Modified trapping technique for the isolation of insect parasitic nematodes

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ABSTRACT: A modified trapping technique for the entomopathogenic nematodes was tested for its efficacy in recovery of naturally occuring insect parasitic nematodes, optimum time of removal of baited traps from the soil and at the same time prevention of attack by predatory ant, *Solenopsis geminata* (Fabr.). The highest nematode recovery (13.5%) and the least ant attack were achieved on fourth day of trapping. Modified trapping technique was found effective in the prevention of predatory ant attack which subsequently increased the efficacy of nematode recovery.

KEY WORDS: Chlorpyriphos, entomopathogenic nematodes, Gellaria mellonella, Solenopsis geminata

Entomopathogenic nematodes (EPN), steinernematids and heterorhabditids in particular, have received much attention in recent years because of their biological control potential against pestiferous insects in agriculture. Isolation of naturally occurring parasitic nematodes form the soil has always been a difficult task. Bedding and Akhurst (1975) came up with two new techniques viz., in situ technique and from the soil sample tested in the laboratory for better isolation of nematodes. Among these two, in situ technique which include, direct placement of Gellaria mellonella (F.) larvae as bait in the soil and recovering the same after one week has been popular and widely used all over the world.

The same method was followed initially during the isolation of parasitic nematodes from Bangalore region during 1995. However, this did not yield anything as *Galleria* larvae were attacked by the predatory ant, *Solenopsis geminata* (F.). Hence, a trapping technique slightly modified over the earlier technique was tested for its efficacy in EPN isolation.

A trapping technique suggested by Bedding and Akhurst (1975) was followed initially for the survey. It involved direct placement of G. mellonella larvae in pits at a depth of 15 cm in soil. However, this did not yield any results due to predation of the bait by ants, and hence a modified technique was tried. Small plastic vials, approximately 25 ml capacity open at both ends were sealed with 80mesh brass sheet. In such vials, four grown up G. mellonella larvae were released along with a piece of wet blotting paper to facilitate easy entry of the nematodes. Even this technique failed to prevent the entry of predatory ants as they could cut the rough brass mesh and enter the vials. Hence, a drop of chlorpyriphos (0.2%) was applied to wet blotting paper to prevent the ants attacking the larvae.

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A field experiment was conducted at the University of Agricultural Sciences, GKVK campus, Bangalore to study the effect of chlorpyriphos on the ant attack, nematode recovery and suitable time of removal of baited traps from the soil. Treatments included a set of plastic vials sealed with 80 mesh brass sheets and treated with chlorpyriphos (0.2%) to a piece of blotting paper and another set treated with plain water. Four healthy, full grown G. mellonella larvae were released in each vial as bait. After covering the lids, each vial was provided with a 20cm long metal wire at the end of which a label was fixed for easy identification. Such vials were placed at a depth of approximately 15 cm in the field at regular interval. Totally 60 traps were used in each treatment and 10 traps from each treatment were removed on every alternate day, up to 12 days after installation of traps. Each trap was examined and the dead and live larvae were separated. The larvae killed by ants were recorded and the remaining cadavers were individually transferred to White's trap for emergence of

nematodes. The per cent mortality of insect larvae caused by EPN and by other causes was recorded. The identity of the nematodes that emerged was confirmed by testing their infectivity against *G. mellonella* larvae under laboratory conditions by the Petri-dish bio-assay method (Woodring and Kaya, 1988). The data transformed to arcsine values were subjected to three factorial Complete Randomized Blocks Design.

Baited traps treated with chlorpyriphos (0.2%) were free from ant attack on *G. mellonella* larvae up to four days and were significantly superior over the traps without chlorpyriphos treatment, which recorded 48.5 per cent ant attack. However, the ant attack started from sixth day onwards and recorded a maximum of 51 per cent on 12^{th} day, which was on par with the untreated check. The highest rate of nematode infection of 13.5 per cent was recorded on fourth day in treated traps compared to the untreated check, which recorded no nematode activity (Table 1). Even though per cent nematode infectivity decreased

Treatment	Per cent mortality (days after)						
		2	4	6	8	10	12
Traps treated a) Ant attack with chlorpyriphos		0.00 (0.57)a	0.00 (0.57)a	26.00 (22.79)b	18.50 (16.89)b	31.00 (28.67)bc	51.00 (45.63)d
	b) Nematode attack	0.00 (0.57)a	13.50 (12.50)a	8.50 (6.46)a	8.50 (6.46)a	8.50 (6.46)a	6.00 (5.01)a
	c) Other cause	18.50 (16.84)b	18.50 (16.84)b	26.00 (24,23)b	41.00 (36.23)c	56.00 (49.56)d	28.50 (32.27)b
Untreated check	a) Ant attack	23.50 (21.34)b	48.50 (44.14)d	23.50 (21.40)b	28.50 (32.27)b	48.50 (43.73)d	51.00 (45.63)d
	b) Nematode attack	3.50 (3.51)a	0.00 (0.57)a	6.00 (6.46)a	6.00 (6.46)a	3.50 (3.51)a	0.00 (0.57)a
	c) Other Cause	11.00 (12.34)a	3.50 (3.51)a	13.50 (13.84)a	13,50 (13,84)a	13.50 (13.84)a	48.50 (44.14.)a

Table 1. Comparative efficacy of traps with and without treatment with chlorpyriphos

Figures in parentheses are arcsine- transformed values.

Values followed by same letters are statistically non-significant.

on subsequent days in treated traps, it was of non-significant.

Source	SEM ±	CD (P= 0.05)		
Treatments	1.022	NS		
Source of infection	2.173	4.26 **		
Days	3.072	6.00 **		

Statistical Analysis

The results revealed that the increased ant attack led to significant decrease in the nematode infectivity. Insect mortality due to other pathogens was almost constant on all the days in both the treatments. It was concluded that for isolation of EPN without interference from ants the traps treated with chlorpyriphos (0.2%) should be kept in the field for four days.

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