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Assessment of the entomopathogenic nematode bacteria against the termite, *Microtermes mycophagus* D. (Isoptera: Termitidae)

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Abstract

The capability of symbiotic bacteria of entomopathogenic nematodes against the termite, *Microtermes mycophagus* D. (Isoptera: Termitidae), was assessed. Different fractions of Pakistani isolates of entomopathogenic bacteria viz., *Xenorhabdus indica* strain (Pak.S.B.50), *X. indica* strain (Pak.S.B.56), *X. stockiae* strain (Pak.S.B. 65), and *X. steinernematis* strain (C.B.10) were assessed against *M. mycophagus* by direct contact method (spraying method) and sand assay in laboratory conditions. Mortality response of cell-free filtrates after 24 h at 20 °C for *X. indica* (Pak.S.B.50), T_2 = *X. indica* (Pak.S.B.56), T_3 = *X. stockiae* (Pak.S.B. 65), and T_4 = *X. steinernematis* (C.B.10) ranged (88.3–100%) as 33 ± 9.34, 98.33 ± 6.22, 88.33 ± 7.22, and 100.00% ± 0.00, respectively. In the case of sand assay, the most effective treatment was T_4, where (100%) mortality rate was recorded 24 h post application of B.S. (bacterial suspension) (4×10^4 CFU/ml) and CFF (cell-free filtrate) (100 μl/10 ml) at 20 and 25 °C.

Keywords: Entomopathogenic bacteria, *Xenorhabdus*, Termite, *Microtermes mycophagus*, Bacterial fractions, Biocontrol

Background

The termite, *Microtermes mycophagus* D. (Isoptera: Termitidae: Macrotermitinae), is a cosmopolitan pest of wood and wood products that can be distinguished by its colonial behavior. Colony members are distinctly varied morphologically, i.e., propagative (king and queen), soldiers, and workers. The head termites, the king and queen, are sexually functional but pheromonal regulation that is responsible for the caste production is only produced by the queen (Noirot and Noirot-Timothee, 1970). Wingless individuals, workers or soldiers, are usually non-reproductive males or females. Soldiers play a major role to defend the colony and represent 1/10th of the population of a colony (Bignell and Eggleton, 1998). Termites are highly devastating and cause damage to furniture, buildings, trees, and agricultural crops, such as cereals, oil crops, pulses, sugarcane, fruits, and vegetables. Estimated losses by this pest are about US$22 billion annually across the world (Govorushko, 2011). The genera viz., *Microtermes*, *Odontotermes*, and *Termes*, are the most prevalent termites in Pakistan (Manzoor and Naeem, 2010).

Chemicals control applications to the wood or to the soil have been determined time by time. Chemical fumigants containing methyl bromide, sulfuric fluoride, or a combination of carbon dioxide and methyl bromide is the suitable procedure of eradicating dry wood termites. Biocontrol agents are environment friendly and proficient in working but its cost effective feature is debatable. Abiotic factors such as warm and moist favored by subterranean termites, which promote epizootics, also have the potential for biological control (Verma et al., 2009). Few studies have reported the potential of entomopathogenic nematodes (EPN) to control termites. EPN exposure to termites

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resulted in significant response of parasitization (47% after 4 days) and 100% mortality after 12 days under lab conditions. Fujii (1975) gained 96% mortality results of *C. formosanus* within 7 days after treating with infective-stage *Steinernema carpocapsae* (Weiser) (Steinernematidae) in laboratory analysis. Mortality rate, more than 95%, was documented within 3 days by Georgis et al., (1982) for both *Reticulitermes* sp. and *Zootermopsis* sp. after laboratory exposure to *S. carpocapsae*; further termites were also serve as vectors for EPN that take back to their colonies. *S. carpocapsae* has shown high rates of infection to *Nasutitermes costalis* and *R. flavipes* obtained under laboratory conditions (Laumond et al., 1979 and Trudeau, 1989).

The present study aimed to assess the efficacy of symbiotic bacteria *Xenorhabdus* species as biopesticide against the termite, *M. mycophagus*, under laboratory conditions.

Materials and methods

Collection of termite

The termite (*M. mycophagus*) was collected from different infested trees in the premises of the University of Karachi, Karachi (24° 56' 21.833" N, 67° 7' 14.869" E), Pakistan.

Bacterial culture (isolation of bacteria from insect hemolymph)

Entomopathogenic nematodes (EPN) were obtained from the storage unit, maintained by Prof. Dr. Shahina Fayyaz at NNRC, University of Karachi, Karachi, Pakistan. All nematodes were propagated in last instar larvae of the greater wax moth, *Galleria mellonella* L., using the method of Dutky (1974). Infective juveniles were collected by White traps (White, 1927), harvested, and stored in sterilized distilled water at 10–15 °C for no more than 2 weeks before they were used.

Isolation of bacteria from insect hemolymph

To isolate bacteria from hemolymph, *G. mellonella* larvae were inoculated by EPN (Table 1) 100 IJs in a Petri dish lined with moistened filter paper. After 48 h the dead larvae were surface sterilized by 75% ethanol for 15 min; then, the cadavers were passed through the flame for further sterilization. Cadavers were dissected with sterilized scissors at the second foot, a loop full of hemolymph streaked onto NBTA agar medium (Akhurst, 1980). The streaked plates were incubated in the dark at 28 °C for 48 h for the development of primary colonies. For further purification, single colonies of bacteria were sub cultured on new plates of agar medium. Then, single colony was transferred to the nutrient broth (0.81 broth + 61 ml water) and kept it for incubation on shaking bath for 2 days at 150 rpm ND 28 °C. The bacterial suspension was used for bioassay.

Biochemical analysis of bacterial isolates

The pure cultures of different species of *Xenorhabdus* were subjected to biochemical test through API 20E test kit of Biomerieux Ltd., USA.

Effect of different application method for controlling the termite

Pakistani isolates of entomopathogenic bacteria viz., *Xenorhabdus indica* (Pak.S.B.50), *X. indica* (Pak.S.B.56), *X. stockiae* (Pak.S.B. 65), and *X. steinernematis* (C.B.10) were assessed against *M. mycophagus* by direct contact method (spraying method) and sand assay in a laboratory experiment.

Spray method

Heavy infested branches of different trees were selected and kept in a plastic shopper after cutting with a hammer or cutter and brought in to the laboratory. Plastic containers about the size of 8 × 6 in. lined with wax at the edges were used for the experiment. Six-inch pieces of tree branches carrying approximately 50 termite individuals were placed in each container and sprayed with 20 ml of each treatment separately. Each container was sealed with a parafilm and each set of experiment incubated at different temperatures 20, 25, and 30 °C. Different EPB formulations were examined viz., B.S. (bacterial suspension) (4×10^4 CFU/ml), CFF (cell-free filtrate) (100 μl/10 ml), B.R. (bacterial residue) (100 μl/10 ml) of T_1 (*Xenorhabdus indica* (Pak.S.B.50)), T_2 (*X. indica* (Pak.S.B.56)), T_3 (*X. stockiae* (Pak.S.B. 65)), and T_4 (*X. steinernematis* (C.B.10)). This combination was replicated 3 times with control treatment, which was sprayed only with water. Mortality rate was assessed each after 24, 48, and 72 h.

Sand barrier assay

A set of 50 termite individuals was placed in 6 × 6 in. Petri dish lined with a filter paper. For sand assay, a

Table 1 Details of symbiotic bacteria used for the study

Strains	Bacterial symbionts	EPN	Accession no.	Authority*
Pak.S.B.50	*Xenorhabdus indica*	*Steinernema abbasi*	MF498486	Shahina F. & Salma J.
Pak.S.B.56	*Xenorhabdus indica*	*S. pakistanense*	MF521953	Shahina F. & Salma J.
Pak.S.B. 65	*Xenorhabdus stockiae*	*S. siamkayai*	MF521964	Shahina F. & Salma J.
Pak.C.B. 10	*Xenorhabdus steinernematis*	*S. maqbooli*	KU097324	Shahina F. & Salma J.

*Accession no. authorized by these persons

Table 2 Biochemical analysis of symbiotic bacteria

Biochemical analysis	***Xenorhabdus indica*** Pak.S.B.50	***X. indica*** Pak.S.B.56	***X. stockiae*** Pak.S.B. 65	***X. steinernematis*** CB.10
Citrate utilization	+	+	-	+
Esculin hydrolysis	++	+	-	+
Catalase	+	++	-	+
Meso-inositol fermentation	+	-	d	+
Salicin fermentation	+	-	-	-
Ribose fermentation	+	-	-	+
Lipase tween 80	+	+	-	+

"+" 90–100% positive, "-" 90–100%, "++" positive 75–89%, "- -" 75–89%, "d" positive 25–74% of strains

thin film of autoclaved sand was spread over the filter paper and then the termites were placed. Twenty milliliters of different formulations B.S. (bacterial suspension) (4×10^4 CFU/ml), CFF (cell free filtrate) (100 μl/10 ml), B.R. (bacterial residue) (100 μl/10 ml) of T_1 (*Xenorhabdus indica* (Pak.S.B.50)), T_2 (*X. indica* (Pak.S.B.56)), T_3 (*X. stockiae* (Pak.S.B. 65)), and T_4 (*X. steinernematis* (C.B.10)) along with 1 ml of 2% Tween 80 (as emulsifier) were dropped on the sand layer under the laminar flow cabinet. Plates were sealed by a parafilm and incubated at different temperatures 20, 25, and 30 °C. Each treatment had 3 replicates. Control treatment only contained water. Mortality rate was assessed after 24, 48, and 72 h of exposure.

Table 3 Influence of different bacterial fractions on the mortality of termites in relation to temperature and time duration by spray method

Treatment	Conc.	Mortality% ± SD								
		20 °C			25 °C			30 °C		
		24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
***T_1, Xenorhabdus indica* (Pak.S.B.50)**	B.S.	83.66 ± 7.44a	100 ± 0.00c	-	80.66 ± 6.98a	100 ± 0.00c	-	71.66 ± 6.44a	78.66 ± 6.75a	78.66 ± 7.83a
	CFF	88.33 ± 9.34a	100 ± 0.00c	-	87.33 ± 7.45a	100 ± 0.00c	-	77.33 ± 8.54a	82.33 ± 7.35a	82.33 ± 8.41a
	B.R.	34.66 ± 5.34b	45.66 ± 5.41b	45.33 ± 7.23b	32.33 ± 7.33b	45.66 ± 6.76b	47.33 ± 9.21b	24.66 ± 5.20b	29.66 ± 5.67b	33.33 ± 5.75b
***T_2, Xenorhabdus indica* (Pak.S.B.56)**	B.S.	85.66 ± 7.34a	100 ± 0.00c	-	80.33 ± 8.34a	83.33 ± 9.38a	92.33 ± 6.39c	74.66 ± 6.78a	77.33 ± 6.78a	81.33 ± 6.39a
	CFF	98.33 ± 6.22c	100 ± 0.00c	-	87.33 ± 8.32a	88.66 ± 7.57a	95.33 ± 9.34c	78.33 ± 7.43a	80.33 ± 7.56a	82.33 ± 8.45a
	B.R.	35.66 ± 4.56b	37.33 ± 5.22b	37.33 ± 5.22b	34.66 ± 8.55 b	35.33 ± 6.56b	39.33 ± 6.98b	28.66 ± 4.56b	32.33 ± 4.87b	35.33 ± 5.43b
***T_3, Xenorhabdus stockiae*(Pak.S.B. 65)**	B.S.	85.66 ± 8.23a	100 ± 0.00c	-	78.66 ± 8.23a	73.66 ± 9.26a	78.97 ± 7.87a	69.66 ± 6.87a	72.66 ± 9.45a	79.66 ± 8.09a
	CFF	88.33 ± 7.22a	100 ± 0.00c	-	88.33 ± 7.22a	100 ± 0.00c	-	72.33 ± 8.49a	75.33 ± 7.54a	82.33 ± 9.34a
	B.R.	33.66 ± 4.22b	35.33 ± 6.22b	38.43 ± 4.10b	29.33 ± 5.35b	30.66 ± 5.67b	38.66 ± 4.56b	25.66 ± 3.86b	35.33 ± 6.22b	38.43 ± 4.10b
***T_4, Xenorhabdus steinernematis* (C.B.10)**	B.S.	100 ± 0.00c	-	-	100 ± 0.00c	-	-	87.66 ± 9.45a	100 ± 0.00c	-
	CFF	100 ± 0.00c	-	-	100 ± 0.00c	-	-	93.33 ± 8.22c	100 ± 0.00c	-
	B.R.	36.33 ± 3.43b	39.33 ± 6.37b	42.33 ± 6.36b	34.33 ± 3.03b	35.66 ± 4.67b	37.33 ± 3.65b	26.33 ± 3.65b	27.33 ± 4.55b	28.33 ± 5.43b
Control	H_2O	0.00 ± .00d	0.00 ± .00d	0.00 ± .00d	0.00 ± .00d	0.00 ± .00d	0.00 ± .00d	0.00 ± .00d	0.00 ± .00d	0.00 ± .00d

B.S. bacterial suspension (4×10^4 CFU/ml), *CFF* cell-free filtrate (100 μl/10 ml), *B.R.* bacterial residue (100 μl/10 ml), "-" experiment complete
The similar alphabets in rows and columns have shown non significant differences at $p = 0.01$

Data analysis

Data are expressed as means, standard deviation and the significance of mean differences was determined with Duncan's multiple range test (SAS Institute, Cary, NC).

Results and discussion

Biochemical analysis

The biochemical test of *Xenorhabdus* species were assessed for the following features. Citrate utilization, esculin hydrolysis, catalase, meso-inositol fermentation, salicin fermentation, ribose fermentation, and lipase tween 80. *X. indica* Pak.S.B.50 showed 90–100% positive expression in all analysis, except for Esculin hydrolysis. *X. indica* Pak.S.B.56 contained Citrate utilization; Esculin hydrolysis and Lipase Tween 80 resulted in 90–100% positive and catalase 75–89% positive, whereas meso-inositol fermentation, Salicin fermentation, and ribose fermentation 90–100% negative. *X. stockiae* Pak.S.B. 65 had 25–74% positive results for meso-inositol fermentation where all remaining factors were found to be 90–100% negative. *X. steinernematis* C.B.10 expressed 90–100% positive for all examined biochemical tests but negative 90–100% for Salicin fermentation (Table 2).

Effect of different application methods for controlling *Microtermes* species

Spray method

All bacterial isolates were found to be significantly effective against termites by spray method. Different fractions of bacterial formulations showed significant differences of mortality rate ($P < 0.001$). Bacterial suspension and cell-free filtrates of all treatments (bacterial isolates) had the potential to control termites at 20 and 25 °C even after 24 h, whereas the bacterial residue of all the bacterial isolates had least potential for controlling termites. Due to direct contact of formulations with termites, effective results were obtained within 24 h in most of the cases. Mortality response of cell-free filtrates after 24 h at 20 °C in *Xenorhabdus indica* (Pak.S.B.50), T_2 (*X. indica* (Pak.S.B.56)), T_3 (*X. stockiae* (Pak.S.B. 65)), and

Table 4 Influence of different bacterial fractions on the mortality of termites in relation to temperature and time duration by sand barrier assay method

Treatment	Conc.	Mortality %								
		20 °C			25 °C			30 °C		
		24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
T_1, *Xenorhabdus indica* (Pak.S.B.50)	B.S.	82.66 ± 6.98a	100 ± 0.00c	-	85.66 ± 8.56a	100 ± 0.00c	-	73.33 ± 5.98a	76.66 ± 7.87a	80.66 ± 6.88a
	CFF	87.33 ± 7.98a	100 ± 0.00c	-	87.33 ± 7.45a	100 ± 0.00c	-	78.33 ± 9.12a	84.33 ± 7.35a	85.33 ± 8.41a
	B.R.	33.66 ± 6.45b	45.66 ± 5.41b	47.33 ± 6.87b	32.33 ± 7.33b	45.66 ± 6.76b	47.33 ± 9.21b	23.66 ± 5.20b	30.66 ± 5.67b	35.33 ± 5.75b
T_2, *Xenorhabdus indica* (Pak.S.B.56)	B.S.	88.66 ± 7.34a	100 ± 0.00c	-	80.33 ± 8.34a	83.33 ± 9.38a	92.33 ± 6.39c	72.66 ± 6.78a	78.33 ± 6.78a	85.33 ± 6.39a
	CFF	93.33 ± 6.22c	100 ± 0.00c	-	88.33 ± 7.76a	88.66 ± 7.57a	95.33 ± 9.34c	75.33 ± 7.43a	82.33 ± 7.56a	85.33 ± 8.45a
	B.R.	37.66 ± 4.56b	38.33 ± 5.22b	39.33 ± 5.22b	34.33 ± 5.34b	35.33 ± 6.56b	39.33 ± 6.98b	26.66 ± 4.56b	36.33 ± 4.87b	39.33 ± 5.43b
T_3, *Xenorhabdus stockiae*(Pak.S.B.65)	B.S.	88.66 ± 7.98a	100 ± 0.00c	-	78.66 ± 8.23a	73.66 ± 9.26a	78.97 ± 7.87a	65.66 ± 6.87a	75.66 ± 9.45a	75.66 ± 8.09a
	CFF	85.33 ± 8.65a	100 ± 0.00c	-	86.33 ± 7.22a	100 ± 0.00c	-	71.33 ± 8.49a	75.33 ± 7.54a	84.33 ± 9.34a
	B.R.	34.66 ± 4.22b	35.33 ± 6.22b	38.43 ± 4.10b	30.33 ± 5.35b	34.66 ± 5.67b	38.66 ± 4.56b	28.66 ± 3.86b	35.33 ± 6.22b	40.43 ± 4.10b
T_4, *Xenorhabdus steinernematis* (C.B.10)	B.S.	100 ± 0.00c	-	-	100 ± 0.00c	-	-	83.66 ± 9.45a	100 ± 0.00c	-
	CFF	100 ± 0.00c	-	-	100 ± 0.00c	-	-	96.33 ± 8.22c	100 ± 0.00c	-
	B.R.	38.33 ± 3.43b	40.33 ± 6.37b	44.33 ± 7.32b	35.33 ± 3.03b	36.66 ± 4.67b	37.66 ± 4.87b	26.33 ± 3.65b	28.33 ± 4.55b	34.33 ± 5.43b
Control	H_2O	0.00 ± .00d	0.00 ± .00d	0.00 ± .00d	0.00 ± .00d	0.00 ± .00d	0.00 ± .00d	0.00 ± .00d	0.00 ± .00d	0.00 ± .00d

B.S. bacterial suspension (4×10^4 CFU/ml), *CFF* cell-free filtrate (100 μl/10 ml), *B.R.* bacterial residue (100 μl/10 ml), "-" experiment complete
The similar alphabets in rows and columns have shown non significant differences at $p = 0.01$

T_4 (*X. steinernematis* (C.B.10)) ranged between 88.3 and 100% as 88.33 ± 9.34, 98.33 ± 6.22, 88.33 ± 7.22, and 100.00% ± 0.00, respectively. No mortality response was found in the control treatment (Table 3).

Sand barrier assay

In a sand barrier assay, Pakistani isolates of EPB were applied 20 ml of different formulations: B.S. (bacterial suspension) (4×10^4 CFU/ml), CFF (cell free filtrate) (100 μl/10 ml), B.R. (bacterial residue) (100 μl/10 ml) of T_1 (*Xenorhabdus indica* (Pak.S.B.50)), T_2 (*X. indica* (Pak.S.B.56)), T_3 (*X. stockiae* (Pak.S.B. 65)), and T_4 (*X. steinernematis* (C.B.10)). Significant differences were observed between control and treatments ($P < 0.001$). Effectiveness of different formulations was dependent on the temperature and time duration. The most effective treatment was T_4, where maximum percentage of mortality found to be 100% after 24 h of application by B.S. (4×10^4CFU/ml); CFF (100 μl/10 ml) at 20 and 25 ° C. After applying T_1 (*Xenorhabdus indica* (Pak.S.B.50)), T_2 (*X. indica* (Pak.S.B.56)), and T_3 (*X. stockiae* (Pak.S.B. 65)) 100% mortality of termites was obtained after 48 h in B.S. (4×10^4 CFU/ml) and CFF (100 μl/10 ml), whereas all insects survived in control treatment (Table 4).

Two different application methods were determined for their proficiency and significantly similar results obtained from both methods.

Different bacterial species have the capability to control termites. The effects of *Bacillus thuringiensis* subspecies was examined under laboratory conditions against *Nasutitermes ehrhardti* (Castilhos-Fortes et al. 2002). They observed that *B. thuringiensis* subspecies *kurstaki* produced 80% mortality of termite species. *B. thuringiensis* proteins having insecticidal properties are highly specific as gut toxins and it has shown a superior safety in reference to the effectiveness for non-target organism (Sarwar, 2015).

Pseudomonas sp., *P.maltophilia*, *Bacillus* strains, and *Paenibacillus* sp., are reported to produce chitinase (Suyal et al., 2015; Verma et al., 2016a; Yadav et al., 2016a). It was also reported that ten bacterial strains along with two control strains have been evaluated as biocontrol against termites. Different bacterial strains having termite-killing ability showed > 80% mortality after 5 days of incubation (Dua, 2014). Four bacterial strains caused 100% killing at 10 days of observation. The cell-free culture filtrate of these cultures showed that the antagonistic substance was extracellular having protein properties. Bacterial strains of *Bacillus subtilis* KBM79 and *Pseudomonas synxantha* KPM35 possessed proteolytic, chitinolytic, and lipolytic enzyme activities and caused 100% killing of termites (Yadav et al., 2016b). In previous studies, *X. nematophila* has been proved as a potential candidate of biocontrol agent against termites (Hiranwrongwera et al., 2007).

Conclusion

The present results proved that different bacterial fractions of *Xenorhabdus* species were found effective against termites in certain adequate conditions and can be a successful candidate for integration in termites' controlling strategy.

Abbreviations

B.S.: Bacterial suspension; CFF: Cell-free filtrate; B.R.: Bacterial residue

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Authors' contributions

EYI planned the research experiment and performed biochemical analysis and mortality response. UAM and SFZA managed data and analyzed and interpreted the results. SR helped in writing the manuscript and reviewed the manuscript. SF provided the EPN material and supervised this research. All authors have read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

N/A

Consent for publication

N/A

Competing interests

The authors declare that they have no competing interests.

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References

Akhurst R (1980) Morphological and functional dimorphism in *Xenorhabdus* spp., bacteria symbiotically associated with the insect pathogenic nematodes *Neoaplectana* and *Heterorhabditis*. J. Gen. Microbiol 121:303–309. https://doi.org/10.1099/ 00221287-121-2-303

Bignell DE, Eggleton P (1998) Termites. In: Calow P (ed) Encyclopedia of ecology and environmental management. Blackwell Scientific, Oxford, pp 744–746

Castilhos-Fortes R, Matsumura ATS, Diehl E, Fiuza LM (2002) Susceptibility of *Nasutitermes ehrhardti* (Isoptera: Termitidae) to *Bacillus thuringiensis* subspecies. Brazi J Microbiol 33:219–222

Dua S (2014). Biological control of subterranean termites (Isoptera: Termitidae) with soil bacte-ria. Ph.D. thesis, Chaudhary Charan Singh Haryana Agricultural University, pp. 225.

Dutky SR, Thompson JV, Cantwell GE (1974) A technique for the mass propagation of the DD-136 nematode. J. Insect Pathol 6:417–422

Fujii JK (1975) Effect of an entomogenous nematode *Neoaplectana carpocapsae* Weiser, on the Formosan subterranean termite, *Captotermes formosanus* Shiraki, with ecological and biological studies on *C. formosanus*. University of Hawaii, Honolulu, Hawaii, USA, Ph.D. dissertation, p 163

Georgis R, Poinar GO Jr, Wilson AP (1982) Susceptibility of damp-wood termites and soil and wood-dwelling termites to the entomogenous nematode *Neoaplectana carpocapsae*. IRCS Medical Science 10:563

Govorushko SM (2011) Biodeterioration: biological processes. In: Natural processes and human impacts: interactions between humanity and the environment. Springer, p 335

Hiranwrongwera C, Adisettakul P, Tansirichaiya S, Piyabun O, Somsuk V(2007). Efficiency of a nematode (*Steinernema carpocapsae*) and its symbiotic bacterium (*Xenorhabdus nematophila*) at eliminating the termite *Coptotermes curvignathus* that infests para rubber (*Hevea brasiliensis*). The 5th international symposium on biocontrol and biotechnology (p. 90). Nong Khai Campus, Nong Khai: Khon Kaen University.

Laumond C, Mauleon H, Kermarrec A (1979) Donn_ees nouvelles sur le spectre d'hoˆtes et le parasitisme du n_ematode entomophage *Neoaplectana carpocapsae*. Entomophaga 24:13–27

Manzoor F, Naeem M (2010). Survey of termite infested houses, indigenous building materials and construction techniques in Pakistan. Pak J Zoology42, 6: 693-696.

Noirot C, Noirot-Timothee C (1970) The digestive system. In: Weesner FM (ed) Biology of termites Krishna, K. Academic, New York and London, pp 49–88

Sarwar, M (2015). Microbial insecticides-an ecofriendly effective line of attack for insect pests management. Inter J Engi Adv Res Technol1, 4-9.

Suyal DC, Yadav A, Shouche Y, Goel R (2015) Bacterial diversity and community structure of Western Indian Himalayan red kidney bean (*Phaseolus vulgaris*) rhizosphere as revealed by 16S rRNA gene sequences. Biologia 70:305–313

Trudeau D (1989) Selection of entomophilic nematodes for control of the eastern subterranean termite, *Reticultermes flavipes* (Kollar) (Isoptera: Rhinotermitidae). University of Toronto, Toronto, Ontario, Canada, Master's thesis, p 93

Verma M, Sharma S, Prasad R (2009) Biological alternatives for termite control: a review. Int. Biodeter Biodegradation 63:959–972

Verma P, Yadav AN, Khannam KS, Kumar S, Saxena AK, Suman A (2016a). Molecular diversity and multifarious plant growth promoting attributes of *Bacilli* associated with wheat (*Triticum aestivum* L.) rhizosphere from six diverse agro-ecological zones of India. Journal of Basic Microbiology56, 44–58.

White GF (1927) A method for obtaining infective nematode larvae from cultures. Science 66:302–303

Yadav AN, Sachan SG, Verma P, Kaushik R, Saxena AK (2016a) Cold active hydrolytic enzymes production by psychrotrophic Bacilli isolated from three subglacial lakes of NW Indian Himalayas. Journal of Basic Microbiology 56:294–307

Yadav AN, Sachan SG, Verma P, Saxena AK (2016b) Bioprospecting of plant growth promoting psychrotrophic Bacilli from cold desert of north western Indian Himalayas. Indian Journal of Experimental Biology 54:142–150

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