14^{ad}	85.54 ± 2.15^{acd}	97.38 ± 2.27^{d}	100.00 ± 0.00^{a}
4.5 x 10^6	10.31 ± 3.23^{a}	20.24 ± 7.43^{ad}	31.70 ± 1.89^{abd}	45.11 ± 3.88^{a}	69.14 ± 4.27^{ad}	77.64 ± 2.09^{abd}	88.10 ± 4.16	98.67 ± 2.31^{a}
4.5 x 10^5	06.86 ± 3.28^{ab}	07.14 ± 3.57^{abc}	18.30 ± 3.72^{abc}	40.30 ± 4.38^{ab}	49.38 ± 2.14^{abc}	63.18 ± 4.24^{abc}	77.59 ± 8.46^{ab}	98.67 ± 2.31^{a}

*Means within columns followed by the same letter did not differ significantly ($P \leq 0.05$).

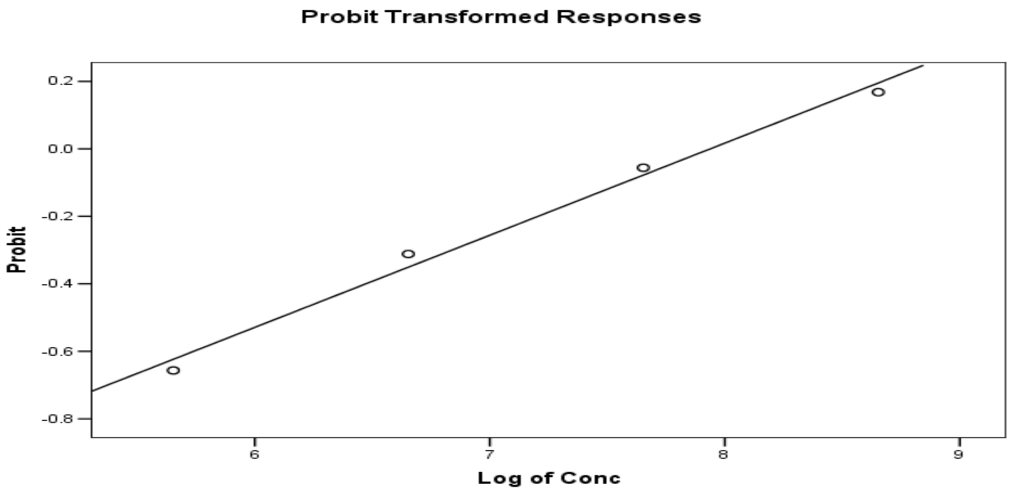
Table 2 . Significance of conidial concentrations of *Beauveria bassiana* (Balsamo) Vuillemin between percent mortality and conidial concentration

ANOVA for worker termite

Concentration	N	Mean	SD	Df	F	Result
4.5 x 10^8	3	52.47	2.83	3, 8	89.61	***
4.5 x 10^7	3	42.68	1.86			
4.5 x 10^6	3	31.70	1.89			
4.5 x 10^5	3	18.30	3.72			

Table 3. Probit analysis [Fiducial limits (95 percent), LC_{50}, LC_{90}, regression equation for mortality of *Odontotermes obesus*].

Probit Equation	LC_{50}	95% Confidence Limit		LC_{90}	95% Confidence Limit	
		Lower	Upper		Lower	Upper
p = -2.156 + .069(Conc.)	$8.67X10^{7}$	$1.22X10^{7}$	$7.35X10^{10}$	$4.52X10^{12}$	$1.61X10^{10}$	$3.96X10^{30}$



Graph 1. Probit Transformed response, of *Odontotermes obesus* treated with *Beauveria bassiana* Concentration v/s Mortality

Table 4

Concentration v/s time

Concentration	LT50(Hrs.)	95% Confidence Limit		LT90(Hrs.)	95% Confidence Limit		Chi Sqr	df	Result
		Lower	Upper		Lower	Upper			
4.5×10^{8}	61.35	48.98	71.52	123.56	110.13	143.98	3.61	6	NS
4.5×10^{7}	76.09	63.97	86.66	146.57	132.01	167.84	2.26	6	NS
4.5×10^{6}	89.47	77.91	100.15	162.83	147.32	185.39	1.11	6	NS
4.5×10^{5}	108.19	97.32	119.14	182.25	165.48	206.86	3.12	6	NS

[a] Pearson chi-square goodness-of-fit test on the Probit model (α= 0.05).

Table 5

Probit Equation for Time

Concentration	Probit Equation
4.5×10^{8}	p = -1.264 + 0.021 (Time)
4.5×10^{7}	p = -1.384 + 0.018 (Time)
4.5×10^{6}	p = -1.563 + 0.017 (Time)
4.5×10^{5}	p = -1.872 + 0.173 (Time)

Figure 1. Developmental changes observed on worke termit after treatment with *Beauveria bassiana* (Balsamo) Vullimen AA– Abnormal Appendages; DB-Demalinized body; ShA– Shrunken Abdoman;