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Improtance of Entomopathogenic Bacteria to Control Termites in Forest Nurseries and Plantations

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The bacterial strains were isolated from the dead termites collected around Mudigere taluk, Western Ghats of Karnataka. The isolated bacterial cultures were inoculated for three species of termites and mortality was recorded. *Odontotermes wallonensis* against entomopathogenic bacteria were screened and the DMRT analysis done for *Bacillus subtilies*, *Bacillus cereus, Pseudomonus florescens, Streptococcus aureus and Bacillus thuringiensis,* with control of termites both castes of worker and soldier at different time intervals of 12 hrs, 24 hrs, 36 hrs, 48 hrs and 60 hrs respectively. After 36 - 48 hrs exposure period mortality was high in *B. thuringiensis* and recorded lowest LT 50 value followed by *B. subtilies, P. fluorescens, B. cereus, S. aureus* among workers and soldier. *Odontotermes brunneus* also showed similar results with high mortality rate at 36- 48 hrs of exposure period among four isolates. At 60 hrs of exposure to *B. thuringiensis* we found 100 % mortality of termites. In *Odontotermes obesus* initially mortality was low but gradually increased at 36-48 hours exposure. Among different isolates *B. thuringiensis* showed 100 % mortality of both workers and soldiers. Hence, *B. thuringiensis* can be used as good biopesticide to control termite population to reduce damage in forest nurseries and plantations.

Key words: Entomopathogens, Bacteria, Termites, Mortality .

Termites are members of the order Isoptera, a relatively small order with approximately 2300 described species (Watson and Gay, 1991). They are soft- bodied insects with cryptic habits, and they are the only social insects in the exopterygota. A colony of termites comprises a reproductive pair, usually a king and a queen, numerous sterile workers and soldiers whose tasks include foraging, nest building, maintenance, defense care of eggs and young once (Creffield, 1996). Termites cause significant damage to the various tree species in the forest nurseries and plantations. According to one of the estimate (Wiseman and Egglebur, 1994) termite causes around US$ 40,000 million of damage per year to building, substantial amount of damage to forest, nursery and agricultural crops. Thus in these days control of termite is gaining greater importance especially the use of biological agents for control of these pests gaining significance due to its efficiency of controlling termite population (Maureen. *et al*., 2005).

The most widely used microbial control agents of bacteria are *Bacillus subtilis, Bacillus papillae, Serratia entomophila, Bacillus sphaericus* and *Bacillus thuringiensis. Bacillus*

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thuringiensis is a gram-positive bacterium that produces crystalline inclusions during sporulation process. These parasporal crystals consist of protein molecules or beta–endotoxin, which is toxic to various insects. These proteins are highly specific for insect gut toxins, with a superior safety recorded regarding their effects on non-target organisms. Varieties of *Bacillus thuringiensis* are currently used to control a broad range of crop and forestry pests. Other species of bacteria are used on a much smaller scale for insect control.

Control of pest insects using chemical pesticides has generated several problems including insecticide resistance; outbreaks of secondary pests normally held in check by natural enemies; safety risks for humans and domestic animals; contamination of ground water; decreased biodiversity; and other environmental concerns. These problems and sustainability of programs based predominantly on conventional insecticides have stimulated increased interest in integrated pest management (IPM). Sustainable agriculture in the 21st century requires an alternative to chemical pesticides for pest management. Those are environmentally friendly and reduce the amount of human contact with pesticides. Hence, the present investigation was taken up to isolate and identify the entomopathogenic bacterial 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 in different forest nurseries and plantations which are located in Western Ghats of Karnataka. Dead and diseased termite species were collected in the forest nurseries and plantations 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; Collee and Miles, 1989; Lacey, 1997) on the medium. The isolated entomopathogenic microorganisms (Bacteria and Fungus) were stored in the refrigerator for further use.

The infectivity and bioassays were conducted with entomopathogenic bacteria 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 natural food collected from termatoria). 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.

Infectivity tests with different bacterial isolates conducted with a uniform dose of 10^7 cell ml^{-1} against the three species of termites. The termites were taken in a Petri dish (9cm) lined by filter paper (Whatman 100) and were directly sprayed with 3ml bacterial suspension using a hand atomizer. Control insects were sprayed with only sterilized distilled water. After air drying the treated filter paper termites was carefully transferred to the petridish (9cm). Small pieces of fresh fungus combs and wood were provided as food. The petridish containing the treated filter papers and insects were maintained at 25+-2° C. For each treatment 25 worker and 25 soldier termites were used with four replications. Observations on the mortality of termites were taken at every 2 hour intervals up to seven days. To confirm the pathogencity by entomopathogenic bacteria reisolated the organism from the infected termites. Fresh healthy termites were again used to the test for the mortality.

In the GKVK forest nursery 2 meter breadth and 5 meter length beds were selected from the termite swarming sites. The entomopathogenic treatments of *Bacillus cereus* and *Bacillus thurengenisis* were used and Control without any entomopathogenic dose. Out of total five treatments four treatments were performed by

distributing 10g of bacteria (5 x 10^9cfu / ml) per bag containing six month old seedlings. Four replications were followed for each treatment.

Treatments

T1=Control without entomopathogen, T2= *Bacillus cereus,* T3=*Bacillus thuringiensis, T4= Pseudomonas fluorescens,* T5= *Streptococcus Aureus,* 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 isolate were subjected to gram staining, spore staining and crystal staining and based on shape they were given isolate number with code of UASB1 to UASB20 further set of biochemical test were conducted to confirm according to Bargies manual (Madigan, *et al*., 2003).

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 bacteria viz; *Bacillus thuringiensis, Pseudomonas fluorescens, Streptococcus aureus , Bacillus cereus* and *Bacillus subtilies.* Mathur *et al.* (1994) reported that the three commercial formulations of *Bacillus thuringiensis* Berliner, *viz*., Dipel wettable powder containing 25x10^9 viable spores/g of the final product of *B. thuringiensis* assessed for their pathogenicity against the insects. Dipel proved to be the most effective, showing the maximum time of 48 hours to initiate to kill and a maximum of 120 hour were required to induce 89.99 % mortality of insect at 0.5 % concentration.

Odontotermes wallonensis against entomopathogenic bacteria were screened and the DMRT analysis for *Bacillus subtilies* , *Bacillus cereus, Pseudomonas florescens, Streptococcus aureus and Bacillus thuringiensis* and with control(without entomopathogens) of termites both caste worker and soldier. After treating different entomopathogenic bacteria the termites are incubated at different time intervals of 12 hrs, 24 hrs, 36 hrs, 48 hrs and 60 hrs respectively. The analysis showed that the termite was initially low mortality and gradually increased and it was more mortality at 36 - 48 hrs of exposure period. Among five bacterial isolates screened *B. thuringiensis* showed very high of 100 % mortality of termite when compare to *B. subtilies*, *B. cereus, P. florescens, S. aureus.* Whereas some of the termites were alive in *B. subtilies*, *B. cereus, P. florescens, S. aureus* and in control all the termites were alive. *B. thuringiensis* showed that there was no significant difference among 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 bacterial isolates viz *Pseudomonas fluorescens*, *Streptococcus aureus*, *Bacillus cereus, Bacillus subtilies* and *Bacillus thuringiensis* was computed among workers, *B. thuringiensis* isolate recorded lowest LT 50 value followed by *B. subtilies, P. fluorescens, B. cereus, S. aureus* and among the soldiers also *B. thuringiensis* isolate recorded lowest LT 50 value followed by *P. fluorescens, B. subtilies, B. cereus, S. aureus.* The results are represented in (Table 1), the lowest LT50 values shows more mortality the LT 50 values high the low mortality of termites. Raquel (2002) reported that the effect of *Bacillus thuringiensis* (Bt) Berliner on the termite *Nasutitermes ehrhardti* (Isoptera, Termitidae) was evaluated under laboratory conditions. From 55 *Bt* subspecies assayed *in vivo* under controlled conditions seven were found to be pathogenic in the subspecies *yunnanensis*, *huazhongiensis*, *brasiliensis*, *colmeri* and *kurstaki* (less than 72 % of mortality), particularly *sooncheon* and *roskildiensis* (100 % mortality at the seventh day after the bacteria application).

Odontotermes brunneus also showed similar result with high mortality rate at 36- 48 hrs of exposure period among four isolates viz: *B. thuringiensis* showed high mortality and at 60 exposure we found 100% mortality in *B. thuringiensis*, and *P. fluorescens, B. subtilies, B. cereus, S. aureus,* we found some of the termites

Table 1. Probit Analysis For Termite Mortality Of Workers And Soldiers Exposed To Different Entomopathogenic Bacterial Isolates

Bacteria	Termite	Workers				Soldiers			
		Chi^2	Regression	LT*50	Fiducial limits (95%)	Chi^2	Regression	LT*50	Fiducial limits (95%)
Pseudomonas fluorescens	*Odontotermes wallonensis*	6.01	1.57632	78.97	68.83-90.01	6.76	1.50273	63.79	42.79-95.82
Streptococcus aureus		5.71	2.53098	96.47	69.44-124.19	5.22	6.26392	83.58	61.39-99.84
Bacillus cereus		11.16	1.03545	79.29	65.28-94.89	7.59	3.74108	68.78	43.29-95.08
Bacillus subtilies		13.52	0.72900	77.46	58.94-98.71	10.73	4.03860	65.32	62.74-98.20
Bacillus thuringiensis		22.76*	1.34894	33.20	21.86-47.32	20.24*	0.77794	30.05	22.18-49.97
Pseudomonas fluorescens	*Odontotermes brunneus*	7.76	1.67584	83.25	73.74-84.14	8.88	3.31414	78.69	63.29-94.61
Streptococcus aureus		5.73	2.25281	95.84	78.17-134.20	9.28	4.06028	91.65	62.93-115.75
Bacillus cereus		6.87	1.45509	79.39	68.51-82.03	13.70	4.03860	85.32	62.74-98.20
Bacillus subtilies		7.05	1.11774	80.69	66.90-86.01	15.98	0.85532	66.32	69.99-85.04
Bacillus thuringiensis		22.94*	0.39022	33.64	29.76-52.25	24.59*	0.81356	21.56	10.77-32.13
Pseudomonas fluorescens	*Odontotermes obesus*	7.05	1.22754	70.96	63.84-92.55	7.82	1.31432	78.69	63.29-92.78
Streptococcus aureus		6.97	1.23589	83.52	62.76-95.41	7.98	1.81132	86.23	75.90-100.11
Bacillus cereus		8.75	2.52341	80.04	68.11-94.50	8.05	2.25649	81.56	60.77-92.53
Bacillus subtilies		15.28	0.43268	85.53	66.12-98.95	14.25	0.65845	91.52	78.25-97.85
Bacillus thuringiensis		22.94*	0.39022	33.64	24.76-40.25	26.59*	0.84256	30.32	22.74-48.58

* Significantly good

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

Entomopathogens	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}
Bacillus cereus	0.0^{ddddd}	0.0^{ddddd}	0.5^{dddd}	1.5^{ddd}	1.5^{dd}	2.0^{d}	2.75^{bbbb}	3.75^{bbb}	4.0^{bb}	4.5^{ba}	4.75^{aa}	4.75^{aa}
Bacillus thuringiensis	0.0^{eee}	0.0^{ee}	0.0^{e}	0.5^{ddd}	0.5^{dd}	0.0^{d}	1.5^{ccc}	1.5^{cc}	2.0^{c}	2.25^{a}	2.5^{aa}	2.75^{aaa}
Pseudomonas fluorescens	0.0^{e}	0.5^{ddde}	0.75^{dde}	1.0^{de}	1.25^{dd}	1.25^{d}	1.5^{dc}	2.5^{cc}	2.5^{c}	4.25^{b}	5.5^{a}	6.25^{aa}
Streptococcus aureus	0.5^{eee}	1.0^{d}	1.5^{ddde}	2.5^{dde}	3.5^{de}	4.25^{dc}	5.0^{bbc}	5.0^{bbc}	6.0^{bba}	7.0^{ba}	8.75^{aa}	9.25^{aaa}

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

alive in both workers and soldiers. In control there is no mortality. The Probit analysis was carried out to know the mortality response of worker and soldiers of *O. brunneus* against bacterial isolates viz *P. fluorescens*, *S. aureus*, *B. cereus, B. subtilies* and *B. thuringiensis* was computed among workers *B. thuringiensis* isolate recorded lowest LT 50 value followed by *B. cereus, B. subtilies, P. fluorescens, and S. aureus* (Table 1) and among the soldiers *B. thuringiensis* isolate recorded lowest LT 50 value followed by *B. subtilies, B. cereus, P. fluorescens and S. aureus* showing LT 50 value highest, there is least effective.

In *O. obesus* initially there was infection and the mortality also low but as the time advanced gradually the mortality of termite also increase and it was peak at 36-48 hours of exposure. And little low at 60 hours of exposure. Among different isolates screened *B. thuringiensis* showed very good mortality at initially and both workers and soldiers showed 100% mortality at 60 hours of exposure period. There was significant difference in workers and soldiers. Where as in other hand *B. subtilies, B. cereus, P. fluorescens and S. aureus* showed low mortality. The Probit analysis was carried out to know the mortality response of worker and soldiers of *O. obesis* against bacterial isolates viz *B. thuringiensis, B. cereus, B. subtilies, P. fluorescens* and *S. aureus*, computed among workers *B. thuringiensis* isolate recorded lowest LT 50 value followed by *B. cereus, B. subtilies., P. fluorescens* and *S. aureus,* LT 50 values are high (Table 1) and among the soldiers *B. thuringiensis* isolate recorded lowest LT 50 value followed by *B. cereus, B. subtilies, P. fluorescens.* The LT 50 values showing highest in *S. aureus* followed by *P. fluorescens*.

Among bacterial isolates *B. thuringiensis* showed good response to in both workers and soldiers and in fungal entomopathogenic isolates of *M. anisopliae* and *B. bassiana* were emerged as efficient isolates against to all the 3 common mound building termites viz- *O. wallonensis, O. brunneus and O. obesus.*

To know the field level efficacy of bacterial entomopathogens, a field level trial was conducted on nursery, each sub-plot was treated with different isolates, where the natural termite activity was present to evaluate a effective strain 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 sub-plot inoculate with *B. thuringiensis* record low infection/damage from termite with comparison to other isolates i.e. plant infection in plot with *B. thuringiensis* isolate was very effective biocontrol for termite damage. The data are represented in (Table 2).

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 all bacterial isolates *B. thuringiensis* showed very good termite mortality rate so, *B. thuringiensis* was effective entomopathogenic isolates, which cause death of termite.

Upon inoculation of entomopathogenic bacterial isolates *Pseudomonas fluorescens*, Streptococcus aureus, *Bacillus cereus,* Bacillus subtilies and *Bacillus thuringiensis* to screened and cultured termites- *Odontotermes wallonensis, Odontotermes brunneus* and *Odontotermes obesus*,. Bacillus thuringiensis was found to be most effective entomopathogenic bacteria. Hence it can be concluded that Bacillus thuringiensis can be used as good bio- pesticide to control termite population there by reduced the damage to the forest nurseries and plantation.

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