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PATHOGENICITY OF ENTOMOPATHOGENIC AGENTS AGAINST TERMITES EMPLOYING INSECTICIDAL BIOASSAY WITH BORAX-TOXICITY METHOD

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A simple bio-assay technique was employed to test the pathogenicity of a few microbial agents against two species of subterranean termites, *Reticulitermes santonensis* and *Zootermopsis*. Borax and Boric acid were used for the toxicity tests on the termites. The effective range and concentrations of the chemical were decided on the basis of the ability of the termites to spread the chemical to others during the first few days and on the level of delayed mortality. Among the four microbial agents tested, the strains of *M.anisopliae* and the nematode species, *Heterorhabditis* and *Steinernema* caused greater mortality of the termites under test.

INTRODUCTION

Termites (Isoptera) are widely distributed in the tropical, semitropical and warmer temperate regions of the world, i.e. between latitude 45° South and 45° North (Harris, 1969; Wood and Johnson, 1986). Though there are almost 3000 species of termites only 300 are considered to be pests that damage buildings and plants of importance to man. However, where they do occur, almost every plant used by man may be attacked at some stage of its growth by at least one species of termite (Sands, 1977). The great number of species and the diversity of their biology and behaviour makes it difficult to device general methods of control.

Biological control techniques are advantageous as they are environmentally friendly and have a low health risk to humans. Termites are eaten by a wide variety of predators (Snyder, 1948; Weber, 1949; Wood and Johnson, 1986) and are also attacked by a number of pathogens, such as fungi, bacteria and nematodes (Hanel, 1982; Khan, Jafri and Ahmed, 1981, 1985; Epseky and Capinera, 1988). These are often more effective as biological control agents of insects as they can be cultured, maintained and applied more easily than the predators.

In this investigation, potential entomopathogenic agents were tested for their pathogenicity against two species of subterranean termites, *Reticulitermes santonensis* and *Zootermopsis*. A preliminary bioassay was conducted to determine the efficacy range of concentration of chemicals and the efficacy period in order to use the information as a guide for the bioassays on entomopathogenic agents. Boron and boric acid were used in this preliminary bioassay as boron compounds are slow acting insecticides of low mammalian toxicity used in wood preservation and development of toxic baits to control subterranean termites.

MATERIALS AND METHODS

Test termites, *Reticulitermes* and *Zootermopsis* were taken from laboratory cultures maintained at the Natural Resources Institute (NRI), U.K. Couplets of Petri dishes formed the test chambers. One filter paper (4.25 cm) was glued to the lid of the couplet and two other filter papers were placed on the base. The paper on the lid was wetted to saturation to maintain humidity conditions inside the couplet. Termites (numbers are given in the tables) collected from laboratory cultures were introduced to each couplet. The Petri dishes were labelled according to the treatments and the replicates. Controlled dishes were prepared with distilled water. All treated Petri dishes as well as a dish of water were placed in a large plastic box and maintained in an incubator at 27±1°C and the mortality was recorded.

Bioassay Ia

A preliminary test was done to determine the effective range of the chemical.

Borax and boric acid were tested at the following concentrations.

a) Borax - 10.0, 1.0, 0.1 and 0.01% with an untreated control.

b) Boric acid - 5.0, 1.0, 0.1 and 0.01% with an untreated control.

Each treatment was repeated 3 times.

Another set of experiments were prepared according to the closer effective range decided upon from the preliminary test (Bioassay Ia).

Bioassay Ib

The selected concentrations were;

a) Borax - 20, 15, 10, 12, 5, 2, 1% and control

b) Boric acid - 2, 1, 0.5, 0.1, 0.05% and control

O.15 ml of the above dilution was applied to a 4.25 cm (diam.) filter paper (% a.i.of filter paper (w/w) taken) placed in a Petri dish couplet and the termites were introduced.

Bioassay II

The following entomopathogenic fungi were tested at the dilutions given in Table 1.

TABLE 1 - *Spore dilutions of entomopathogenic fungi tested on termites (Bioassay II)*

			Entomopathogenic fungi		
			M.anisopliae		*B.bassiana*
			Ma 101-82	*299982*	*298058*
			Fungal dilutions of spores ml-^{1}		
Termite sp.	*No.of termites / dish*	*Repli-cates*			
Reticulitermes	10	3	7.55×10^{7}	7.39×10^{7}	13.2×10^{7}
			5×10^{7}	5×10^{7}	5×10^{7}
			5×10^{6}	5×10^{6}	5×10^{7}
			5×10^{5}	5×10^{5}	5×10^{5}
			Control	Control	Control
Zootermopsis	3	3	7.55×10^{7}	–	13.2×10^{7}
			5×10^{7}	5×10^{7}	5×10^{7}
			5×10^{6}	5×10^{6}	5×10^{6}
			5×10^{5}	5×10^{5}	5×10^{5}
			Control	Control	Control

Metarhizium anisopliae isolate 101-82 of Glasshouse Crop Research Institute, Little Hampton, U.K.

Metarhizium anisopliae isolate 299982 of IMI Jan.1986 (freeze-dried sample).

Beauveria bassiana isolate 298058 of IMI Nov.1985 (freeze-dried sample).

All the test samples were received from IIBC/Silwood Park, Ascot, U.K.

The number of spores were counted using a Hemacytometer.

Bioassay III

Two nematode species, *Steinernema carpocapsae* and *Heterorhabditis* species (Bacterophora) were tested with the concentrations given in Table 2.

TABLE 2 - *Concentrations of nematodes tested on termites (Bioassay III)*

			Entomopathogenic nematodes	
			Steinernema sp.	*Heterorhabditis sp.*
			Concentrations of nematodes/dish	
Termite sp.	*No.of termites / dish*	*Repli-cates*		
Reticulitermes	20	5	T1-25	T1-35
			T2-50	T2-70
			T3-75	T3-105
			T4-105	T4-140
			T5-Control	T5-Control
Zootermopsis	3	5	T1-26	
			T2-52	
			T3-78	
			T4-104	
			T5-Control	

Dead termites were washed and kept on a wet filter paper to allow the nematode population to develop. Termite dissections were done in Ringer's solution.

Bioassay IV

Higher concentrations of nematodes were tested as given in Table 3.

TABLE 3 - *Higher concentrations of nematodes tested on termites (Bioassay IV)*

Termite sp.	*No.of termites / dish*	*Repli-cates*	*Entomopathogenic nematodes* *Steinernema sp.* *Heterorhabditis sp.* *Concentrations of nematodes/dish*
Reticulitermes	10	3	T1-522
			T2-870
			T3-1218
			T4-1566
			T5-Control
Reticulitermes	10	4	T1-522
			T2-870
			T3-1218
			T4-1566
			T5-Control
Reticulitermes	20	3	T1-778
			T2-2334
			T3-3112
			T4-4668
			T5-7000
			T6-9336
			T7-11670
			T8-14000
			T9-16338
			T10-Control

Inverted large Petri dish couplet was used as the test chamber. Twenty termites were placed on the filter papers which were kept wet and the number of dead and live termites were counted. The dead termites were not removed to allow reinfection.

To obtain the LD_{50} values log dose probit mortality data was used. The percentage response observed for each dose used in this study was first calculated and converted to probits by means of the Probit Table. It is to be noted that since very extreme probits carry little weight they can be disregarded, according to this estimation.

RESULTS AND DISCUSSION

Mortality of the target insect is the commonly used factor in bioassays since the desired effect of the pathogens is the death of the target pest.

The LD_{50} values obtained from the bioassays are given below;

Tests with fungi

Bioassay	*Fungi species*	*Termite sp.*	*Day*	*LD_{50}(spores ml^{-1})*
2	Ma 101-82	*Reticulitermes*	7	5.511×10^{8}
	Ma 101-82	*Reticulitermes*	14	$.073 \times 10^{8}$
2	Ma 299982	*Reticulitermes*	5	7.592×10^{7}
	Ma 299982	*Reticulitermes*	14	7.72×10^{7}
2	Ma 299982	*Zootermopsis*	4	5.068×10^{8}
	Ma 299982	*Zootermopsis*	6	9.0656×10^{4}
2	Bb 298058	*Reticulitermes*	4	0.1×10^{-3}
	Bb 298058	*Reticulitermes*	14	1.145×10^{57}

Tests with nematodes

Bioassay	*Nematode sp.*	*Termite sp.*	*Day*	*LD_{50}(spores ml^{-1})*
3	*Steinernema*	*Reticulitermes*	4	1.6342×10^{-12}
	Steinernema	*Reticulitermes*	14	8.1959×10^{4}
3	*Heterorhabditis*	*Reticulitermes*	8	0.256×10^{-1}
	Heterorhabditis	*Reticulitermes*	14	5.84×10^{5}
4	*Steinernema*	*Reticulitermes*	3	3.455×10^{3}
	Steinernema	*Reticulitermes*	14	3.1573×10^{3}
4	*Heterorhabditis*	*Reticulitermes*	3	7.673×10^{1}
	Heterorhabditis	*Reticulitermes*	14	3.9616×10^{3}
4	*Steinernema*	*Reticulitermes*	3	5.55×10^{8}
	Steinernema	*Reticulitermes*	14	3.44×10^{4}

The results of the data of the bioassay I carried out using borax and boric acid method is given in Figures 1-4. A promising bioassay should show a mortality of less than 10% within first 3 days but achieve 100% within 14 days (Stringer,Lofgren and Bartlett, 1964). This requirement has been satisfied by both the borax and boric acid, bioassay I (Figs 1 and 3).

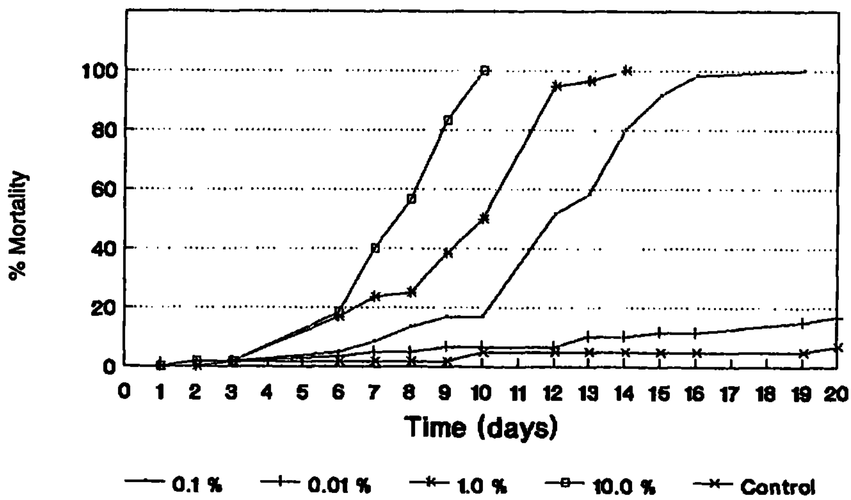

Fig. 1 – *Percentage mortality of* ***Reticulitermes*** *with selected concentrations of Borax.*

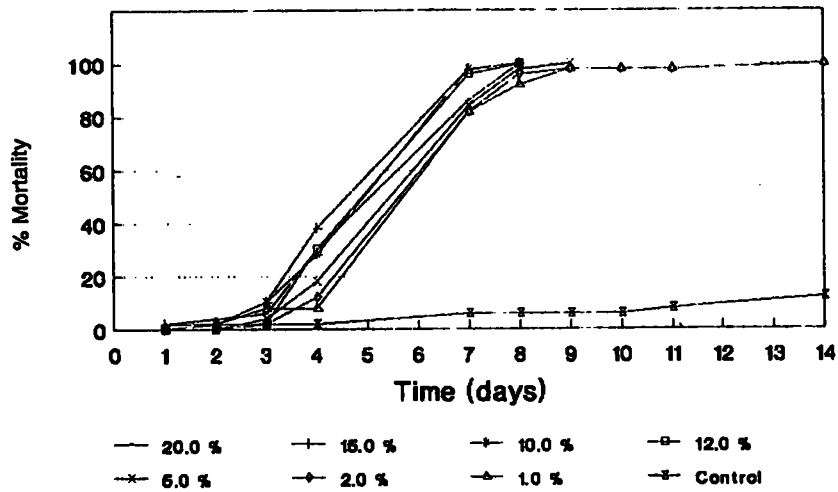

Fig. 2 – *Percentage mortality of* ***Reticulitermes*** *with effective range of Borax.*

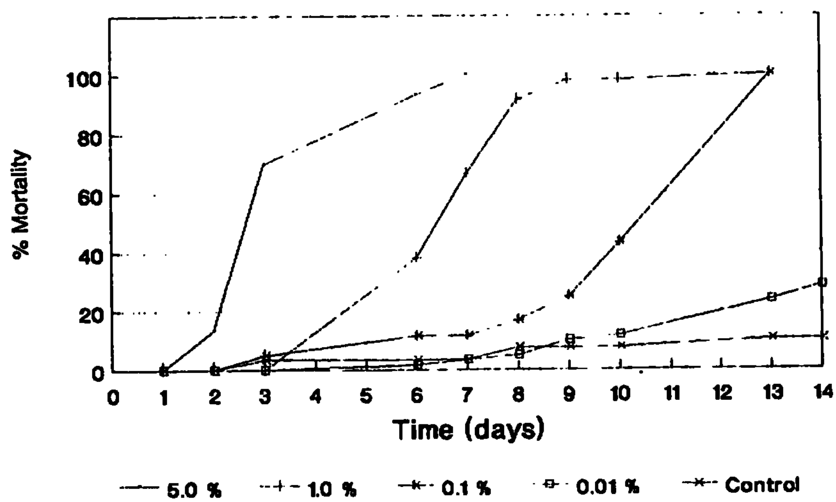

Fig. 3 – *Percentage mortality of* ***Reticulitermes*** *with selected concentrations of Boric acid.*

With borax all the concentrations above 1% behaved similarly (Fig.2). Thus, if we add 20% concentration of this chemical, though it isdiluted with other materials in the environment it has an ability to kill the colony.

As seen in Fig.4 with boric acid, 1% concentration is effective while the lower concentration (0.05%) required a longer period to bring mortality.

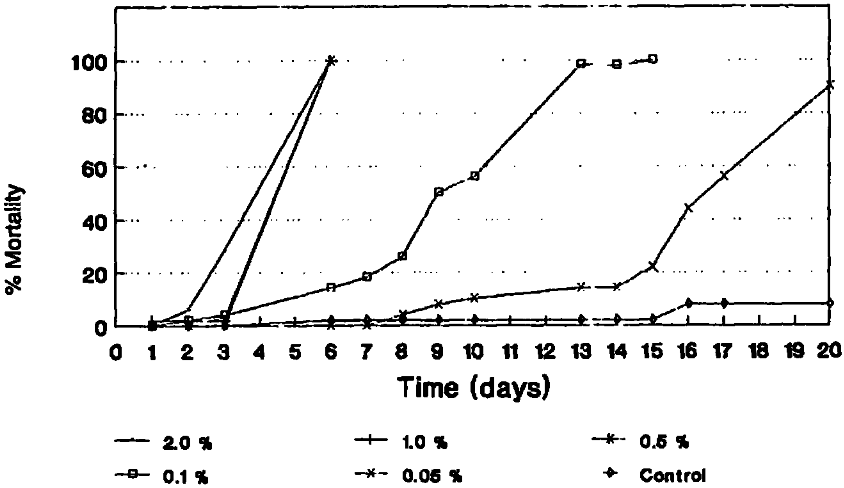

Fig. 4 – *Percentage mortality of **Reticulitermes** with effective range of Boric acid*

In bioassay II, three fungal isolates as seen in Fig.5 were tested on both species of termites. The mortality due to *M.anisopliae* 101-82 isolate on *Reticulitermes*, increased steadily up to 7 days with each concentration and the highest mortality achieved was around 70% (Fig.6). With *Zootermopsis* the mortality increased rapidly within the first few days and achieved 100% mortality (Fig.7).

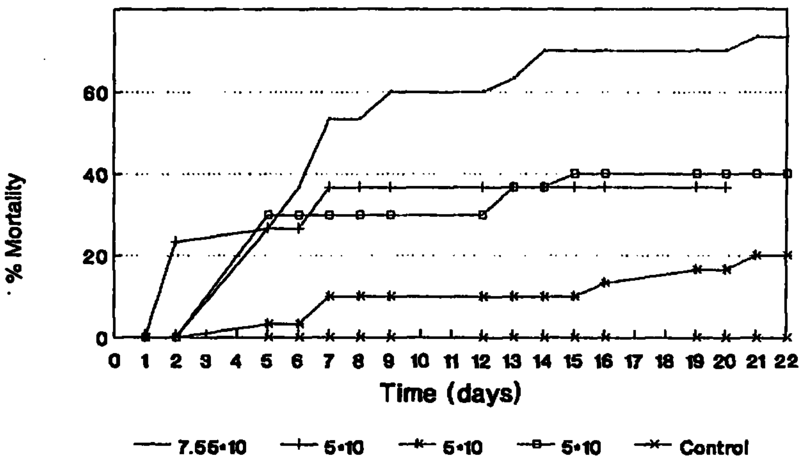

Fig. 6 – *Percentage mortality of **Reticulitermes** with given concentrations of 101-82 **M. anisopliae***

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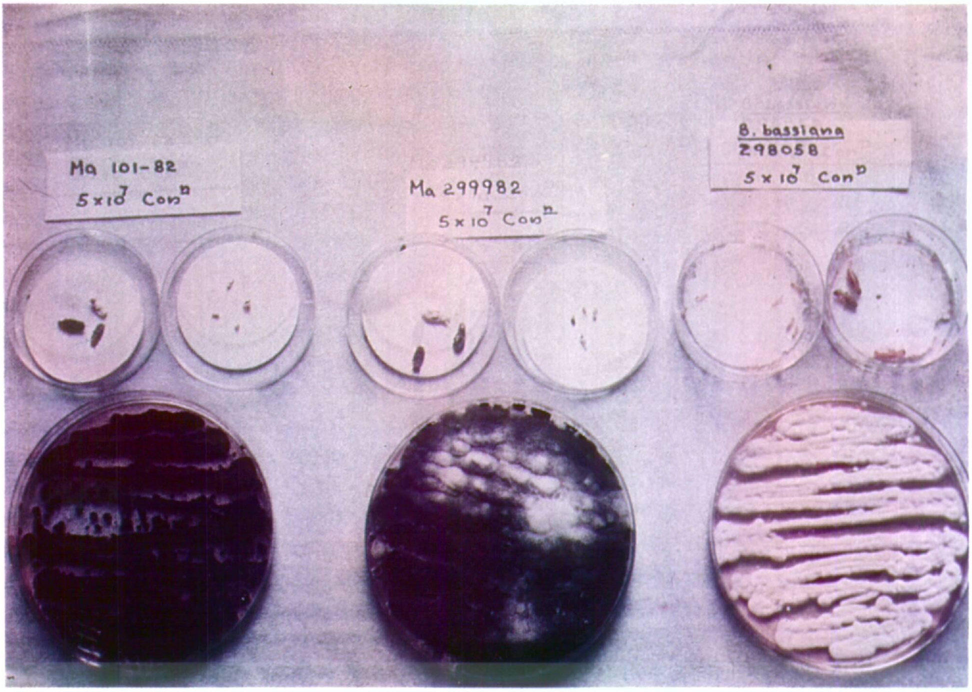

Fig. 5 – *Termites (**Zootermopsis** and **Reticulitermes**) killed by **Metarhizium anisopliae** (Ma 101 – 82 and Ma 299982,) unaffected by **B. bassiana**, with culture plates of these isolates*

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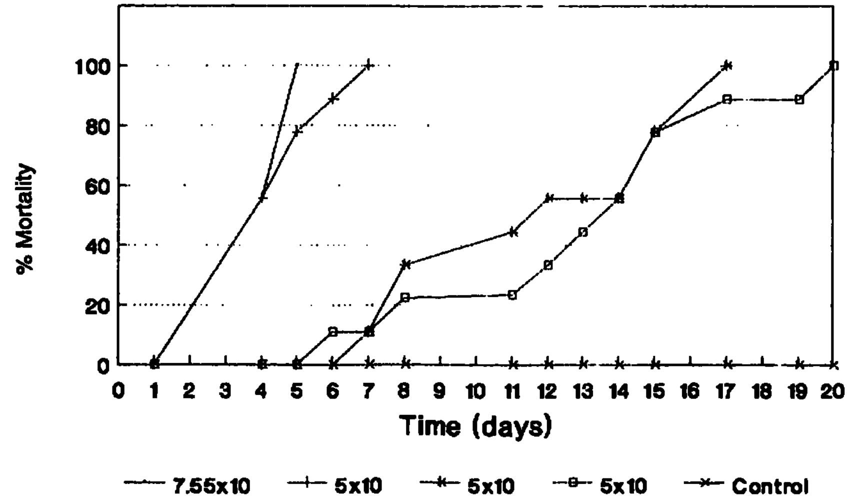

Fig.7 – *Percentage mortality of* ***Zootermopsis*** *with given concentrations of 101-82* ***M. anisopliae.***

M.anisopliae 299982 gave 86.67% and 100% mortality on *Reticulitermes* and *Zootermopsis* respectively within 19 and 7 days (Figs.8 and 9). LD_{50} values show that 299982 isolate of *M.anisopliae* is more virulent than that of 101-82 isolate.

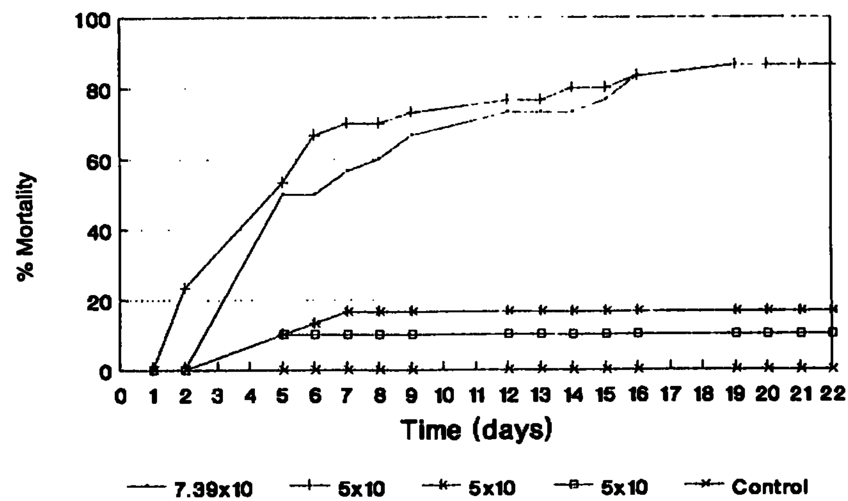

Fig.8 – *Percentage mortality of* ***Reticulitermes*** *with given concentrations of 299982* ***M. anisopliae.***

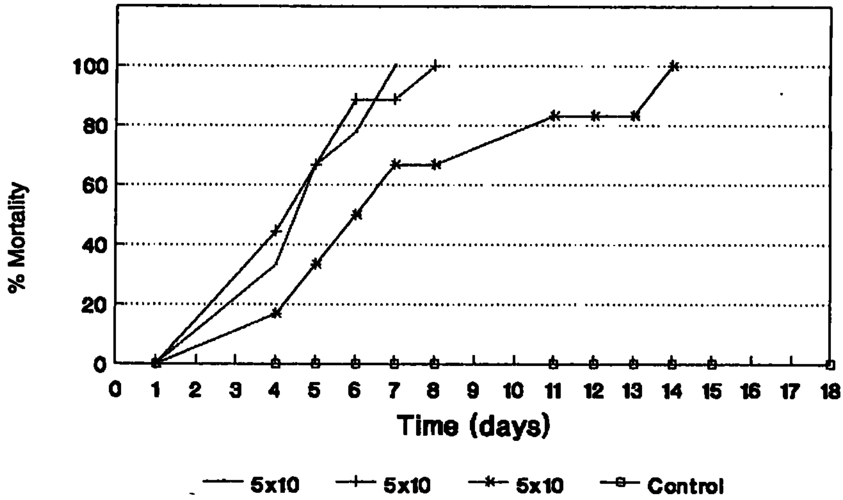

Fig. 9 – *Percentage mortality of **Zootermopsis** with given concentrations of 299982 **M. anisopliae.***

Even with higher spore concentrations the mortality due to *B.bassiana* 298058 isolate did not exceed 30% (Fig.10). With lower concentrations of spores the mortality was partly increased due to cannibalism.

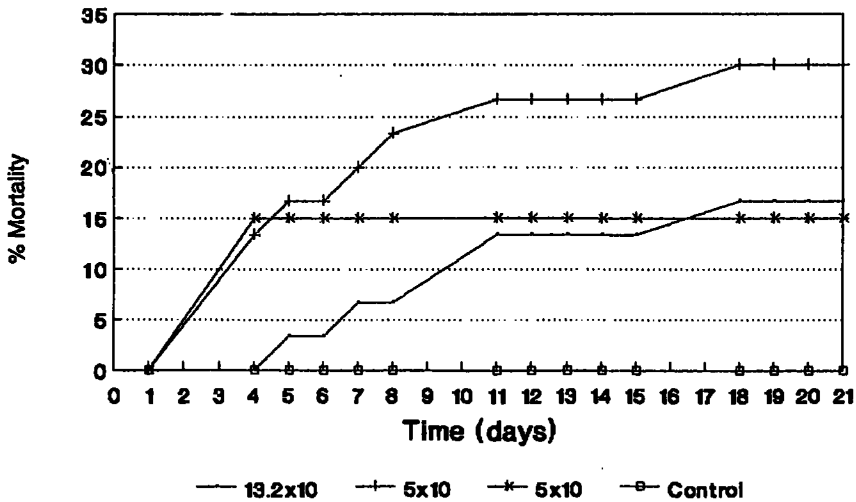

Fig. 10 – *Percentage mortality of **Reticulitermes** with given concentrations of 298058 **B. bassiana.***

In the case of *B.bassiana* tested on *Zootermopsis,* all concentrations except 5 x 10^7 concentration behaved like the control. In this case, the fungus did not have any effect on the termite species. There was mortality observed but death was due to cannibalism (see Fig.11).

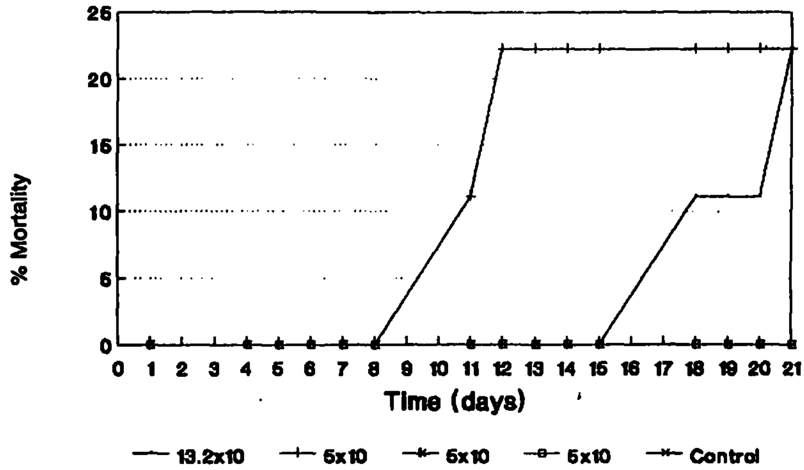

Fig. 11 – *Percentage mortality of **Zootermopsis** with given concentrations of 298058 **B. bassiana***

With nematodes, lower concentrations did not bring about adequate mortality, the mortality remaining at less than 30% (Figs. 12, 13 and 14). When the concentration was increased, the mortality increased (Figs. 15 and 16) but did not reach the required levels. The termites were able to escape the nematodes by getting underneath the filter papers. Even here, cannibalism was unavoidable, nor was it possible to introduce additional termites.

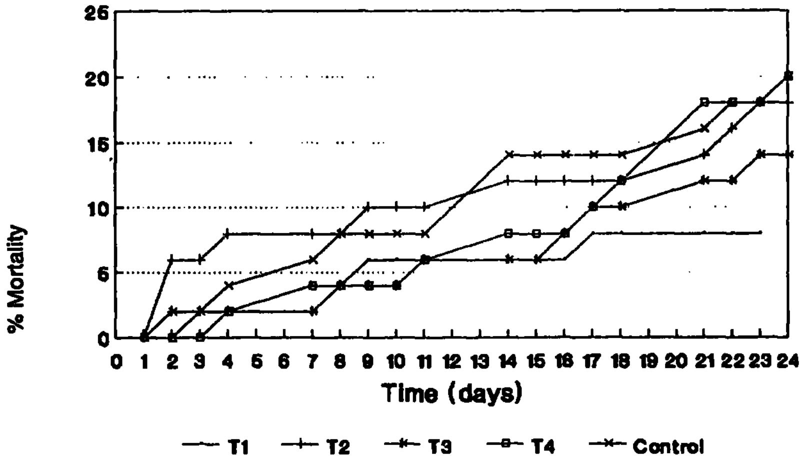

Fig. 12 – *Percentage mortality of **Reticulitermes** with given concentrations of **Steinernema** sps.*

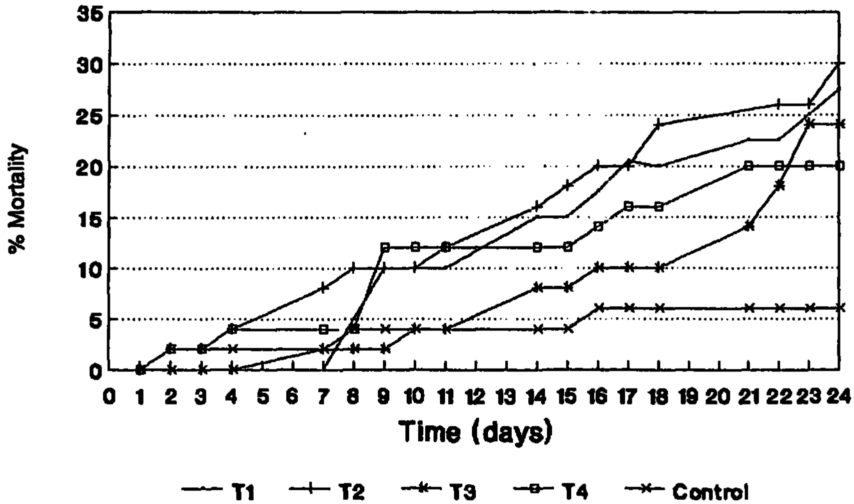

Fig. 13 –*Percentage mortality of* ***Reticulitermes*** *with given concentrations of* ***Heterorhabditis*** *sps.*

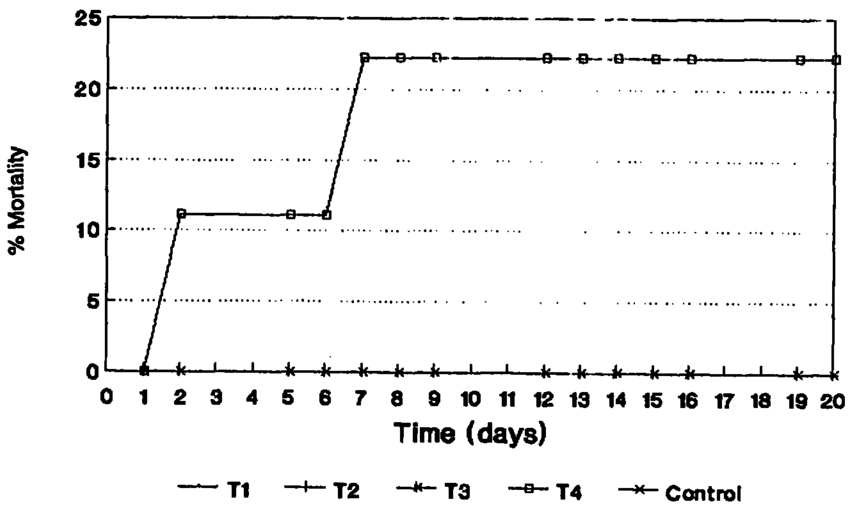

Fig. 14 – *Percentage mortality of* ***Zootermopsis*** *with given concentrations of* ***Steinernema*** *sps.*

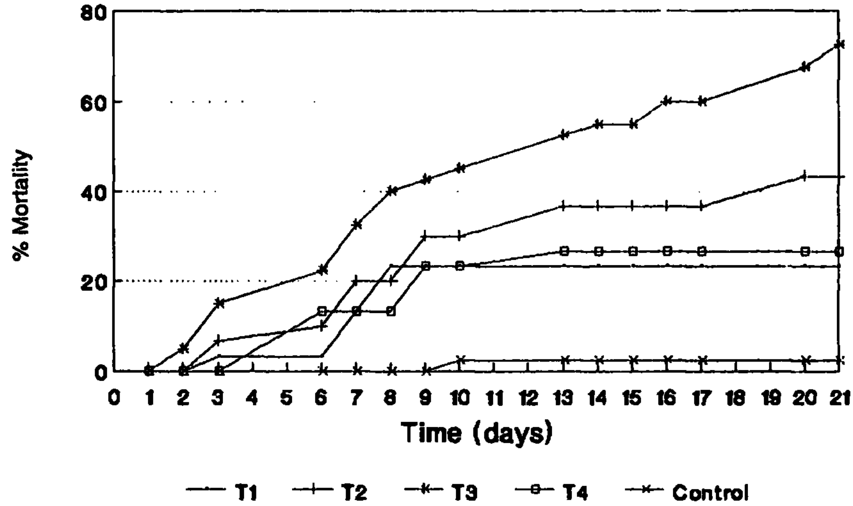

Fig. 15 –*Percentage mortality of* ***Reticulitermes*** *with given concentrations of* ***Steinernema*** *sps.*

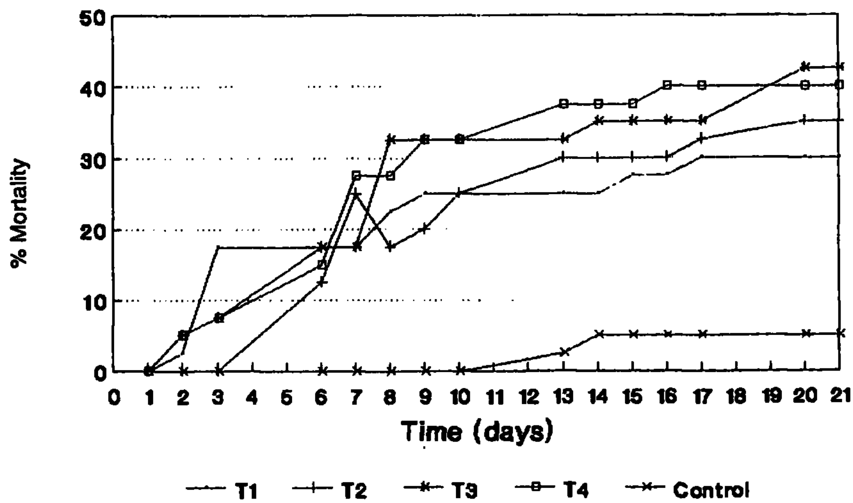

Fig. 16 –*Percentage mortality of* ***Reticulitermes*** *with given concentrations of* ***Heterorhabditis*** *sps.*

According to bioassay IV, large number of nematodes per termite have to be introduced. In the highest concentration 100% mortality was achieved at one month (16,338 nematodes per 20 termites) (Figs. 17 and 18).

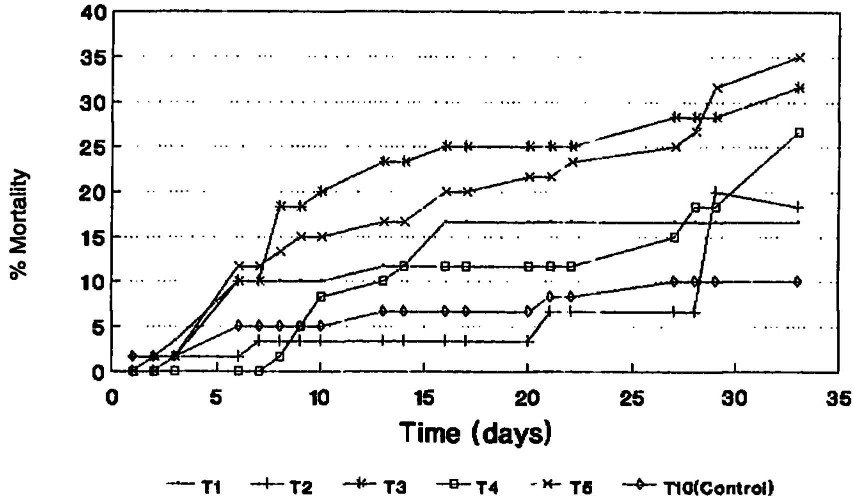

Fig. 17 – *Percentage mortality of* ***Reticulitermes*** *with given concentrations of* ***Steinernema*** *sps.*

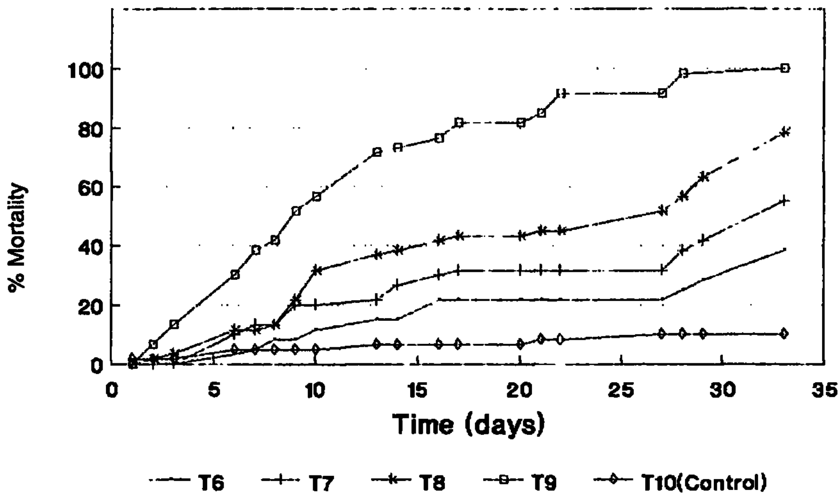

Fig. 18 – *Percentage mortality of* ***Reticulitermes*** *with given concentrations of* ***Steinernema*** *sps*

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