Determination of LD_{50} and LT_{50} of entomopathogenic nematodes against shoot borer (*Conogethes punctiferalis* Guen.) infesting ginger (*Zingiber officinale* Rosc.)

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ABSTRACT

Study on the pathogenicity of four native entomopathogenic nematodes, *Heterorhabditis* sp. (IISR-EPN 01), *Steinernema* sp. (IISR-EPN 02), *Oscheius* sp. (IISR-EPN 08) and *O. gingeri* against shoot borer larva (SBL), by dose response and time exposure assay and determination of lethal dosages (LD_{50}) and lethal time (LT_{50}) by regression analysis. LD_{50} were calculated using four densities of test EPNs *viz.*, 25, 50, 75 and 100 IJs/SBL and LT_{50} was calculated using one nematode density 100 IJs to mortality of SBL at 24, 48 and 72 hrs. Although the SBL was susceptible to test EPNs, there were differences among these EPNs in their ability to kill the insect. Among test EPNs, *Steinernema* sp. (IISR-EPN 02) and *O. gingeri* appears to be the most promising. The per cent mortality of SBL increased with the increase the dosages as well as exposure time. A positive correlation was found between dosages as well as exposure time and mortality. Probit analysis indicated that *Oscheius* sp. (IISR-EPN 08) required less number (48 IJs/larva), whereas *Steinernema* sp. (IISR-EPN 02) took less time (29 hrs) for desired mortality of SBL. This study will be helpful in the shoot borer management under field conditions.

Key words: *Conogethes punctiferalis,* EPN, LD_{50}, *Zingiber officinale.*

Among the insect pests, shoot borer, *Conogethes punctiferalis* is one of the most important pest infesting Zingiberaceous crops specially ginger, turmeric and cardamom resulting in yield losses (Champakam *et al.*, 2006 & Venugopal *et al.*, 2006). The only effective method to manage this pest is the use of insecticides resulted residues in the produce affecting human health and also causing other ecological hazards. There has been a renewed interest in developing environment- friendly pest management schedules in agriculture. Entomopathogenic nematodes (EPNs) are effective biocontrol agents of a variety of economically important insect pests (Seal *et al.*, 2010) and they have been used in controlling insect pest for about a decade, extending their usage from high value markets to large area crops including spices (Pervez *et al.*, 2012). Estimation of lethal concentrations and time are a relative measure of susceptible of insect and is convenient and commonly used index of relative efficacy (Girling *et al.*, 2010). Hence, study on the pathogenicity of entomopathogenic nematodes, *Heterorhabditis* sp. (IISR-EPN 01), *Steinernema* sp. (IISR-EPN 02), *Oscheius* sp. (IISR-EPN 08) and *O. gingeri* (Pervez *et al.*, 2013) against shoot borer larva, *C. punctiferalis* by dose response and time exposure assay and determination of lethal dosages and lethal time by regression analysis.

Materials and Methods

Infective juveniles (IJs) of test EPNs were obtained from stock cultures which maintained in the Nematology laboratory, I.I.S.R., Kozhikode. All EPN species were cultured on fully grown *Galleria mellonella* larvae as per the procedure described by Woodring and Kaya (1988). The IJs were surface sterilised in 0.1% hyamine solution and stored in distilled water in tissue culture flasks for study. *Galleria* larvae were obtained from cultures maintained on artificial diet. The shoot borer larvae were collected from ginger fields of the IISR-Experimental Farm, P'muzhi, Kozhikode. The larvae were sorted and those of similar size were select for the study.

Lethal dosages were calculated using four densities *i.e.* 25, 50, 75 and 100 IJs/SBL in 100 µl of distilled water. Suspension of each density was inoculated into six well plates (3.5 cm dia.) lined with filter paper at the bottom of the plate. To each well was introduced one shoot borer larva and the mortality of the larvae recorded at 72 hrs. The experiment was conducted at 28 °C in a BOD incubator and replicated 12 times along with controls. The mortality calculated into percentage and converted to probit mortality and values were transformed to log. LD_{50} values were calculated using regression analysis. Lethal time was calculated using one density 100 IJs/SBL in 100 µl of distilled water. All experimental conditions were same as to determine LC_{50} except the mortality of the larvae recorded at 24, 48 and 72 hrs of post inoculation and mortality calculated into percentage. The mortality was converted to probit mortality and exposure times were transformed to log values. LT_{50} values were calculated using regression analysis.

Results and Discussion

All test EPNs were found pathogenic against SBL but the rate of mortality was vary. However, dosages and exposures time had a significant effect on mortality of the SBL. Per cent mortality of SBL increased with the increase the dosages of IJs. At 25 IJs/larva, mortality was first observed at 72 hrs for three test EPNs except *O. gingeri*, whereas it was observed at 50 IJs/larva (Fig. 1). No larval mortality was recorded in control. A significant and positive correlation was found between dosages and mortality. Probit analysis indicated that, *Oscheius* sp. (IISR-EPN 08) and *Steinernema* sp. (IISR-EPN 02) required less number (48 & 50 IJs/larva, respectively) to provoke 50% mortality of SBL at 72 hrs post inoculation (Fig. 3). Bano *et al.* (2003) reported variable response of EPN species against red weevil.

The mortality induced by test EPNs was first observed at 24 hrs. SBL mortality % increased with the increase the exposure time (Fig. 2). A significant

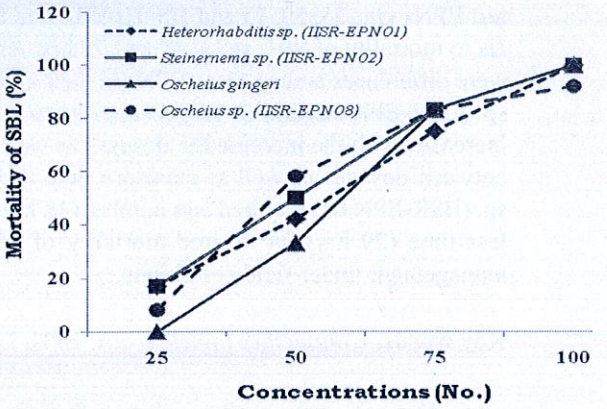

Fig. 1. Percent mortality of shoot borer larva (SBL) at different concentrations

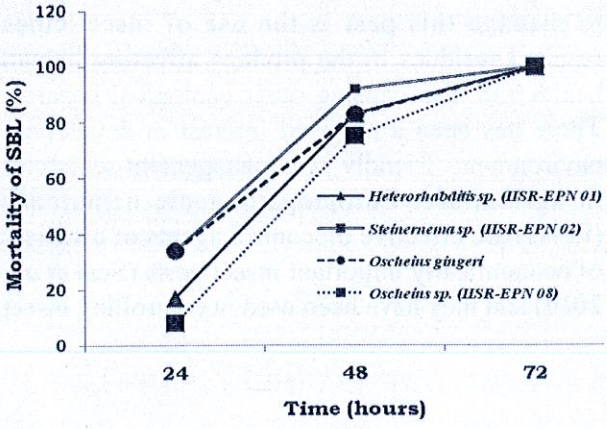

Fig. 2. Percent mortality of shoot borer larva (SBL) at different exposure time.

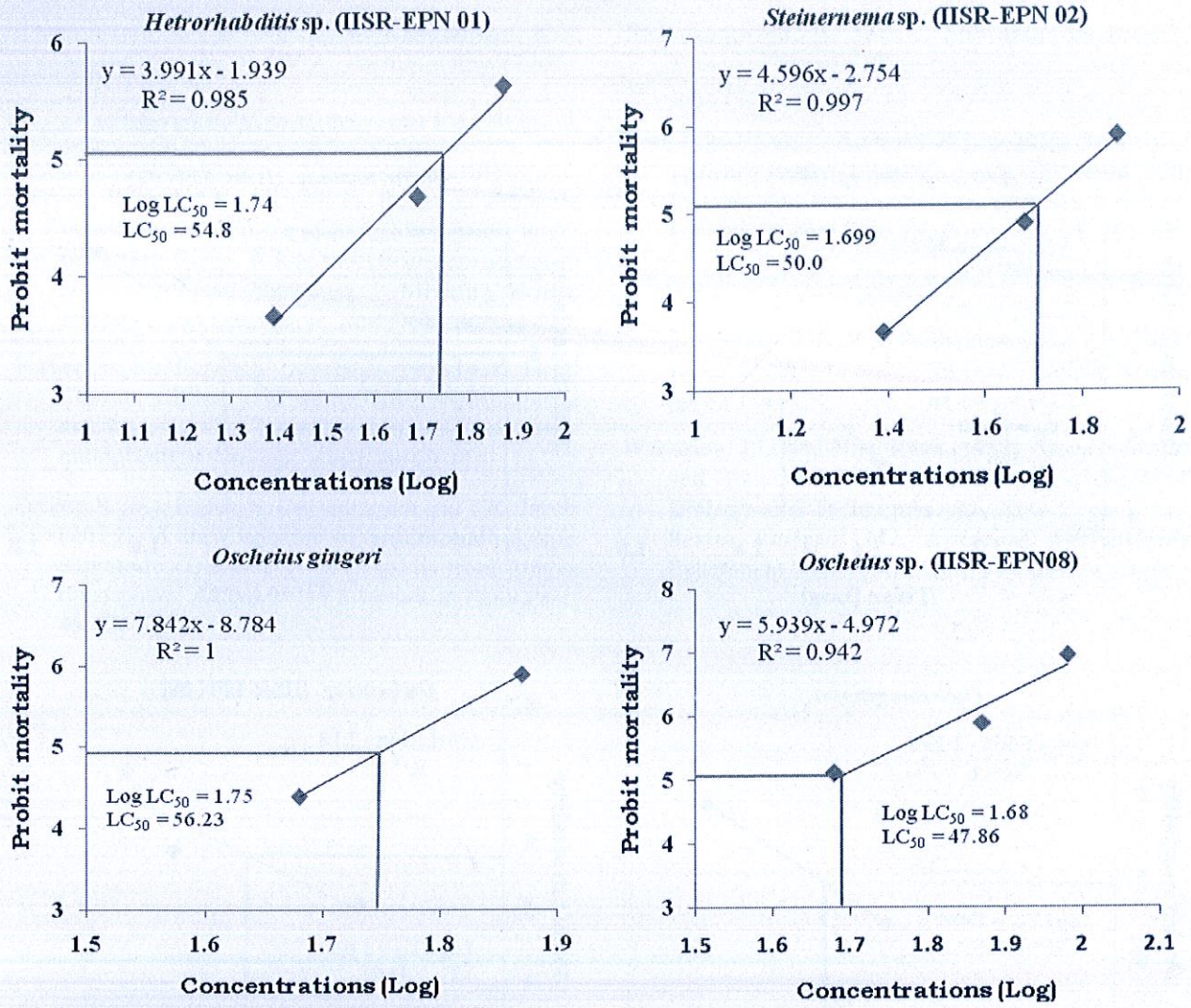

Fig. 3. LD_{50} value of entomopathogenic nematodes against shoot borer larva.

and positive correlation was found between exposure time and mortality. Time assay response revealed that required *Steinernema* sp. (IISR-EPN 02) and *O. gingeri* took less time (29 & 31 hrs, respectively) to kill 50% SBL (Fig. 4). No larval mortality was observed in control. No statistical difference in mortality of SBL obtained among test EPNs at 100 IJs dosage. Therefore 25 and 50 IJs/larva was distinctive rates to differentiate EPNs biological efficacy on the shoot borer. Similarly, not much difference was recorded in the mortality of SBL among test EPNs at 72 hrs time exposure, whereas mortality at 24 hrs were distinctive rates to differentiate infectivity of test EPNs against SBL. Saravanapriya and Subramanian (2007) reported dosage mortality response against stages of four lepidopterans insects. Although the shoot borer larva was susceptible to test EPNs, there were differences among these EPNs in their ability to kill the SBL. Among test of EPNs, *Steinernema* sp. (IISR-EPN 02), and *O. gingeri* appears to be the most promising EPN against SBL as it was reflected in the LD_{50}, LT_{50} and % mortality. The mortalities were higher, LD_{50} values were lower and LT_{50} values were shorter for these EPNs. These differences may be due to difference of the origins

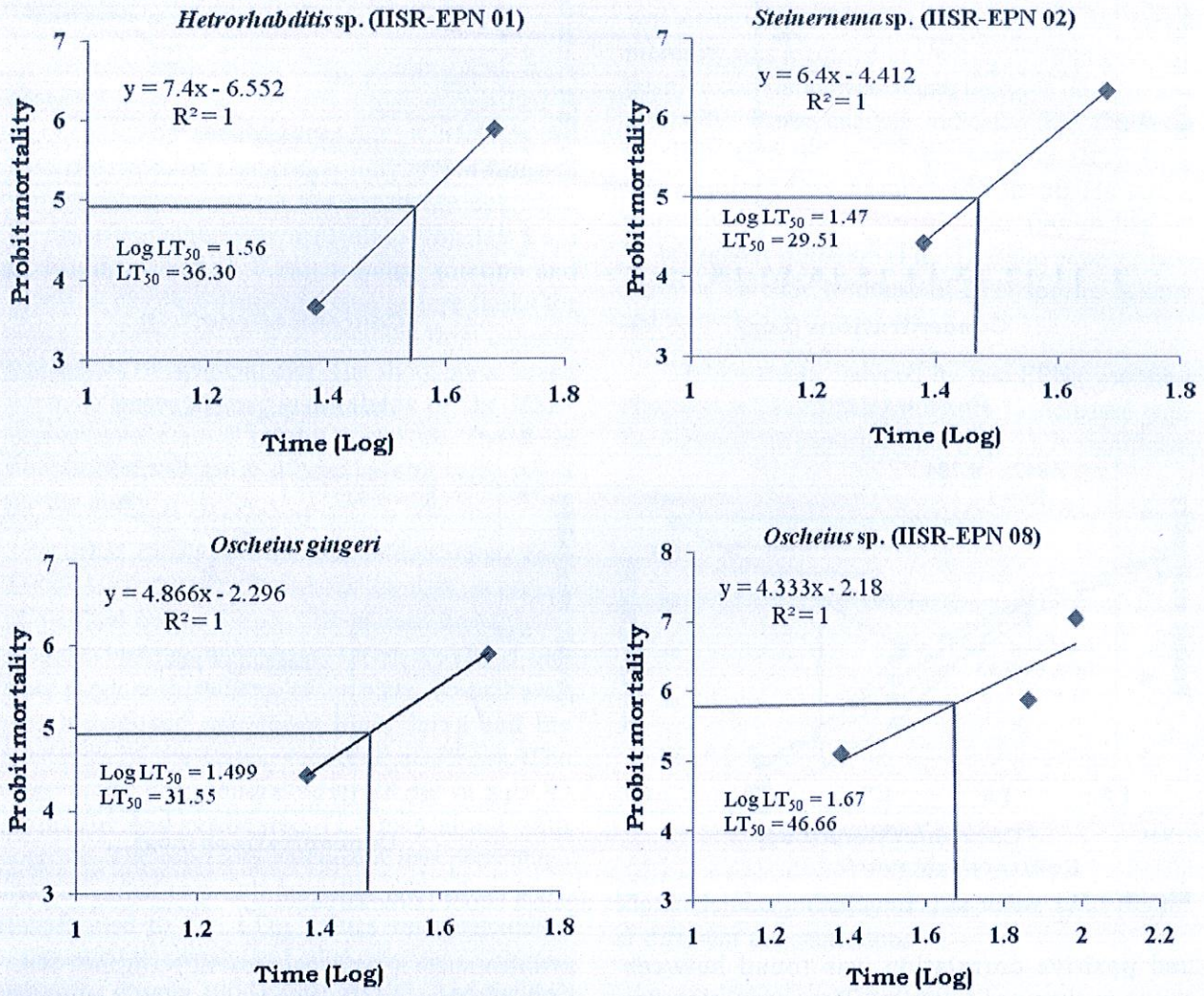

Fig. 4. LT_{50} value of entomopathogenic nematodes against shoot borer larva.

of the strains (Canhilal, 2012). All strains of EPNs tested were effective in controlling *Agrotis ipsilon* and LD_{50} was calculated to be 634 IJs (Seal *et al.*, 2010).

Apparently, they are effective biocontrol agents against shoot borer. However, environmental factors such as temperature, humidity and host density under green house and field conditions have huge impact on the efficacy of EPNs (Pervez & Ali, 2013). Therefore, future studies need to be directed to the green house and field conditions with these EPNs against shoot borer.

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