

SHORT COMMUNICATIONS**FIELD EVALUATION OF ENTOMOPATHOGENIC NEMATODES AGAINST WHITE GRUB *HOLOTRICHIA SERRATA* F. IN SUGARCANE****C. Sankaranarayanan*, B. Singaravelu and M. Rajeshkumar****Abstract**

Two separate field trials were conducted to evaluate two native entomopathogenic nematode (EPN) isolates of, *Heterorhabditis indica* viz., DSM78 and BNR against white grub *Holotrichia serrata* F. (Coleoptera: Scarabaeidae) in sugarcane. In field trial I, three to four days-old *H. indica* (BNR) infected cadavers of *Galleria mellonella* were applied manually near the root zone at five inch depth. In field trial II, talc formulated *H. indica* (DSM78) was applied @ 4 billion IJs/ac. Field efficacy of EPN, *H. indica* (BNR) against white grub in sugarcane in the field trial I revealed considerable reduction of white grub population due to EPN application compared to initial grub population. About 51% reduction of grub population was reported at 15 days after EPN inoculation and increasing trend (87.3%) of grub mortality was observed at 30 days after EPN inoculation. In field trial II, *H. indica* (DSM78) treated field recorded 46.6% control of white grub population compared to the initial grub population.

Key words: Entomopathogenic nematodes, *Heterorhabditis indica*, white grub, *Holotrichia serrata*, sugarcane

Introduction

White grub *Holotrichia serrata* F. (Coleoptera: Scarabaeidae) is a widespread and serious pest of sugarcane in India. The damage caused by this pest is observed in patches but during epidemics the entire crop may be wiped out. Insecticides have been the primary means of managing white grub for many years but the method is often ineffective because of the difficulty of insecticides reaching the root zone (Potter et al. 1996). Also, increasing concern about the environment and human safety has created a need for alternative control strategies. Biological control is a suitable alternative and entomopathogenic nematodes (EPN) belonging to the families of Heterorhabditidae and Steinernematidae offer an environmentally safe and IPM compatible alternative to insecticides for the control of several white grubs including *H. serrata* (Karunakar et al. 2000, Sankaranarayanan

and Singaravelu 2012, 2013). EPN occurring naturally, can be mass produced, and are safe to use. Nematodes that are native to a region are expected to be better for biological control programs in that region because they are adapted to the local soil and environmental conditions. In addition, regulatory issues and fears regarding the impact of exotic EPNs on non-target organisms are minimized when indigenous strains or species of these biological control agents are used. Therefore, it is recommended that such native nematodes must be developed as new biological control agents (Ehlers 2005, Lewis et al. 2006). The search for local EPN strains is important, and researchers in different countries continue to isolate local EPN strains from countrywide surveys (Malan et al. 2006, Hatting et al. 2009, Noosidum et al. 2010). One potential method of EPN delivery would be to apply the EPN to the target site in

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the form of EPN infected hosts. Pest suppression would then be achieved by the nematodes that emerge from the host cadavers. Effective pest suppression has been reported in field trials using this method (Jansson et al. 1993, Parkman et al. 1993). Laboratory experiments indicated greater nematode dispersal (Shapiro and Glazer 1996) and infectivity (Shapiro and Lewis 1999) when the nematodes were applied in cadavers than as aqueous application. To ascertain the efficacy of these EPN against *H. serrata* in sugarcane under field conditions, two separate experiments were conducted with two virulent native strains (DSM78 and BNR) of the EPN *Heterorhabditis indica* and the results are presented here.

Materials and methods

EPN strains and culture conditions

Two native virulent strains of *Heterorhabditis indica* viz., DSM78 and BNR were used in this study. The two strains were isolated in our earlier EPN surveys in white grub endemic sugarcane areas of Tamil Nadu (DSM78 was isolated from sugarcane soils of Palacode, Dharmapuri, Tamil Nadu and BNR from EPN infested dead cadaver of *H. serrata* from Bodinayakanur, Theni, Tamil Nadu. These nematodes were earlier identified on the basis of morphological characteristics and genomic sequence of the internal transcribed spacer (ITS) region (GenBank Accession Numbers KF937809 and KF937812). The nematode strains were cultured and maintained in the laboratory on fifth instar larvae of greater wax moth, *Galleria mellonella* Linnaeus (Lepidoptera: Pyralidae), using standard methods (Kaya and Stock 1997). Infective juveniles (IJs) were harvested using White's traps (White 1927) and stored in culture flasks at room temperature ($26 \pm 2^\circ\text{C}$).

Mass production and formulation of EPN

Mass production of the EPN isolates was done *in vivo* on *G. mellonella*, reared in laboratory conditions on artificial medium as per the method described by David and Kurup (1988). The grown up larvae of *G. mellonella* were collected after 25 to 30 days for *in vivo* mass production of EPN based on the method outlined by Woodring and Kaya (1988). The infective juveniles were harvested in 0.01% formaldehyde, and EPN suspension was mixed with talc powder in such quantity to maintain 20% moisture content. The talc formulated EPN was sealed and kept at room temperature ($26 \pm 2^\circ\text{C}$) for further studies.

Field evaluation of EPN against white grub in sugarcane

Two separate experiments were conducted in farmers' sugarcane fields naturally infested with *H. serrata* at two different locations in Tamil Nadu. Before the experiment, soil samples were collected randomly in the experimental field for enumeration of natural EPN population present in the soil. The collected soil samples were baited with *G. mellonella* larvae (Bedding and Akhurst 1975). But no EPN could be recovered from the soils of the experimental fields.

Trial I was conducted in Anaikarapatti Village, Bodinayakanur, in June 2015 in a field with sugarcane cv. Co 86032 of five months age. The initial population density of white grub/m² was recorded in 30 randomly selected places and all the white grub stages were observed in the field at the time of treatment. EPN strain, *H. indica* (BNR) was applied by placing two EPN infected *G. mellonella* cadaver per sugarcane clump. Three to four days-old EPN infected cadavers were applied @ 4000 cadavers/acre manually near the

root zone at five inch depth. Total area selected for EPN treatment was one acre. The observation on mortality of the grubs was recorded on 15th and 30th day after EPN application. The grub population was observed by counting the number of grubs/m² in 30 randomly selected sites in the experimental field.

Trial II was conducted in Theganari village of Thalavady, Tamil Nadu during August 2015. The initial population density of white grub was recorded in 30 randomly selected places as in Trial I. The grubs were mainly in 3rd instar stage at the beginning of the experiment. The field selected had six month old sugarcane cv. Co 86032. Total area selected for EPN treatment was 0.5 acre and the talc formulated EPN strain, *H. indica* (DSM78) was applied @ 4 billion IJs/acre. The EPN formulation was mixed with 100 kg of soil and broadcasted in the field. The observation on mortality of the grubs was recorded 10 days after EPN application. The grub population was

observed by counting the number of grubs/m² in 30 randomly selected sites in the experimental field.

Statistical analysis

For the comparison of pre- and post-populations of white grubs, non-parametric tests for dependent samples were employed using individual sample units as replications or blocks and time of observation as treatments. Wilcoxon matched pairs rank test was used to compare two observations and Friedman ANOVA by ranks & Wilcoxon matched pairs rank test was used to compare three or more observations. The analyses were performed using StatSoft Inc (2004).

Results and Discussion

Field efficacy of EPN strain, *H. indica* (BNR) against white grub in sugarcane in the first trial revealed considerable reduction of white grub population due to EPN application compared to initial grub population (Table1). About 51%

Table 1. Effect of *Heterorhabditis indica* treatment on white grub *Holotrichia serrata* populations in two different locations

Observation	Field trial I		Observation	Field trial II	
	Mean No. of grubs/ m ²	Z-value [#]		Mean No. of	Z-value [#]
Pre-treatment	6.83 ± 1.39 a ¹	-	Pre-treatment	3.43 ± 0.90 a ¹	-
Post-treatment I	1.70 ± 1.24 b	-	Post-treatment	1.83 ± 0.87 b	-
(15 days)					
Post-treatment II	0.87 ± 1.07 c	-	Pre-treatment vs Post-treatment		4.10**
(30 days)					
Pre-treatment vs Post-treatment I		4.70***	-	-	-
Pre-treatment vs Post-treatment II		4.70***	-	-	-

1 Means followed by the same letter in a column are not significantly different by Friedman ANOVA based by Wilcoxon matched pairs rank test significant with a Bonferroni correction (P<0.0001)

Wilcoxon matched pairs test;*** P<0.00001;

reduction of grub population was observed at 15 days after EPN inoculation and increasing trend (87.3 %) of grub mortality was observed at 30 days after EPN inoculation (Friedman ANOVA: $\chi^2 = 49.42$; $df=2$; $N=30$; $P>00001$) (Table 1). *H. indica* infected *G. mellonella* cadavers has shown promising results in terms of a 25-66% reduction in white grub population and an increase in sugarcane yield to up to 60-80 quintals/acre (Mohan 2013).

In trial II at Theganari village of Thalavady also suppression of white grub population observed due to EPN application. The pre treatment grub population was 3.43 ± 0.90 and the post treatment grub population was 1.83 ± 0.87 . The suppression of grub population due to *H. indica* (DSM78) was 46.6%. Comparison of periods of observation by Wilcoxon matched pairs test was highly significant (Pre-treatment vs Post-treatment: $Z=4.10^{***}$; $P=0.0001$) (Table 1).

The results of the present study demonstrated the field efficacy of EPN against white grub *H. serrata*. *Steinernema glaseri* and *H. indica* caused mortality of different instars of white grub *H. serrata* (Karunakar et al. 2000). In our earlier studies (Sankaranarayanan et al. 2011, Sankaranarayanan and Singaravelu 2012, 2013), we found increased susceptibility of white grub to native EPN. *H. heliothidis* under field trials provided 60% control of white grubs at 47 days after treatment and was equivalent to the control achieved by Chlorpyrifos, Trichlorfon and Isofenfos (Villani and Wright 1988). The results of the present findings are similar to the findings of Gao et al. (2015) who observed *Steinernema longicaudum* and *H. bacteriophora* effective against *Holotrichia oblita* larvae in the laboratory and field in peanut.

The findings from present study revealed that, field trial I at Bodinayakanaur recorded 87.3% reduction of white grubs which was higher when compared to the trial II at Thalavady (46.6%). The reasons might be i) due to longer contact period of EPN (30 days) in field trial I than trial II (10 days) with white grub in the soil and field trial I was conducted during June 2015 during which time the grubs present in the field were early instar grubs which were very susceptible to EPN infection while in the field trial II, the experiments was conducted during August 2015 against older 3rd instar grub stage. Karunakar et al. (2000) tested *H. indica* and *S. glaseri* against different instars of *H. serrata* and found that the mean time taken to cause host mortality of white grubs increased as the instar stages increased. ii) EPN infected *G. mellonella* cadaver application might have enhanced the mortality of white grubs in trial I compared to talc application EPN in trial II. The superior pest suppression observed in cadaver applications may have been due to metabolites present in the cadavers that enhance dispersal or infection (Shapiro and Lewis 1999, Shapiro Me Coy. 2000).

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