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Entomopathogenic Fungus of Termites and their Potential in Management of Forest Nurseries and Plantations

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The fungal strains were isolated from the dead termites collected around Mudigere taluk, Western Ghats of Karnataka. The isolated fungal cultures were inoculated for three species of termites and mortality was recorded. Initially *Odontotermes wallonensis* termite was exposed to entomopathogenic fungus at different time intervals. The analysis showed the termite mortality was initially low but gradually increased and highest was found at 36 - 48 hours of exposure period. Among four fungal isolates screened *B. bassiana* and *M. anisopliae* showed 100 % of mortality of termites, compared to *Penicillium notatum* and *Aspergillus flavus lowest* LT 50 values. *Odontotermes brunneus* also showed similar results with high mortality at 36- 48 hrs of exposure period, among four isolates *B. bassiana* and *M. anisopliae* showed high mortality at 60 hours exposures, with lowest LT 50 values. In *Odontotermes obesus* initially the mortality was low but as the time advanced gradually the mortality of termites were also increased and it was peak at 36-48 hours of exposure. It is concluded that among fungal isolates *B. bassiana* and *M. anisopliae* is effective entomopathogenic fungus which cause death of termite, hence can be used as good bio- pest to control termite population.

Key words: Entomopathogens, Fungus, Termites, Mortality

Termites cause significant damage to the various tree species in the forest nurseries and plantations. At times, the infestation is quite alarming, and even it threatens survival of the nursery stock in many forest areas. In well managed nurseries, the problem of termite damage is almost negligible. However under unfavourable conditions the termite attacks have resulted in abandonment of nurseries and total loss in plantations (Thakur, 1990). The increased use of conventional chemical pesticides to control termites has adverse effects on the environment and non-target organisms. Hence the necessity for eco-friendly pest management technique is being largely felt in the recent times. Entomogenous fungi are potentially the most versatile biological control agents, due to their wide host range that often results in natural epizootics.

The temperature and humidity vary little in these nests, are suitable for the type of pathogens (Fernandes and Alves, 1991; Fernandes and Alves, 1992a; Alves *et al*., 1995). Biological control of subterranean termite using entomopathogenic bacteria and fungus are promising alternative to chemical control against termites (Krutmuang, 2005).

The study of termite pertaining to the relationship which exists between the termites and their environment has got practical utility. But so

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far very little work has been done in India on ecological aspects of termites. Termites are the social insect which has many advantages to ecosystem but major disadvantage is damage caused by termites to timber and timber products. (Andrew. C. Rath, 2000). Hence, the present investigation was taken up to isolate and identify the entomopathogenic fungi and their potential to control termites in forest nursery and plantation in Western Ghats of Karnataka.

MATERIALS AND METHODS

The research work was conducted in the Department of Agriculture Microbiology laboratory, College of Horticulture; Mudigere (Chikmagalur district). The survey work was carried out and dead and diseased termite species were collected in different forest nurseries and plantations which are located in the places such as Mudigere taluk, Chikamagalore district in Western Ghats of Karnataka. The entomopathogens were isolated from the termites with standard procedures of serial dilution and plate count method was followed (K. R. Aneja, 2003).Based on the visual appearance and morphological characteristics of the isolated colonies were studied following the standard microbiological methods (K. R. Aneja, 2003) on the medium. The isolated entomopathogenic Fungus were stored in the refrigerator for further use.

The infectivity and bioassays were conducted with entomopathogenic fungi on the three common mound building termites, *Odontotermes wallonensis, Odontotermes brunneus* and *Odontotermes obesus*. Under each termite species two casts namely workers and soldiers were utilized. For collecting test termites for the study, uniform sized termite castes were collected from mound. The assorted populations of collected worker and soldier termites were maintained in the laboratory, on fungal comb. The culture was maintained at 25 ± 2° C with relative humidity of 80 to 90 %. Due care was taken for acclimatization of worker termites before proceeding for the bioassay (Gurusubramanian *et al*., 1999). Every time the samples were drawn from the same mound for a given species.

Bioassay tests were carried out with four isolates of *Beauveria bassiana, Aspergillus flavus*, *Penicillium notatum*, *Metarhizium anisopliae*, as they were effective mycopathogens among the various fungi tested against termites for their infectivity tests at concentration of 10^{10} conidia per ml, for each fungal isolate were standardized. The termites were sprayed with the inoculum as described in section. Each treatment had 25 insects and there were four replications. Observations on the mortality were recorded at 2 hour interval up to seven days. The four isolates of *Beauveria bassiana, Aspergillus flavus, Penicillium notatum*, *Metarhizium anisopliae*. Optimum conditions (temperature, cell concentration, growth period and different media) required for growth and yield of conidia was determined in a series of growth experiments.

In the GKVK forest nursery 2 meter breadth and 5 meter length beds were selected from the termite swarming sites. The entomopathogenic treatments of *Beauveria bassiana, Aspergillus flavus*, *Penicillium notatum*, *Metarhizium anisopliae* were used and Control without any entomopathogenic dose. Out of total five treatments four treatments were performed by distributing 10g of fungal (5×10^9 conidia ml^{-1}) per bag containing six month old seedlings. Four replications were followed for each treatment. **Treatments:** T1=Control without entomopathogen, T2= *Aspergillus flavus*, T3= *Penicillium notatum*, T4= *Beauveria bassiana*, T5= *Metarhizium anisopliae*, and the observations were recorded termite damage was assessed by recoded the number of infected seedlings or extent of damage caused due to termite activity on plants. Observations were recorded from one week after treatment at weekly intervals up to three months and data was analyzed statistically.

RESULTS AND DISCUSSION

The isolates which are brought after survey were taken to lab study to identified the strain based on morphological; characteristics with the help of Department of Entomology, UAS, GKVK, Bangalore. The survey samples of fungal isolates were analyzed under laboratory conditions in the Department of Agricultural Microbiology, UAS, GKVK, Bangalore. The samples were cultured on Petri plates, the colony characters (Spores and mycelial) were observed under the microscope and

they are identified and codes were given; UASBF (24) 1 to UASBF (24) 18. Among eighteen fungal isolates five fungal isolates namely *Metarhizium anisopliea*, *Beauveria bassiana, Penicillium notatum* and *Aspergillus flavus*, were confirmed based on conidial morphology based on standard manual (. Aneja, K. R. 2003, Purohit, 2003, and Singh, R. P. 2005). Three commonly available mound building termites viz; *Odontotermes wallonensis, Odontotermes brunneus* and *Odontotermes obesus* were utilized for pathogenicity and bioassay studies. In the preliminary pathogenicity assay all four isolate of fungus viz; *M. anisopliae*, *B. bassiana, Penicillium notatum,* and *Aspergillus flavus* were used.

Initially *Odontotermes wallonensis* termite was taken for study and exposed to entomopathogenic fungus at different time interval of 12 hrs, 24 hrs, 36 hrs, 48 hrs and 60 hrs respectively. The four isolates of fungus vize; *Penicillium notatum*, *Aspergillus flavus*, *Beauveria bassiana*, *Metarhizium anisopliae* and with control (without entomopathogenic fungus) for both castes worker and soldier. The analysis showed that termite mortality was initially low and gradually increased and was more at 36 - 48 hours of exposure period. Among four fungal isolates screened *B. bassiana* and *M. anisopliae* showed very high of 100 % mortality of termites when compared to *P. notatum* and *A. flavus,* where as some of the termites were alive. In control treatment all the termites were alive. *B. bassiana* and *M. anisopliae* were found non significant difference among both the workers and soldiers of *O. wallonensis* termites. The Probit analysis was carried out to know the mortality response of worker and soldiers of *O. wallonensis* against fungal isolates viz; *A. flavus*, *P. notatum*, *B. bassiana* and *M. anisopliae* was computed. Among workers of *M. anisopliae* isolate recorded lowest LT 50 value (Table-1) followed by *B. bassiana, A. flavus, P. notatum* and among the soldiers *B. bassiana* isolate recorded lowest LT 50 value followed by *M. anisopliae, A. flavus, P. notatum.*

The O. brunneus also showed similar result with high mortality rate at 36- 48 hrs of exposure period among four isolates of entomopathogenic fungus, But *B. bassiana* and *M. anisopliae* showed high mortality rate. At 60

Table 1.

Fungus	Termite	Workers				Soldiers			
		Chi^2	Regression	LT*50	Fiducial limits (95%)	Chi^2	Regression	LT*50	Fiducial limits (95%)
Aspergillus flavus	*Odontotermes wallonensis*	6.04	1.62721	81.63	71.79 - 92.47	5.22	6.48712	68.30	61.63 - 83.42
Penicillium notatum		7.87	2.61248	87.56	80.55 - 95.39	7.69	3.86415	9.10	66.29 -112.92
Beauveria bassiana		44.52**	0.66821	15.23	10.39 - 42.79	45.84**	1.93104	25.09	15.70 - 42.77
Metarhizium anisopliae		46.52**	0.92462	11.44	12.59 - 57.32	40.36**	1.15030	29.90	17.27 - 45.21
Aspergillus flavus	*Odontotermes brunneus*	15.75	2.23421	73.71	66.85-98.86	10.88	3.19070	67.47	52.48-97.40
Penicillium notatum		8.48	2.53613	81.46	6.56-98.96	7.76	3.32563	65.18	56.66-93.07
Beauveria bassiana		72.40**	1.25566	27.77	21.54-40.93	57.54*	1.85248	21.08	11.52-32.42
Metarhizium anisopliae		80.21**	0.98476	29.25	22.05-48.77	55.05*	1.44483	24.57	14.30-37.06
Aspergillus flavus	*Odontotermes obesus*	7.08	2.93515	72.09	66.82-90.89	6.48	4.01376	66.46	62.80-95.83
Penicillium notatum		7.91	2.91283	84.57	73.98-92.00	7.91	3.38076	69.28	60.53-90.78
Beauveria bassiana		66.23**	0.90107	28.69	18.13-36.23	43.80**	1.84672	25.09	15.08-36.05
Metarhizium anisopliae		70.28**	1.03634	22.84	16.14-34.26	31.38**	1.30722	28.79	18.37-32.12

* Significantly good

Table 2. Termites Infestation Of Forest Nursery Seedlings In The Analysis Of Field Efficacy Of The Evaluated Entomopathogens

Entomopat hogens	Plants infected by termites											
	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8t0 week	9th week	10th week	11th week	12th week
Control	0.5^{eee}	1.25^{ee}	2.25^{de}	2.75^{ddd}	3.25^{dd}	5.00^{dc}	6.75^{bc}	7.25^{bb}	9.00^{b}	10.75^{ab}	12.75^{aa}	14.00^{aaa}
Aspergillus flavus	0.0^{e}	0.5^{ee}	1.0^{d}	1.0^{d}	1.5^{dc}	2.0^{ccc}	2.0^{ccc}	2.0^{cc}	2.0^{c}	2.25^{ac}	2.75^{a}	3.0^{aa}
Penicillium notatum	0.0^{ddddd}	0.5^{ddddd}	1.0^{dddd}	1.5^{ddd}	1.5^{dd}	2.5^{d}	3.0^{cd}	4.75^{cbb}	4.75^{cbb}	5.0^{cb}	5.5^{b}	6.5^{a}
Beauveria bassiana	0.0^{ccc}	0.0^{cc}	0.0^{cc}	0.0^{cc}	0.5^{bba}	0.5^{bba}	0.5^{bba}	0.5^{bba}	0.5^{bba}	1.0^{ba}	1.5^{a}	1.5^{aa}
Metarhizium anisopliae	0.0^{dddd}	0.0^{dddd}	0.0^{dddd}	0.0^{dddd}	0.0^{dddd}	0.5^{bc}	0.75^{bb}	1.00^{ba}	1.00^{ba}	1.00^{ba}	1.0^{ba}	1.25^{aa}

Note: Means with the same letter are not statistically significant (P=0.05) according to the new Duncan's multiple range test,

hrs exposure, we found 100% mortality in *B. bassiana* and *M. anisopliae*, where as in case of *P. notatum, A. flavus* were found some of the termites were found alive in both the workers and soldiers. Were as in control there was no mortality of termites. The Probit analysis was carried out to know the mortality response of worker and soldiers of *O. brunneus* against fungal isolates viz *Aspergillus flavus*, *Penicillium notatum*, *Beauveria bassiana* and *Metarhizium anisopliae* was computed among workers *B. bassiana* isolate recorded lowest LT 50 value (Table-1) followed by, *M. anisopliae, A. flavus, P. notatum* and among the soldiers *B. bassiana* isolate recorded lowest LT 50 value followed by *M. anisopliae, P. notatum. A. flavus.*

In *O. obesus* initially there was infection and the mortality also was low but as the time advanced the mortality of termite also increase gradually and it was peak at 36-48 hours of exposure. And little low mortality at 60 hours exposure. Among different isolates screened *B. bassiana* and *M. anisopliae* showed very good mortality initially and both showed 100% mortality at 60 hours of exposure period. Where is in other hand *P. notatum* and *A. flavus* compare to *B .bassiana* and *M. anisopliae* showed lower mortality. There was significant difference in workers and soldiers. The Probit analysis was carried out to know the mortality response of worker and soldiers of *Odontotermes brunneus* against fungal isolates viz *Aspergillus flavus*, *Penicillium notatum*, *Beauveria bassiana* and *Metarhizium anisopliae* was computed among workers *M. anisopliae* isolate recorded lowest LT 50 value followed by, *B. bassiana, A. flavus, P. notatum* and among the soldiers *B. bassiana* isolate recorded lowest LT 50 value followed by *M. anisopliae, P. notatum. A. flavus,* and the results are presented in (Table-1).

The dose at which microbial insecticide controls the insect pest depends on the susceptibility of the pest population and the inherent potency of the microbial insecticide. In the present study, the susceptibility of termites to different fungal isolates was done by estimating and comparing the LT50 values. Since, LT50 values were most useful in comparing the infectivity of different fungal isolates (Burges and Thonpson, 1971). Various estimates of the regression based on Probit analysis of dosage mortality and time

mortality response of the host species to fungal isolates are also discussed. The Chi square test in all the bioassays with the three species of termites showed homogeneity of the test population, which was a reflection of a good fit of the regression also was a reflection of the precision of the techniques and procedures adopted in the bioassay test (Ignoffo *et al.,* 1982).

Among the fungal isolates tested *M. anisopliea* and *B. bassiana* were the most effective against all the tree species of termites (recording the lowest LT50). The Probit analysis results showed *M. anisopliea* and *B. bassiana* showed most effective against all the three species of termites, recording lowest LT50 followed by *Penicillium notatum,* and *Aspergillus flavus* showed *M. anisopliea* and *B. bassiana* most effective fungal strains to control termite (Table 1). The virulence in different isolates within the single fungal species might have been varied due to heterokaryanosis, somatic recombination from the anastomosis of huphae and saprobic growth the fungus has undergone in the environment prior to encountering insects as reported by Roberts Yendol (1972). Among the three species of termites *Odontotermes brunneus* was the most susceptible to fungal isolates followed by *Odontotermes wallonensis* and *Odontotermes obesus*

Field evaluation of potential entomopathogens on termites after analysis, a field trial was conducted on nursery, each plot was treated with different entomopathogenic isolates, where the natural termite activity was present to evaluate a effective damage/infection to seedling in nursery was considered as a check point and the observation was taken upto 12 weeks after analysis the infection/damage to plot inoculate among fungal isolates *B. bassiana* and *M. anisopliae* showed enhanced cause of mortality of termite (Table 2) when compared to other fungal isolate. *B. bassiana*. *M. anisopliae* are most effective entomopathogenic isolates, It is concluded that among fungal isolates *B. bassiana* and *M. anisopliae* is effective entomopathogenic fungus which cause death of termite and which can be used as good bio- pest to control termite population.

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