

EFFICACY OF ENTOMOPATHOGENIC NEMATODE (*STEINERNEMA Karii*) IN CONTROL OF TERMITES (*COPTOTERMES FORMOSANUS*)

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Abstract

Entomopathogenic Nematodes (EPNs) are soil parasites that infect different types of arthropods e.g. larva of butterflies, moth, beetles and grasshopper thus affecting them in various ways. This is through reducing their fertility or causing sterility, delaying development and shortening longevity of the arthropods. Termites also sometimes suffer chance infections from the *Steinernematids* that become naturally dispersed in ordinary soil. Often, this result in the death of the affected termite but the impact on the termite colony itself is generally minor and of limited duration. The main aim of this study was to determine the susceptibility of termites to EPNs (*Steinernema karii*) in a laboratory set up as a candidate for bio-control. The method used involved trapping the termites in petri dishes and infecting them with EPNs. Termites were also exposed to EPNs in a simulated natural habitat and the efficacy determined. It was found that infection in petri dish resulted in as high as 100% mortality especially in worker termites. There was little impact of EPNs on termites in the soil although workers showed higher mortality than soldier termites. At $P=0.05$, significant mortality difference was noted between EPNs-infected termites and control group in petri dish bioassays. Moreover, there was significance difference between soldiers and workers in terms of mortality rate. Infection in simulated natural habitat yielded less significant results compared to control group. It was concluded that *S. karii* has the potential to control termites, but methods have to be devised to allow maximum exposure of termites to the EPNs in their natural habitat.

Key words: Entomopathogenic nematodes, *Steinernema karii*, biocontrol, termites

1.0 Introduction

Termites sometimes suffer chance infections from the *Steinernematids* that become naturally dispersed in ordinary soil (Fasulo, 2002). Though such infections often result in the death of the affected termite, the impact on the termite colony itself is generally minor and of limited duration, unless conditions are right for the EPN (Entomopathogenic Nematode) involved to mount repeated, massive intrusions into the termite colony's workings (Lenz, 2005). Since EPN species are host specific, they can be used selectively for the target organisms. Furthermore,

although they succeed in infecting many of the insect hosts found in the soil they both inhabit naturally, they are unable to mount a significant attack to succeed in eradicating such hosts from that common habitat under normal conditions (Kaya, 1990). As occurs with parasites in general-including those with free-living propagules, for example, spores, and infective juveniles that are capable of giving rise to new, fully functioning organisms in kind - nematode tends to be different in their virulence (Ehlers *et al.*, 1990).

In nature, EPN-insect interactions involving Steinernematidae though invariably lethal to the individual insects they manage to infect, typically establish a kind of uneasy equilibrium (Khuong & Nguyen, 2010). Thus the EPN and their insect hosts manage to coexist, though the hosts are somewhat fewer in number than would be the case in absence of the EPN. While a degree of insect suppression occurs, insect "control", to the extent that the insects are unable to carry out their characteristic patterns of behavior, does not. (Bednarek & Gaugler, 1997). By way of contrast, EPN-insect interactions involving the *Steinernematidae* that are bolstered artificially by man often produce excellent control of many important insect populations (Lacey *et al.*, 2001). The subterranean termite is considered by many authorities to be a major exception to that rule. Termite and nematode biologists have fretted for years about the difficulties they've encountered trying to get EPN to work as well in the field as they do in a laboratory environment (Kaya & Koppenhofer, 1996).

2.0 Materials and Methods

2.1 Entomopathogenic Nematodes and Termites

EPNs were collected from Kenya Agricultural & Livestock Research Organization–Kandara and mass produced at the Technical University of Kenya, Microbiology Laboratory using late in-star larvae of *Galleria mellonella*. Termites were collected from around the University compound and maintained in the laboratory.

2.2 Study Site

The research was conducted at the Microbiology Laboratory, Technical University of Kenya, Nairobi-Kenya.

2.3 Mass Production of EPNs

Ten Petri dishes were lined with Whatmans filter paper and the nematodes *Steinernema kariii*, applied at the rate of 100 ij per dish (9 x 3.5). The nematodes were applied in 1ml distilled water and given 30 minutes to distribute on the filter. Third instar larvae of *G. mellonella* were applied in ten replicates to Petri dishes treated with 100 ij of *S. kariii*. The treatments were left on laboratory benches at room temperature (18-25°C) and 60% relative humidity for three days. The cadavers from each petri dish were placed in own White traps (Woodring & Kaya, 1988) for extraction of emerging entomopathogenic nematodes. Nematodes were

harvested and cleaned by sedimentation and decantation. Nematodes from each treatment were counted under a binocular microscope.

2.4 Termite Collection

In a well aerated bowl, soil and the wooden material being fed on by termites were collected with as many termites as possible from around the study site. In the laboratory, termites were kept at room temperature in well aerated place. Little water was be sprinkled regularly on the soil to dampen it to allow termites 'construct their shelters' to maintain them as in natural condition (Nan-Yao, 2005). One day before testing, termite workers (at least third instar) and soldiers were taken from rearing containers, counted, and transferred to 9.0 by 1.5-cm plastic petri dishes lined with a wet filter paper.

2.5 Infection with EPNs in Petri Dishes

Five petri dishes were sterilized using 70% ethanol and labeled 1-5 and filter paper placed such that they cover the inside of the dishes. With gloves and with the help of a sterile forceps, 10 ml of *Steinernema kariii* isolate was placed in each of petri dishes 1, 2, 3 and 4. In petri dishes 4 and 6, 10 ml distilled water was added. Exactly 10 soldier termites were placed in each of the 1, 2 and 5 dishes, 10 worker termites in dishes 3, 4 and 6 and covered. They were monitored after 12, 24, 48, 72 and 96 hours.

2.6 Infection of Termites In The Soil at a Laboratory Set-Up

Collected termites in 5 bowls were maintained in the laboratory for three days to allow them to stabilize as in natural habitat. In bowl 1, 2 and 5, ten soldier termites were put, in 3, 4 and 6, ten worker termites. EPN concentrates was sprayed except in bowl 5 and 6 where distilled water was added. Death due to *S. kariii* infection was determined by dissecting the cadavers to confirm presence of infective juveniles. Mortality rate was also determined through counting the number of dead termites and calculating the percentage in reference to the N value which was 10 termites per bowl.

2.7 Data Analysis

Data was analysed using SAS statistical package. Descriptive statistic, measures of central tendency (mean), was used to describe results from triplicate of experiments. Before performing any statistical analysis data was explored graphically using histograms to gain a greater understanding of the variables. The data was then subjected to analysis of variance (ANOVA) using SAS. A significant level of $P \leq 0.05$ was used.

3.0 Results

3.1 Mass production of EPNs

Galleria mellonella mortality occurred between 24 and 72 hours in the treatments. Infected larvae were floppy and maintained the pale color (Plate 1). After

harvesting the nematodes, approximately 20,000 infective juveniles were obtained determined by counting under binocular microscope.

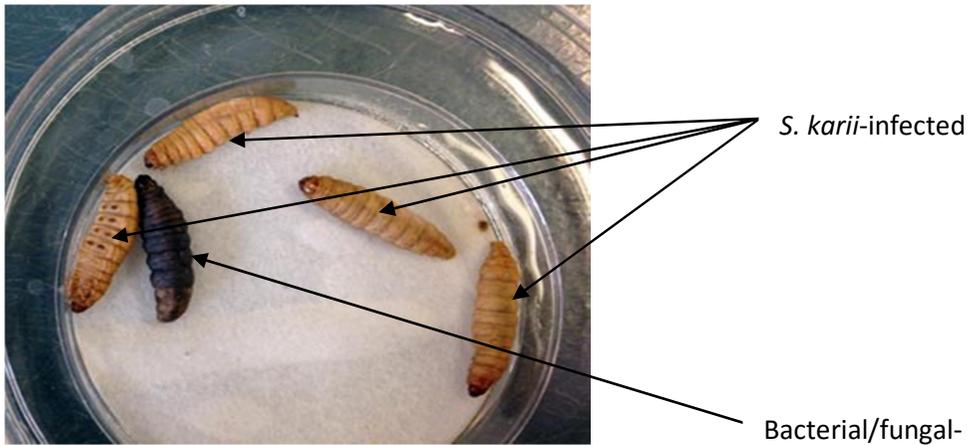


Plate 1: *S. kariii* infected larvae and bacterial/fungal infected *G. Mellonella* larvae

3.2 Termite Collection

Termites remained alive and healthy for the full period they were maintained in the laboratory (Plate 2A). They exhibited no mortality thus regarded as viable for the bioassays (Plate 2B).

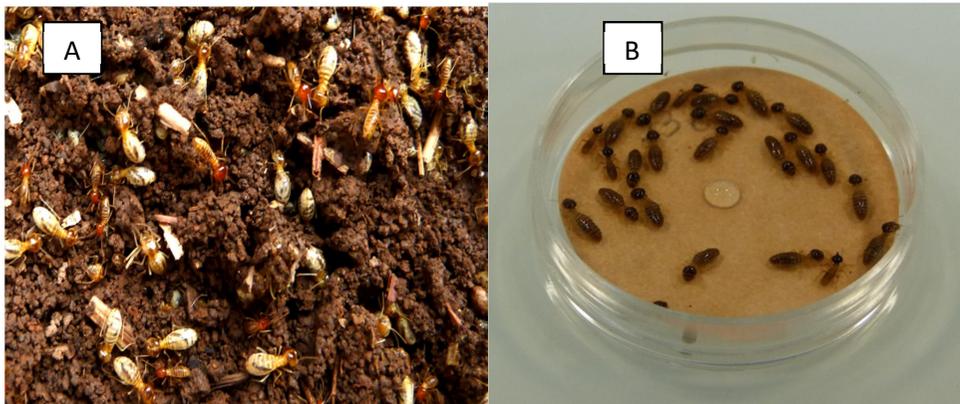


Plate 2: **A** termites maintained in a bowl containing soil and feeding material; **B**: termites in a petri dish during the bioassay in the laboratory

3.3 Efficacy of EPNS against termites in petri dish

Table 1: Mean mortality rate of soldiers and worker termites infected with EPNS and controls

Time(hrs)	Mean Mortality rate (%)			
	Soldier N=10	Control N=10	Worker N=10	Control N=10
12	10	0	4	0
24	15	0	57	0
48	28	0	73	3
72	37	0	97	13
96	48	0	100	20

Mortality of termites was observed after 12 hours, where 10% of soldiers and 40% of workers died. The highest mortality was observed in the worker termites after 96 hours at 100%, while 50% mortality was achieved after 24 hours. Highest mortality in soldiers was 48% which was achieved in 96 hours. Soldier termites used as control did not have any mortality, but 20% mortality was observed in worker termite control (Table 1). The hypothesis was that there was no significance difference between termites infected with EPNS and those not infected. Soldiers infection in the petri dish showed significance difference (ANOVA; $F=15.65$, $df=1$; $P=0.004197$). EPN infected and control worker termites also showed significant difference (ANOVA; $F=29.53$, $df=1$; $P=0.00062$) in mortality. Mortality of workers differed significantly with that of soldier in petri dish bioassay (ANOVA; $F=11.58$, $df=1$; $P=0.009317$).

3.4 Efficacy of EPNS against termites in the soil at a laboratory set up

Table 2: Mean mortality rate of termites infected with EPNS in the soil and controls (N=10)

Time(hrs)	Mean Mortality rate (%)			
	Soldier N=10	Control N=10	Worker N=10	Control N=10
12	0	0	0	0
24	0	0	0	0
48	0	0	15	0
72	10	0	15	0
96	20	0	35	10

Soldier termites showed the least mortality at 20% within 96 hours, which was way below the mortality in the petri dish. Workers infected with EPNS in the soil

showed higher mortality at 35% compared to the soldiers. Only 10% worker termites in the control group died (Table 2). It was hypothesized that there was no significant difference in mortality between infected termites and control group. The null hypothesis was accepted since there was no significant difference (ANOVA; $F=2.25, df=1; P=0.172003$) between infected soldier termites and control, as well as infected worker termites and control (ANOVA; $F=2.66, df=1; P=0.14159$). Unlike termites in the petri dish, the mortality rate of soldiers and workers in the soil did not differ significantly (ANOVA; $F=0.85, df=1; P=0.382936$).

3.5 Comparative Analysis

Infection in petri dishes produced mortality much earlier, within 12 hours (figure 1), compared to mortality in the soil, which occurred after 48 hours (figure 2). Moreover, it is noted that there was no death of termite soldiers in the control group. Few workers died in the control group both in the soil and petri dish. The death of the control worker termites only was attributed to the symbiotic protozoa they harbour in their guts. The suppression of their immunity by the *S. kariii*'s symbiotic bacteria allowed most of protozoa to attack the worker as they are only found in them and not the soldiers. Moreover, the workers perform all the tasks including building shelter and feeding the soldiers and thus slight change of environment and exhaustion may have led to the mortality rate witnessed. Standard error was included to cater for the death of termites in the control groups during data analysis.

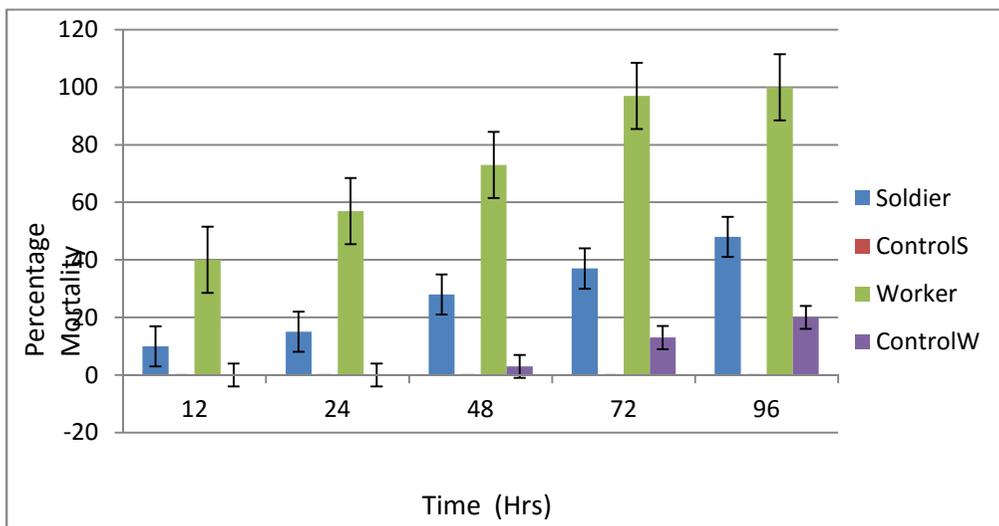


Figure 1: Percentage mortality of soldier, worker termites and their controls within 96 hours of infection with *S. kariii* in petri dishes. No Soldier termites in control died (ControlS does not appear)

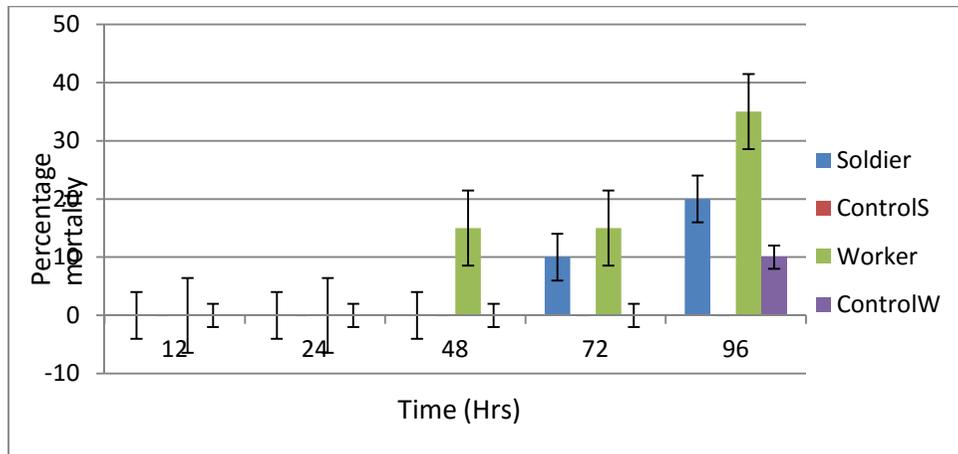


Figure 2: Percentage mortality of soldier, worker termites and their respective controls within 96 hours of infection with *S. kariii* in the soil at a laboratory setting. No soldier termites in control died (ControlS does not appear)

4.0 Discussion and Conclusion

In figure 1 & 2, it shows that termite workers were more susceptible to EPNs than soldier termites. The higher infection and thus mortality rate in worker termites could have resulted from their morphological position. This is because much of their body surface is in contact with the ground while moving giving nematodes opportunity to enter through the orifices (Suszkiw, 1998). Soldiers are large in size thus their body is elevated from the ground as they move.

It also shows that in the petri dishes (figure 1 & 2) mortality of the termites is high compared to infection in the soil, the high numbers of termites in a colony and the wide foraging range are obstacles for nematodes to eliminate termite colonies (Su & Tamashiro, 1987). The limited mobility of nematodes and their low rate of reproduction inside the dead termites make it unlikely that nematodes will reach and maintain a large enough density to eliminate a termite colony in the field, it may not be feasible to use these entomopathogenic nematodes in classical biological control for subterranean termites (Su & Scheffrahn, 1998).

Inundative release of these nematodes will only be useful for short-term protection and local control until means are developed to enhance survival and pathogenicity in systems such as bait matrices (Nguyen & Smart, 1994). There is a lot to learn about nematode biology, ecology, and relationships with their hosts. Termites stressed by sub-lethal doses of chemical or pathogens probably are more susceptible to entomopathogenic nematodes (San-Blas & Gowen, 2008). Entomopathogenic nematode (*Steinernema kariii*) that was first isolated in Kenya (Waturu et al, 1997) has shown the capability to infect and kill termites within 96

hours. Although mortality is more in petri dishes than in the soil, with repeated inoculations, it can yield reliable results.

Since termites were collected from the field within the study site and not reared in controlled environment, their health could not be determined and could have led to death of some. Some of the termites could have encountered EPNs before collection since nematodes are widely dispersed in the soil thus producing false positive results.

Experiments should be conducted in open fields to check their effect. By doing so, better understanding of their economic viability will be determined.

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