FFICACY OF TWO ENTOMOPATHOGENIC NEMATODES STRAINS *STEINERNEMA SIAMKAYAI* AND *S. ABBASI* AGAINST THE 3RD INSTAR LARVAE OF *CHILOLOBA ACUTA*

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ABSTRACT

Larvae of scarabaeid beetle Chiloloba acuta (Coleoptera: Cetoninae) was found high densities in Khulekhani VDC, Makawanpur district. These insects are major pest of flower. The efficacy of two species of entomopathogenic nematodes, Steinernema siamkayai (CD1) and S. abbasi (CS1) was tested against third instar of Chiloloba acuta. In a dose response experiments, 0, 125, 250, 500, 1000, 2000 and 4000 infective juveniles (IJs) were inoculated in 50 ml plastic vial containing 40g silt loam soil (45.3%sand, 33.5% silt, 12.2% clay 4.913 organic matter and 5.5pH) and a single C. acuta larva. Mortality of C. acuta exposed to series of increase dose of two nematodes strains was analysed two days intervals upto 14th days after the inoculation by time dose mortality regression .Between these strains, S. abbasi found more effective (LD_{50} 44.9IJs/ml) as compared to S. siamkayai (LD_{50} 98.1IJ/ml) after 14th days. At initial days both strains had high LD_{50} value and it was gradually decreased with increase time.

Key words: Entomopathogenic nematodes, scarabaeid beetle, *Steinernema siamkayai, Steinernema abbasi, Chiloloba acuta*

INTRODUCTION

White grubs are the soil inhabiting root feeding larvae of scarab beetles. They cause significant damage to many agricultural, horticultural and plantation crops, and also to ornamentals, lawns, turfs, pastures and forest trees in different parts of the world (Jackson, 1992; Potter *et al.*, 1992; Koppenhofer and Fuzy, 2003a). *Chiloloba acuta* (Coleoptera: Cetoninae) is one of the major white grubs species found in Makawanpur district. They are called Green rose chafers; larvae move ventrally and dorsoventrally and have well developed hairs on the body. They are major pest of flower.

Due to the unavailability of successful biologic control agents to manage these pests, Nepalese farmers mainly rely on chemical pesticides. However it poses a risk of environmental pollution and is a threat to human health. Application of insecticides to control soil dwelling pests like white grubs is even more harmful to soil health. The most effective and persistent insecticides used to control soil dwelling insects are banned. There is an increased desire for safer and more environmentally sound methods of control.

Entomopathogenic nematodes (EPNs) from families Steinernematidae and Heterorhabditidae are important natural enemies of insects (Kaya, 1990). They are soil organisms, which live in mutualistic relationship with bacteria from the genera Xenorhabdus and Photorhabdus (Burnell and Stock, 2000). Once inside the infected insect, symbiotic bacteria are released from the bodies of infective juveniles (third larval stage of EPNs) to the host hemocoel system. And with the excretion of several toxins they cause its death within 24 to 72 hours (Forst and Clarke, 2002). Entomopathogenic nematodes are an attractive biological control alternative for white grubs, often providing suppression comparable to that achieved with chemical pesticides (Klein, 1993).

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These nematodes may offer an environmentally safe and IPM compatible option for curative white grub control (Grewal *et al.,* 2005). The development of techniques of in-vitro mass production involving solid substrates (Bedding, 1984) and liquid media (Ehlers, 2001), and formulation (Grewal, 2002) led to important progress for the use of these nematodes as biologic control agents. Many qualities make them excellent biocontrol agents: they have a broad host range, possess the ability to search for hosts actively, present no hazard to mammals, and were made exempt from registration and regulation requirements by the US Environmental Protection Agency (EPA) (Gaugler, 1988; Georgis and Manweiler, 1994). At present, they are used commercially against soil-inhabiting pests (Georgis and Manweiler, 1994).

The aims of our research was to study the efficacy of indigenous strain, *S. siamkayai* (CD1), in a comparison to other strain *S. abbasi*(CS1) when controlling third stage-larvae of Green rose chafers *C. acuta* and on the other hand to determine concentration of suspension on the activity of studied biological agents.

MATERIALS AND METHODS

NEMATODES PRODUCTION

A survey of entomopathogenic nematodes (EPN) was conducted for the first time in Nepal during June–December 2007 (Khatri-Chhetri *et. al.*, 2010) and identified. Those identified strains used in this study were as follows.

1. *Steinernema siamkayai* (CD1)
2. *Steinernema abbasi* (CS1)

These two nematodes species were produced in-vivo by using the *Galleria* larvae. *Galleria* larvae killed by nematodes were placed in modified White traps (White, 1927) to allow the emergence of infective juveniles (IJ). Harvested nematodes were stored in incubator at 12-15 °C (Woodring and Kaya, 1988). In all experiments, fresh nematodes(less than two weeks older) were used.

MAINTENANCE OF WHITE GRUB HOST INSECTS

The grubs were collected from damaged maize field from different locations of Makawanpur district during June 2010 *Chiloloba acuta* were mainly collected from field. Naturally infected larvae were discarded and only non infected larvae were used in bioassay experiments.

EXPERIMENT DESIGN AND BIOASSAY PROCESSES

The experiments were carried out at laboratory of Entomology Division, NARC, Khumaltar. Two bioassays were conducted at different dates. 1st bioassay was done at 8 July 2010. In 1st bioassay *Steinernema siamkayai* was used for 350 white grub larvae. Seven treatments were used in this bioassay (125IJs, 250IJs, 500IJs, 1000IJs, 2000IJs, 4000IJs and 1 control used dichloranated water (0IJs) per larvae of white grub). In each treatment 50 white grub larvae were used.

Second bioassay was conducted at 9 July 2010. In this bioassay *Steinernema abbasi* was used for 350 white grub larvae. Seven treatments were used in this bioassay as mentioned as bioassay 1st. In each treatment 50 white grub larvae were used. The bioassay was carried out using EPNs in a complete randomized design (CRD).

BIOASSAY

Separate bioassays were conducted for each nematodes species. Two bioassays were conducted at room temperature (23.96 °C to 29.75 °C) in aerated plastic vials filled with 40 g sterilized soil. Initially, soil contained the moisture 21 g by volume. Soil was found a silt loam (45.3% sand, 42.5% silt, 12.2% clay, 4.913% organic matter and 5.5pH) for 1st and 2nd bioassays. Individual larvae that had been maintained for bioassay were released into the aerated plastic vials. Larvae that did not

enter into the soil with in half hour were replaced. Treatments were prepared and applied in each vials with required number of nematodes in 1ml dechlorinated tap water. Control treatment received the same amount of dechlorinated tap water only. To maintain the moisture in nematodes treated vials, two ml of dechlorinated tap water added in each vials. Potato slice also added in vials for food. Larval mortality was monitored at 2 days interval after treatments up to 14 days for survival. Dead larvae were removed from the experiment and dissected two days after death for checking nematode infections using the microscope at 10x magnitude. Individuals were recorded during each evaluation as "survived", "death due to EPN infection", "death due to other reasons". For surviving larvae a potato slice (about 1 g) was provided as food after each observation.

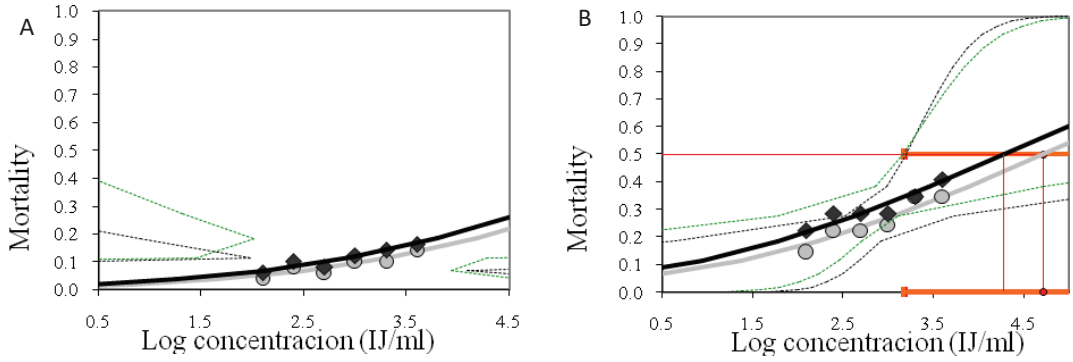
STATISTICAL ANALYSIS

The data were further analyzed to assess the dose-time mortality curves for each EPN strain tested. First, survival data from each evaluation date were submitted to Probit analysis according to Finney (1977) to determine the dose-mortality relationship for each date individually. LD_{50}-values and the slope of the regression were determined for each date individually with their corresponding standard errors and confidence bands. Parallelism was evaluated using a Likelihood Chi^2-test (G-test).For calculating EPN-caused mortality observed mortalities were adjusted using Abbott's formula (Abbott, 1925). Established functions were used to establish an overall dose-time-mortality model that predict LT_{50} –values for a given EPN dose.

RESULTS

VIRULENCE OF THE TWO STRAINS OF EPNS AGAINST *Chiloloba acuta*

Regression based on the Probit model resulted always P-values above 0.97 (according to Chi^2) indicating high acceptability of model fit over all evaluation dates (Table 1). Individual regression lines for each evaluation interval are presented in Figure 1(A-G).



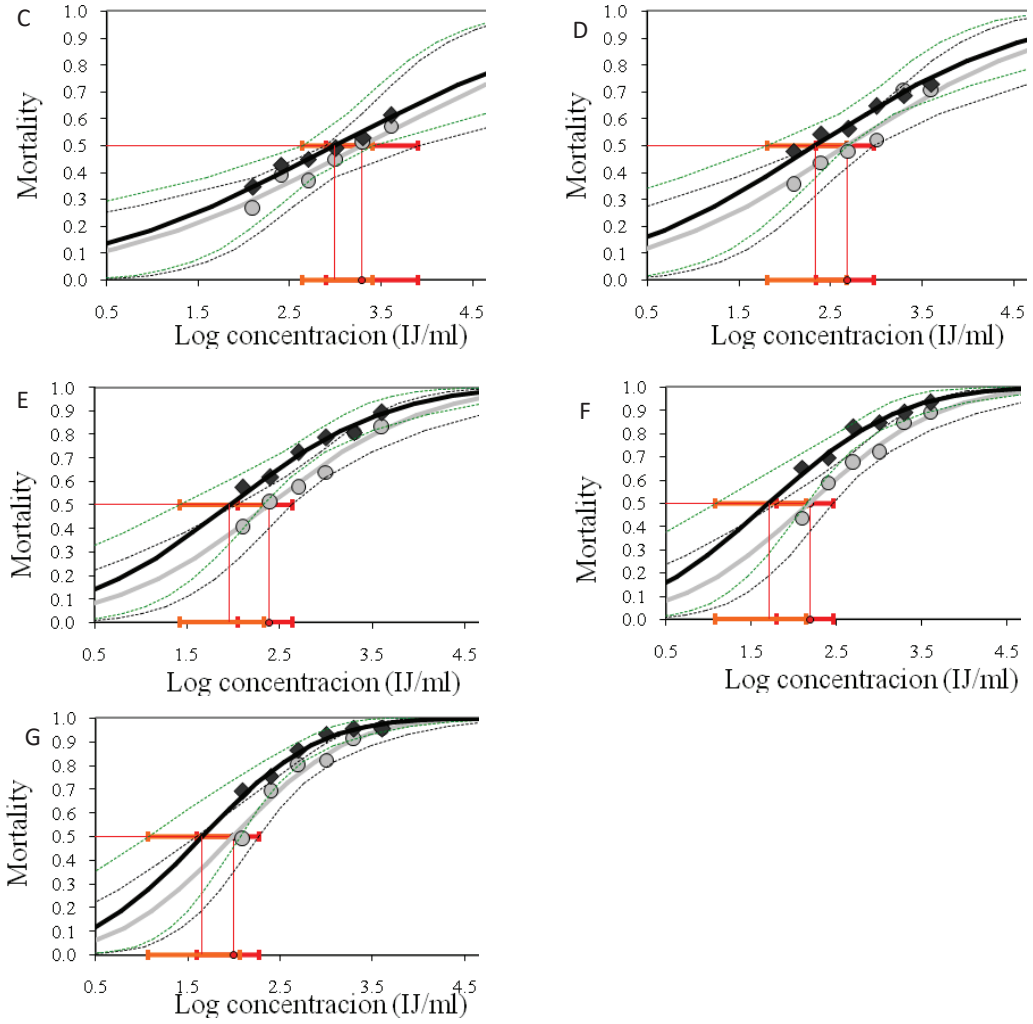


Figure 1. Comparing the regression line between the *S. siamkayai* and *S. abbasi* treated third instar larvae of white grub (*Chiloloba acuta*) showing the LD_{50} values at 2 (A), 4 (B), 6 (C), 8 (D), 10 (E), 12 (F) and 14 (G) days after inoculation.

Filled diamonds: observed mortality in *S. abbasi* Black line: resulting probit model for *S. abbasi*, filled circle: observed mortality in *S. siamkayai*, gray line: probit mod for *S. siamkayai*, scatter lines indicate 95% confidence bands for the probit models; bars: confidence band (95%) for LD_{50}-values.

Table 1. Details of the probit statistics resulting from 1st experiment.

DAI	Strain	Intercept	Slope	Heterogeneity		Parallelisms		LD_{50}	CL95		Potency	CL95	
				chi^2	P	chi^2	P		Lower limit	Upper limit		Lower limit	Upper limit
2	*S.s.*	-2.38	0.35	0.93	0.99	0.01	0.92	$5.28x10^{6}$	$7.68x10^{-4}$	$2.79x10^{53}$	1		

		(±0.115)											
	S. a.	-2.24 (±0.105)						$2.16x10^{6}$	$6.05x10^{-1}$	$1.31x10^{46}$	2.44	0.18	∞
4	*S.s.*	-1.69 (±0.082)	0.35	1.35	0.99	0.31	0.58	$5.19x10^{4}$	$1.56x10^{3}$	$2.86x10^{8}$	1		
	S. a.	-1.53 (±0.079)						$1.92x10^{4}$	$1.46x10^{3}$	$1.28x10^{7}$	2.70	0.62	37.3
6	*S.s.*	-1.46 (±0.075)	0.44	1.03	0.99	0.16	0.68	$1.92x10^{3}$	$7.76x10^{2}$	$7.93x10^{3}$	1		
	S. a.	-1.33 (±0.075)						$9.70x10^{2}$	$4.33x10^{2}$	$2.54x10^{3}$	1.98	0.66	8.37
8	*S.s.*	-1.46 (±0.076)	0.54	1.54	0.992	0.90	0.35	$4.79x10^{2}$	$2.16x10^{2}$	$9.27x10^{2}$	1		
	S. a.	-1.27 (±0.077)						$2.15x10^{2}$	$6.43x10^{1}$	$4.80x10^{2}$	2.23	0.90	7.13
10	*S.s.*	-1.78 (±0.080)	0.75	1.51	0.993	0.37	0.541	$2.40x10^{2}$	$1.10x10^{2}$	$4.27x10^{2}$	1		
	S. a.	-1.47 (±0.084)						$9.08x10^{2}$	$2.66x10^{1}$	$2.12x10^{2}$	2.64	1.31	6.30
12	*S.s.*	-1.816 (±0.084)	0.83	1.14	0.997	0.37	0.545	$1.54x10^{2}$	$6.28x10^{1}$	$2.88x10^{2}$	1		
	S. a.	-1.421 (±0.091)						$5.14x10^{1}$	$1.20x10^{1}$	$1.41x10^{2}$	2.98	1.51	7.05
14	*S.s.*	-2.028 (±0.092)	1.01	2.22	0.974	0.21	0.643	$9.81x10^{1}$	$3.91x10^{1}$	$1.86x10^{2}$	1		
	S. a.	-1.682 (±0.102)						$4.49x10^{1}$	$1.18x10^{1}$	$1.16x10^{2}$	2.18	1.187	4.45

DAI Days after treatment, *S. a. Steinernema abbasi*, S. s. *Steinernema siamkayai*

Between the two nematode species tested against the third instar of white grub (C. acuta), S. abbasi was more effective (LD50, 44.9 IJs/ml/40g soil) as compared to S. siamkayai (LD50, 98.1 IJs/ml/40g soil) after 14 days of inoculation. LD50 values obtained from each observations of both nematodes strain clearly indicated S. abbasi had low LD50 value than S. siamkayai. At initial days, both strains had high LD50 value and gradually decreased with increased time.

NATURAL MORTALITY

Natural mortality within the experiment increased from 2% at 2 DAT to 10% at 14 DAI. The estimated for natural mortalities from the Probit model resulted quite similar from 1.99% (±0.019) for 2 DAI and 9.88% (±0.038) for 14 DAI. The increase in natural mortality over time could be well described by a linear regression model (R^2 = 0.971) with a daily increase rate of 0.62% (SE 0.044%) which is significantly different from zero (F=201, df = 6, p <0.0001) (Figure 2).

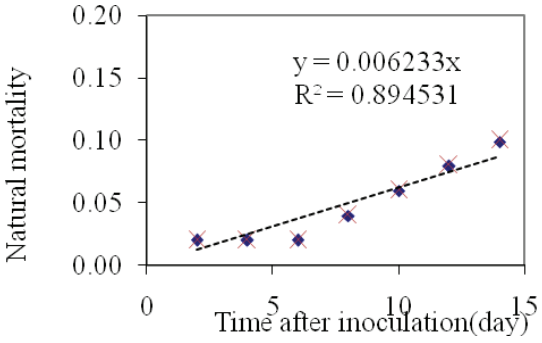


Figure 2. .Control mortality in *Chilolobaacuta* observed during experiment

Crosses indicate observed mortality, blue symbols indicate the estimated mortality by the probit model, and line represents the linear model fitted to the data.

Probit regression lines for each evaluation date were fitted in a parallel assay. The slope of regression lines for the two strains were not significantly different between the two stains of nematodes (*S. siamkayai and S. abbasi*) throughout the evaluation period (see chi^2 values for parallelism in Table 1) Common slopes obtained for each evaluation was increased but it was lower than 0.5 at 2, 4 and 6 DAI and higher than 0.5 at 8, 10, 12 and 14 DAI. Slope obtained at 14 days after treatment was higher than 1, which indicates that the variability in insect response to the nematodes reduces with incubation time. The increase in slope over time could be well described by an exponential model; i.e. $y = a \times \exp(b \times x)$, where "y" is the Slope and "x" is the incubation period in days after treatment, and "a" and "b" are fitted parameters. The model explained 97.3% of the variation in slopes by DAI (Figure 3).

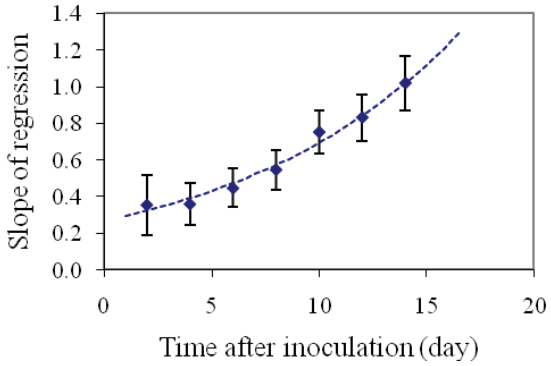


Figure 3. Common slopes of the probit regression lines for the two nematodes (*S. siamkayai* and *S. abbasi*) against *C. acuta* at different days after inoculation.

Blue symbols indicate observed slope, Bar: confidence bands for the slope scattered line: linear model fitted to the data.

Between the two nematode species/strains tested against the third instar of white grub (*Chilolobaacuta*), *S. abbasi* found more effective (LD_{50}, 44.9 IJs/ml/40g soil) as compared to *S. siamkayai* (LD_{50}, 98.1IJs/ml/40g soil) after 14 days of treatment. LD_{50} values obtained from each observations of both nematodes strain clearly indicated *S. abbasi* had low LD_{50} value than *S. siamkayai*. At initial days both strains had high LD_{50} value and it was gradually decreased with increased time (Table 1).

DIFFERENCES IN BIOLOGICAL ACTIVITY BETWEEN STRAINS

Relative potencies of LD_{50}-values with their 95% confidence limits were used to compare the virulence between *S. siamkayai* and *S. abbasi* of nematode strains using *S. siamkayai* as reference (activity = 1). Relative potencies, which were in the range between 1.981 and 2.989 values (Table 1), did not change for different evaluation dates. This was verified by linear regression,where the regression coefficient resulted not significantly different from zero ($F = 0.047$, $df = 5$, $P = 0.83$). Due to the low activities of pathogens at the beginning of the evaluation period obtained potencies were more variable than at the end of the experiment and accordingly confidence interval for the relative potencies resulted much wider than at the end of the experiment. Therefore, the relative potency for *S. abbasi* resulted not significantly different compared to *S. siamkayai* after 2, 4, 6 and 8 days of incubation; however, from 10 to 14 days the potency became significantly different; i.e. higher, due to more precise estimates of the LD_{50}-values (Table 1).

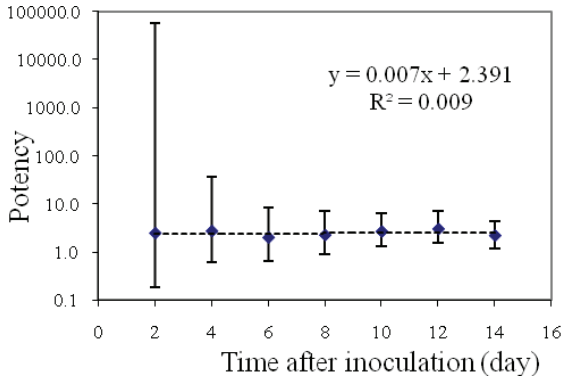


Figure 4. Relative potencies obtained for comparing *S.siamkai* against *S. abbasi* throughout the course of experiment. Symbols: calculated potencies obtained form probit analysis. Bar: confidence bands for the relative potencies Scattered line: linear model fitted to the data.

DISCUSSION

White grub need to be controlled because of their negative impact on agricultural crops and ornamental plants. The present results as well as those of Gharty Chhetry (2006) indicate the important role of biological control agents. Insect pathogenic fungi (*M. anisoplia* and *B. bassiana*) and insect pathogenic nematodes Steinernematidae and Heterorhabditidae are the major microbial pesticides to control the white grub. Entomopathogenic nematodes are the most extensively studied parasites of white grub (Klein, 1993; Georgis *et al.*, 2006). So, different nematode strains *S. siamkayai* and *S. abbasi* were assessed against third instar white grub (*C. acuta*) to determine the biological activity of these pathogens. These nematodes were first recovered and described from Asia: *S. abbsi* from Oman (Elawad *et al.,* 1997) and *S. siamkayai* from Thailand (Stock *et al.,* 1998). In Nepal these nematodes were isolated and identified first time from inner terai region (silt loam soil) 160 and 145 masl respectively (Khatri-Chhetri, 2010).

Our study established the mortality of *C. acuta* treated with entomopathogenic nematodes *S. siamkayai* and *S. abbasi* at different days with the various concentration levels. Mortality of *C. acuta* treated with *S. siamkayai* ranges from 26.5-%57% and 49 -95% at 6 and 14 days after treatment treated with 125 and 4000 IJs/grub/40gm soil respectively. In case of *S. abbasi* treated *C. acuta* mortality became higher ranged from 34.7-61.2% and 68.9-95% at same dose and days. Several researchers demonstrated that most effective strains which controls white grub were *H. bacteriophora* GPS11 (83-96%), *H. zealandica* X`1 (96-98%) and *S. scarabaei* (100%)(Cappaert and Koppenhofer, 2003; Koppenhofer and Fuzy, 2003; Grewal *et al.*, 2004). This study shows that the entomopathogenic nematode *S. siamkayai* and *S. abbasi* can provide effective curative control of third instar white grub *C. acuta*.

The dose response bioassay has been used many times previously (Morris *et al.*, 1990; Mannion and Jansson, 1992) and probit analysis has been used to analyze the data to calculate LD_{50} values. In the assays conducted in 50 ml plastic vial, nematodes and insects were kept in close contact and the influence of foraging strategies was limited. High mortality was received by treating the nematodes strains.

The efficacy of entomopathogenic nematodes depend upon various factor. Soil texture, soil pH, soil moisture are the major factor. Soil used in bioassay is silt loam having the pH 6.4 which is favorable

for nematodes. Observations of other studies suggest that *S. scarabaei*, *H. bacteriophora*, and *H. zealandica* becomes less effective in acidic soils than in more pH-neutral soils Koppenhofer and Fuzy (2006).Grewal *et al.*, (2004) reported that optimum soil moisture has vital role in nematodes movement and efficacy, the grub control provided by the two strains always exceeded 80% (83–97% for H.b.-GPS11 and 96–98% for H.z.-X1 strain). Entomopathogenic nematodes require moisture film to prevent desiccation and in which to move, they are more typically used in soil environments. Interestingly, our results also indicate that optimum soil condition may have positive effect on nematode efficacy.

Luan *et al.*, (1996) computed the LD_{50} values as 417 IJs of *Steinernema glaseri* NC 34 for the second instar larvae of *Holotrichia parallela*. In our study, a positive correlation between the inoculation dose and the host mortality was observed in the case of all the test nematode species/strains evaluated with the soil inoculation method. Between the two nematode species/strains tested against the third instar of white grub (*C. acuta*), *S. abbasi* was more effective (LD_{50}, 44.9 IJs/ml/40g soil) as compared to *S. siamkayai* (LD_{50}, 98.1 IJs/ml/40g soil) after 14 days of inoculation. LD_{50} values obtained from each observations of both nematodes strain clearly indicated *S. abbasi* had low LD_{50} value than *S. siamkayai*. At initial days, both strains had high LD_{50} value and gradually decreased with increased time.

CONCLUSION

In bioassay experiment, mortality of *C. acuta* exposed to series of increase dose of two nematodes strains was analyzed at two days intervals up to 14 days after the treatment by time- dose mortality regression. Between the two nematode species/strains tested against the third instar of white grub (*C. acuta*), *S. abbasi* was more effective (LD_{50}, 44.9 IJs/ml/40g soil) as compared to *S. siamkayai* (LD_{50}, 98.1 IJs/ml/40g soil) after 14 days of inoculation. LD_{50} values obtained from each observations of both nematodes strain clearly indicated *S. abbasi* had low LD_{50} value than *S. siamkayai*. At initial days, both strains had high LD_{50} value and gradually decreased with increased time. It was found that both entomopathogenic nematodes were effective in lab conditions so it is suggested to test them in field for the management of white grub.

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