Pakistan Journal of Nematology (2017) 35 (1): 65-71

ISSN 0255-7576 (Print)
ISSN 2313-1942 (Online)
www.pjn.com.pk

http://dx.doi.org/10.18681/pjn.v35.i01.p65-71

Infection of *Galleria mellonella* larvae by *Steinernema affine* and production of infective juveniles

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Abstract

The information regarding relationship between nematode dosage and infective juveniles (IJ) production are available for *Steinernema affine*. Therefore, in the present study the effect of inoculation doses of *S. affine* and extraction methods was investigated on the production of IJ in *Galleria mellonella*. There was significantly greater emergence of IJ of *S. affine* from the *Galleria mellonella* using White traps than with modified Baermann extraction trays. In Baermann extraction trays, the maximum emergence of IJ of *S. affine* was observed at an inoculation dose of 400 IJ followed by the dose of 50 IJ. The minimum emergence was found with an inoculation dose of 100 IJ. On the other hand, in case of White traps, the maximum emergence of IJ was recorded with doses of 100 and 50 IJ. The minimum emergence of IJ was observed with a dose of 200 IJ. The relationships between inoculation doses and emergence of IJ from the cadavers for both the methods were found to be non-significant.

Keywords: Entomopathogenic nematodes, emergence, reproduction, *Steinernema affine*, *Galleria mellonella*.

Entomopathogenic nematodes (EPN) are presently used against soil-dwelling insects attacking vegetables, fruits and ornamentals (Georgis, 1990). More than a dozen companies are presently producing and selling nematodes in the USA, Australia, Japan and Europe (Georgis & Manweiler, 1994) but these companies are not yet producing EPN for use in the warmer countries of the tropics and sub-tropics. Despite the increasing commercial and scientific interest in steinernematids and heterorhabditids, a universal standard infectivity assay has not been established. The need to evaluate nematode insecticidal activity in the laboratory has resulted in the development of a variety of assays that measure nematode infectivity by recording host mortality.

EPN are now established as potent biological control agents because the ability to mass produce them has allowed the development techniques of inundative application (Griffin *et al*., 2005). The mass production technique based on fermentation technology is an industrial process (Gaugler & Han, 2002; Ehlers & Shapiro-Ilan, 2005a). Such technologies are not yet available in countries such as Pakistan where the use of EPN is in its infancy. In these countries the development of use of EPN will depend initially on low technology mass production techniques such as use of host insects for *in vivo* production (Ehlers & Shapiro-Ilan, 2005b). These techniques are labor intensive but are feasible where labor costs are low. In Pakistan initial field evaluation of EPN are

likely to be done with *in vivo* produced nematodes in hosts such as in *Galleria mellonella* (Rahoo *et al*., 2011).

Infective juveniles (IJ) are applied as inundative biological control agents for the management of soil-borne insect pests on a variety of crops (Kaya, 1990; Kaya & Gauglar, 1993; Kaya, 2002; Shapiro-Ilan & Gaugler, 2002; Shapiro-Ilan *et al*., 2002; Rahoo *et al*., 2016a & b). *Steinernema affine* is found commonly in many areas and grasslands. However, this species is not currently being mass produced for commercial purposes. The reason may be that IJ productivity in culture is low. Production rate *in vivo* cultures is affected by the number of nematodes to which the host is exposed. Cabanillas & Raulston (1996) showed that there is a decrease in number of IJ produced with increasing inoculum level.

Results on the relationship between nematode dosage and IJ production are available for different nematode species (Zervos *et al*., 1991; Selvan *et al*., 1993; Cabanillas *et al*., 1994; Cabanillas & Raulston, 1996). However, no such data exist for *S. affine.* Therefore, in the present study *S. affine* was assessed for its reproduction potential in relation to the dosage of IJ applied.

Materials and Methods

Nematode inoculums: The stock culture of entomopathogenic nematode *Steinernema affine* obtained from CAB International was maintained in the laboratory at the Department of Agriculture, University of Reading, UK. The *S. affine* was mass cultured on last instar larvae of *Galleria mellonella* (Dukty *et al*., 1964). The infective juveniles (IJ) were harvested in White traps (White, 1927). Only one-week old IJs of *S. affine* were used in the experiment.

Effect of inoculation doses of *S. affine* on production of IJ: Fifty six late instar larvae of *Galleria mellonella* with individual weights of 0.28 to 0.32 g were selected to investigate whether the inoculum levels of *Steinernema affine* affect the infection of *G. mellonella*. Each larva was transferred to Whatman No.1 filter paper in a 30 mm Petri dish. The larvae were divided into four groups of fourteen. The larvae of four groups were each inoculated with 50, 100, 200 and 400 IJ contained in 0.1, 0.2, 0.4 and 0.8 ml suspension, respectively (and the total volume was made up to 1 ml). The dishes were stored in an incubator at 20°C for 4 days in which time all larvae succumbed to nematode infection. Twenty eight Petri dishes (30 mm dia.) containing 5 g of dry silver sand were prepared, divided into four groups and 1 ml of tap water was added. Half of the infected larvae (cadaver) from each treatment cohort were added to each dish of each group. A small piece of Netlon was used to support each cadaver and the Petri dishes were sealed. Each of the seven remaining infected larvae from each treatment was placed in each small dish (55 × 20 mm) diameter which was used as a modified White trap. All the Petri dishes and White traps were kept in an incubator at 20°C.

One week after inoculation each cadaver was moved on the supporting Netlon and transferred to new Petri dish containing 5 g silver sand plus 1.0 ml water, these Petri dishes were then sealed and returned to the incubator. Nematodes that had migrated into the sand from the original dish were extracted as described previously. This procedure was repeated every 3 days until no more nematodes were recovered. Each Petri dish was monitored daily to observe when nematodes first emerged from cadavers. To facilitate case of counting the nematodes the two groups were evaluated on different days.

Data analysis: The data were found normally distributed and did not require transformation. All the data were subjected to Analysis of Variance (ANOVA) using GenStat package 2009, (12th edition) version 12.1.0.3278 (www.vsni.co.uk). The means were compared by Fisher's Protected Least Significant Difference Test at 5%. Standard errors of means were calculated in Microsoft Excel 2003. Data were also subjected to regression analysis.

Emergence of IJ from cadavers was regressed as the dependent variable with the inoculation dose of IJ as the independent variable for both extraction methods.

Results

The extraction methods have significant effect ($P<0.001$) on emergence of IJ from the cadavers. There was significantly greater emergence of IJ of *S. affine* from the *Galleria mellonella* using White traps than extracted with modified Baermann extraction trays as shown in Fig. 1.

In the Baermann extraction trays, the inoculation doses have significant effect on the emergence of IJ of *S. affine*. The maximum emergence of IJ of *S. affine* was observed at an inoculation dose of 400 IJ followed by the dose of 50 IJ. The minimum emergence was found with an inoculation dose of 100 IJ (Fig. 2).

On the other hand in case of White traps, the maximum emergence of IJ was recorded with a dose of 100 IJ followed by the dose of 50 IJ. The emergence at these two doses was statistically non-significant. The minimum emergence of IJ was observed with a dose of 200 IJ as shown in Fig. 3.

The relationships between inoculation doses and emergence of IJ from the cadavers for both the methods were found to be non-significant. These relationships have been shown by trend lines and regression equations and are given in Fig. 4 and 5.

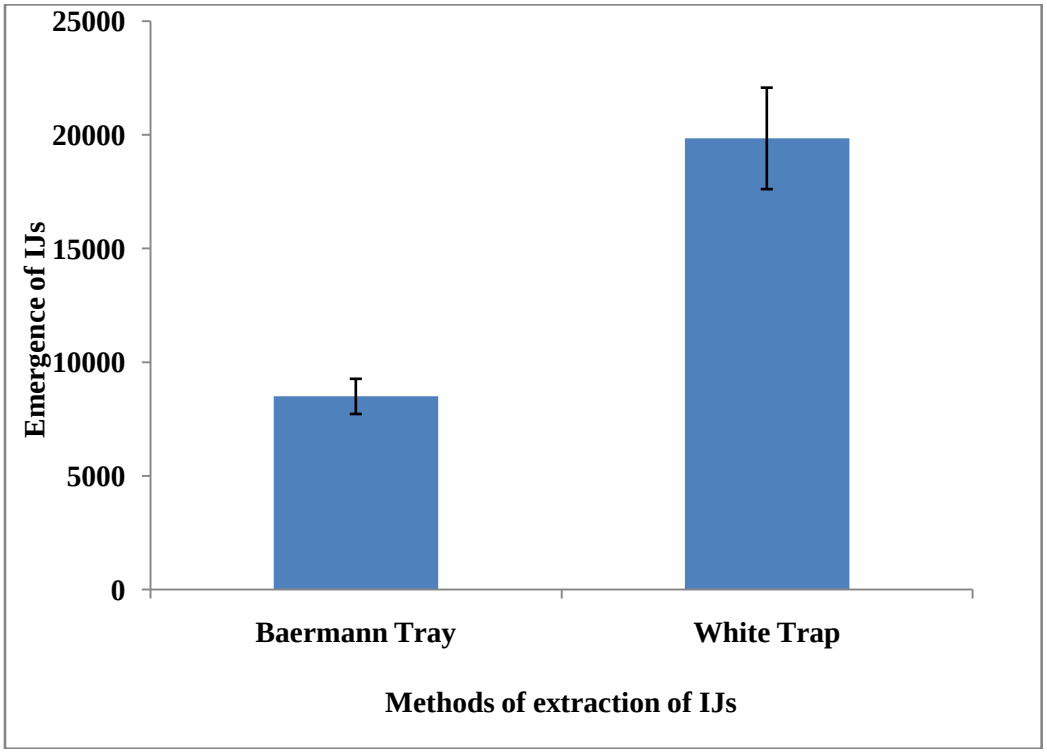

Fig. 1. Comparison of emergence of infective juveniles of *Steinernema affine* from *Galleria mellonella* cadavers using Baermann extraction tray and modified White trap.

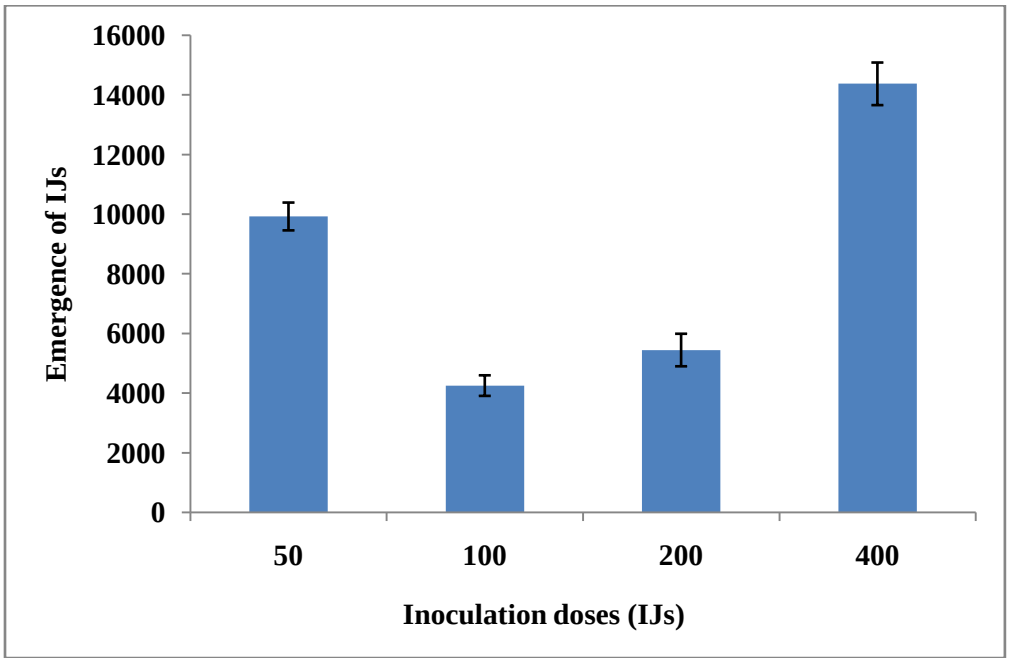

Fig. 2. Emergence of infective juveniles of *Steinernema affine* from *Galleria mellonella* using Baermann extraction tray.

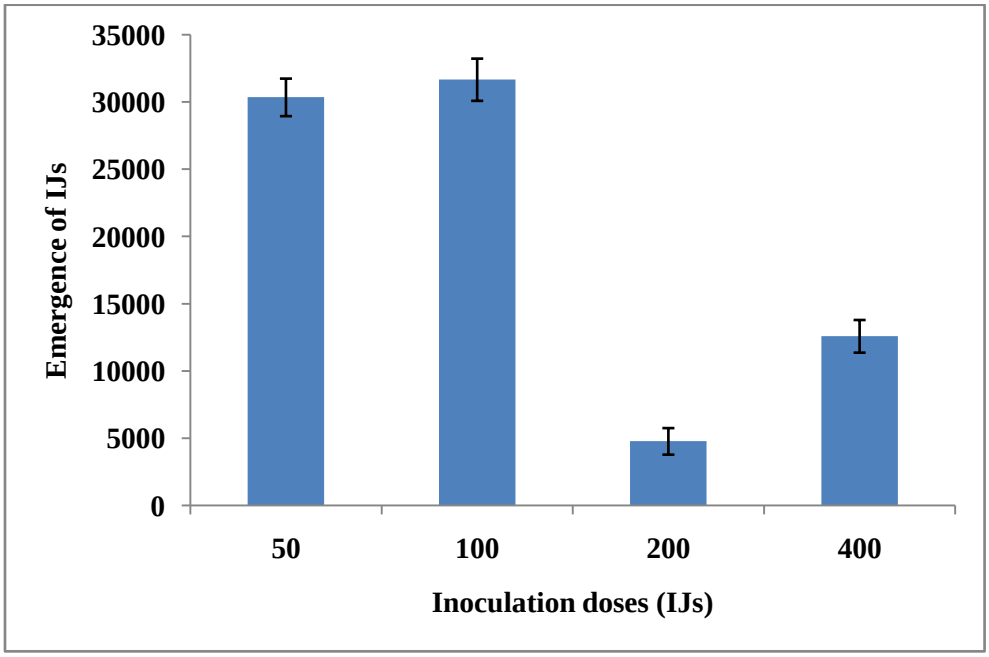

Fig. 3. Emergence of infective juveniles of *Steinernema affine* from *Galleria mellonella* cadavers using modified White trap.

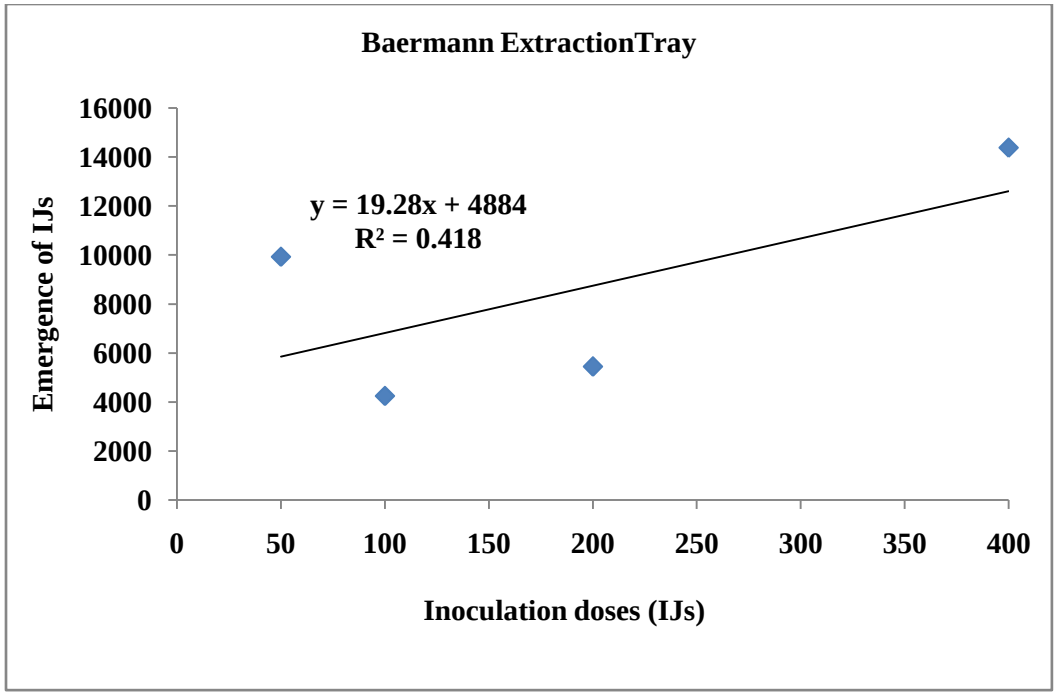

Fig. 4. Relationship between nematode dosage and emergence of IJs of *Steinernema affine* using Baermann extraction tray.

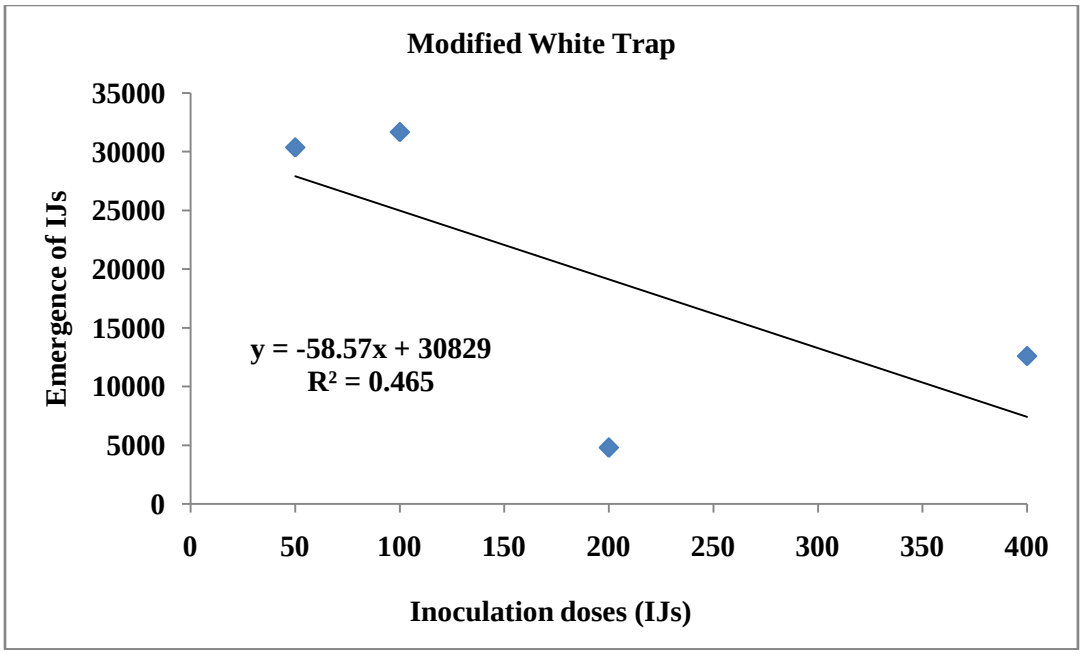

Fig. 5. Relationship between nematode dosage and emergence of IJs of *Steinernema affine* using modified White trap.

Discussion

For *S. affine* the relationship between nematode dosage and IJs production was non-significant (see Fig. 4 & 5). Similar to the results by Zervos *et al.*, (1991), the variability in the number of juvenile production per host was high. *In vivo* production of IJs depends on the nematode dosage applied (Zervos *et al*., 1991; Selvan *et al.*, 1993; Cabanillas & Raulston, 1996). EPNs have different optima for the nematode dosage in relation to IJs production. *Steinernema riobravae* showed highest reproduction rates in prepupae and pupae of *Helicoverpa zea* at low application rates of 5 to 25 nematodes whereas *Steinernema glaseri* and *S. carpocapsae* reached optimum reproduction rate in *G. mellonella* after exposure to 100 to 500 and 800 IJs, respectively (Zervos *et al.*, 1991; Selvan *et al*., 1993).

Results for *Heterorhabditis bacteriophora* are variable and may be influenced by the nematode isolate. Zervos *et al.*, (1991) observed highest reproduction for *H. bacteriophora* in *G. mellonella* larvae after exposure to 5 and 25 nematodes whereas Selvan *et al*., (1993) reached optimum IJ production with 800 IJs applied per *G. mellonella* larva.

Differences between nematode species in the response of nematode inoculum on production rate might be due to different metabolism rates (Selvan *et al*., 1993) and differences in the processing of the host tissue by the symbiotic bacteria (Akhurst & Smith, 2002). Zervos *et al.*, (1991) suggested that the decrease in production rate at high inoculum level is due to a crowding effect which affects nematode species differently. At very low application rates production of *Steinernema* species might be negatively affected by their need to find a mate in the first generation. Low nematode density within the host may cause difficulties for nematode finding a mate or possibly only one sex is present within host (Zervos *et al.*, 1991). This might be the reason that in the present experiment the numbers of IJ per *G. mellonella* did not exceed those in the Baermann extraction. IJ production was not significantly affected by the number of nematodes applied (Fig. 3). Inoculum levels might have been too low to result in a crowding effect within host and consequently lower production levels. Results have proved that any nematode dosage of up to 400 IJs per host can be applied without a detrimental effect resulting in reduced IJs production.

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(Accepted: Oct 10, 2016)