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Biocontrol potential of entomopathogenic nematodes against greater wax moth *Galleria mellonella*

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Abstract
The present investigation was carried out on "Biocontrol Potential of Entomopathogenic Nematodes Against Greater Wax Moth *Galleria Mellonella",* under Post Graduate Laboratory, Entomology Section, College of Agriculture, Nagpur, during 2020-2021, with the aim to evaluate the effectiveness, pathogenicity and multiplication potential of Entomopathogenic nematodes (EPN) against *Galleria mellonella*. *Heterorhabditis indica* (CICR-Guava), *Steinernema bicornatum* (CICR-W) and *Steinernema siamkayai* (SAM 4) EPN strains were used in experiment for the study of biocontrol potential. The results of this investigation explored that, *Heterorhabditis indica* (CICR-Guava) was most pathogenic than other EPNs.

Keywords: biocontrol, entomopathogenic nematode, *Heterorhabditis indica, Steinernema bicornatum, Steinernema siamkayai,* LC_{50}

Introduction
Losses due to insect pests in Indian agriculture have been estimated from time to time, extensive surveys carried out during 2015 reveal that cotton crop suffers the maximum losses 30%, followed by rice 25%, sugar cane and rapeseed mustard each 20%, maize 18%, groundnut and pulses 15%, other oilseeds 12%, coarse cereals 8% and wheat 5%. In all, the Indian agriculture suffers annual losses to the tune of about US $ 36 billion due to the ravages of insect pest in field. India is an agricultural country and about seventy five per cent population is based on Agriculture. An all out efforts are made to bring these colossal losses to the minimum so that the access to food is increased for the expanding population. Since few decades chemical origin insecticides are used for controlling the losses caused by the insect pests. At early stage use of pesticides in agriculture resulted in fast controlling of pests in low cost and therefore increase in production, this resulted in favourism towards use of pesticides rather than traditional pest control methods by the farmers. Due to the greedy tendency of human beings for more and more production they started more and more use of pesticides due to this high intensity production system, Not so early but after some period of time now we are enforced to see the disappointing negative impacts of chemical pesticides on nature i.e residues in soil, water, air, food chain, food web, now in human beings also. Major problem faced by the agriculturists now is the pest resistance, pest resurgence, secondary pest outbreaks, destruction of biodiversity which includes natural enemies and other helpful living organisms in the farm vicinity etc, these problems are results due to the improper use of chemical pesticides. Therefore rigorous and continued efforts are being made to identify alternate methods, which are economical and environmentally safe. Biological control is one such alternative which is good pest control option without any harm to human beings and natural enemies. Entomopathogens as biological control agents are giving very good results and therefore this work reports the best performing EPN amongst some most used EPNs viz. *Heterorhabditis indica* (CICR-Guava), *Steinernema bicornatum* (CICR-W) and *Steinernema siamkayai* (SAM 4).

Material and Methods
- Infective juveniles (IJs) of the three isolates were taken into separate beakers.
- Serial dilutions as per the treatments were prepared in separate beakers.
- The count of the infective juveniles was taken for 100 µl and the count was repeated five times.

- Each treatment was replicated thrice.
- The known IJs were placed in the petri dish lid with the moistened filter paper and in each treatment and replication five larvae of *Galleria mellonella* were taken per petri dish and twenty larvae were used in each replication.
- After treatment, the petri dishes were sealed and kept in captivity.
- After 24 hours the observations for larval mortality in each replication and treatment were recorded up to 72 hours.
- Larval mortality data were recorded and larvae were placed on white trap in a petri dish where water was added.
- Then these petri plates and white traps were observed under stereozoom binocular microscope for nematode emergence.
- The nematodes emerged in the water of petri dish were taken out in a beaker.
- The suspension taken out was subjected to check for nematode population count, which was taken by observing 100 □l suspension under stereozoom binocular microscope for number of IJs in the droplet.
- Total count of nematode suspension taken out from petri plate was noted and total population count was calculated.
- These observations for population count were taken for all treatments and replications separately.
- In the control larvae were treated with plain distilled water.

Larval mortality was calculated by using the following formula,

$$\text{Larval mortality (\%)} = \frac{\text{Number of larvae died}}{\text{Total number of larvae}} \times 100$$

Larval Bioassay Studies for Determination of lethal concentration of EPN isolates

Based on mortality rates obtained in first experiment, the virulent strains were selected to determine their lethal concentrations (LC_{50}). The larval mortality bioassay was carried out in petri dishes of measured size with double layer Whatman No.1 filter paper, following the methods of Kaya and Stock (1997) [8]. 6 to 7 concentrations (measured as IJs /larva) of EPN isolate cultures in 0.5 ml of sterile distilled water were added to filter paper in separate petri dishes. After 30 minutes a single 3rd instar fully fed larva of *Galleria mellonella* were placed in each of Petri dish. Larval mortality was checked at every 24 h up to 120 h period. The cause of larval death was confirmed by body color change of cadaver which being evident due to presence of symbiotic bacteria.

LC_{50}

The LC_{50} values were calculated as per Finney (1971) [4] using Probit EPA software available in Section after computation of corrected percentage mortalities as per Abbott (1925) [1].

Reproduction of EPNs on *Galleria mellonella*

In this experiment the larva of *Galleria mellonella* were exposed to 10, 20, 30, 40, 60, 80 and 100 IJs/larvae concentration of each EPN isolates (Yadav and Lalramliana, 2012) [20] in separate rearing tray. Total number of IJs produced/larva up to a period of 20 days were counted and collected daily till the emergence of IJs stopped from insect cadavers.

Treatment details

Sr. No	Treatments	Dose
1	T1	10 IJs/100 µl
2	T2	20 IJs/100 µl
3	T3	30 IJs/100 µl
4	T4	40 IJs/100 µl
5	T5	60 IJs/100 µl
6	T6	80 IJs/100 µl
7	T7	100 IJs/100 µl
8	T8	Control (distilled water)

Statistical analysis

The data, thus, obtained were statistically analysed by using one factor analysis with the help of online software (OPSTAT) available on Hissar Agricultural University, Hissar.

Results and Discussion

The investigation of pathogenicity and LC_{50} of locally isolated three EPNs *Steinernema bicornatum, Heterorhabditis indica* and *Steinernema siamkayai* by using *Galleria mellonella* as laboratory host under the research entitled as "Biocontrol potential of entomopathogenic nematodes against greater wax moth, *Galleria mellonella"* recorded following discussions with corresponding literatures available as under.

Pathogenicity of EPN isolate *Heterorhabditis indica* (CICR-Guava) against *Galleria mellonella*

Data depicted in Table no. 1 states that, After 24h exposure of galleria larvae to EPN at the dose of 100Ijs/100µl showed maximum mortality i.e, 36.67% which is significantly superior over other doses and also found at par with 80Ijs/100µl with 35.00% mortality. Next effective treatments 60IJs/100µl, 40IJs/100µl and 30IJs/100 µl recorded 36.67%, 25.00% and 23.33% mortality and these were at par with each other. Further least mortality was seen of 18.33% and 15.00% at 20 IJs/100µl and 10 IJs/100µl doses and both were at par with each other. After 48h of exposure to EPN to *Galleria* larvae at 100Ijs/100µl dose expressed maximum mortality (73.33%), this was significantly higher than others and at par with 80Ijs/100µl, 60Ijs/100µl resulting 68.33% and 65% mortality. From 40IJs/100µl, 30IJs/100µl and 20IJs/100µl; 61.67%, 56.67% and 51.67% mortality were noted and these were at par to each other. Comparing to others lower mortality of 41.67% was recorded at dose of 10IJs/100µl.

After 72h of exposure of *G.mellonella* to EPNs, dose of 40IJs/100 µl, 60IJs/100 µl, 80IJs/100 µl and 100IJs/100µl resulted highest mortality i.e, 100%, and were found significantly superior to 30IJs/100 µl, 20IJs/100 µl and 10IJs/100 µl which recorded 95%, 85% and 81.67% mortality of *G. mellonella* at 4th instar stage. Amongst these 20 IJs/100 µl and 10IJs/100 µl doses of the treatment were at par with each other.

Pathogenicity of EPN isolate *Steinernema bicornatum* (CICR-W) against *Galleria mellonella*

The data presented in Table 2 illustrated that as the concentration of dose and exposure time of *S.bicornatum* over 4th instar larval stage of *G.mellonella* was increased, larval mortality of *G.mellonella* also increased.After 24h of inoculation of infective juveniles treatment of 100 IJs/100µl

obtained highest mortality i.e, 31.67% and found at par with 80 IJs/100µl showed 26.67% mortality. 60 IJs/100µl, 40 IJs/100µl and 30 IJs/100µl treatment recorded 25%, 23.33% and 20% mortality were in next order of merit and are at par with each other. And these were followed by the treatments 20 IJs/100µl and 10 IJs/100µl which recorded 18.33% and 13.33% mortality. After 48h of inoculation of EPNs treatment 100 IJs/100µl obtained highest mortality (71.67%) and was at par with 80 IJs/100µl which expressed 65% mortality. Next effective treatment was 60 IJs/100µl recorded 60% mortality and was found at par with 40 IJs/100µl (56.67%) and 30 IJs/100µl 53.33% mortality. These treatments were followed by 20IJs/100µl and 10 IJs/100µl showed 45% and 41.67% mortality and were at par with each other. After 72h exposure of 100 IJs/100µl and 80IJs/100µl to *G.mellonella* larvae it showed 100% mortality. Next superior treatments were 60 IJs/100µl and 40 IJs/100µl which exhibited 96.67% and 91.67% mortality and were at par with each other. Next effective treatment 30 IJs/100µl recorded 88.33% and mortality was at par with 20 IJs/100µl and 10IJs/100µl treatments and achieved 85%, 80% mortality.

Pathogenicity of EPN isolate *Steinernema siamkayai* (SAM 4) against *Galleria mellonella*

Results present in the Table 3 revealed that as the time of exposure and concentration of inoculation was increased against 4th instar larvae of *G.mellonella,* mortality of larvae also increased gradually. After 24h of inoculation of EPNs, at 100IJs/100µl the highest mortality of 30% was recorded and was significantly higher and at par with 80 IJs/100µl, 60 IJs/100µl, and 40 IJs/100µl with 26.67%, 23.33% and 21.67% mortality respectively. Treatment 30 IJs/100µl achieved comparatively lower mortality 18.33% which was at par with 20 IJs/100µl and 10 IJs/100µl exhibiting 15% and 10% mortality. After 48h of exposure larvae of *Galleria* at 100IJs/100µl achieved highest mortality (66.67%) which was significantly superior over control and at par with 80 IJs/100µl and 60 IJs/100µl treatments exhibiting 61.67% and 56.67% mortality. Next effective treatments were 40 IJs/100µl, 30IJs/100µl and 20IJs/100µl observed 53.33% 51.67% and 43.33% mortality and they were at par with each other. The lowest mortality (14%) was observed at 10IJs/100µl.After 72h of exposure, 100 IJs/100µl achieved 100% mortality of *Galleria mellonella* larvae and was at par with 80 IJs/100µl with 98.33% mortality. In next order of merit treatments 60 IJs/100µl, 40 IJs/100µl, 30 IJs/100µl and 20 IJs/100µl recorded 95%, 91.67%, 90%, and 88.33% mortality respectively and which were at par to each other. The lowest mortality was observed of 80% at 10 IJs/100µl.

Reproductive potential of EPN Isolates

Observation data depicted in Table no. 4 illustrate the number of infective juveniles of respective strains emerged from cadavers of *Galleria mellonella*. In CICR-G population emergence was highest of 2708.1×10^2 IJs/larva at 100 IJs/100µl and then in descending order it goes like 2603.5×10^2 IJs/larva, 2487.7×10^2 IJs/larva, 2366.8×10^2 IJs/larva, 2209.3×10^2 IJs/larva, 2045.9×10^2 IJs/larva, 1886.5×10^2 IJs/larva which were gained at concentrations of 80 IJs/100µl, 60 IJs/100µl, 40 IJs/100µl, 30 IJs/100µl, 20 IJs/100µl and 10 IJs/100µl respectively. In CICR-W maximum number of infective juveniles 2082.6×10^2 IJs/larva was obtained at 100IJs/100µl followed by 1975.8×10^2 IJs/larva, 1883.4×10^2IJs/larva, 1792.5×10^2IJs/larva, 1582.6×10^2 IJs/larva, 1355.1×10^2IJs/larva, 1213.8×10^2IJs/larva obtained at concentrations of 80 IJs/100µl, 60 IJs/100µl, 40 IJs/100µl, 30 IJs/100µl, 20 IJs/100µl and 10 IJs/100µl respectively. SAM 4 maximum population of infective juveniles was 2384.7×10^2IJs/larva obtained at 100IJs/100µl followed by 2266.6×10^2IJs/larva, 2143.6×10^2IJs/larva, 2051.2×10^2IJs/larva, 1928.2×10^2IJs/larva, 1822.9×10^2IJs/larva, 1714.8×10^2IJs/larva at inoculum level of 80 IJs/100µl, 60IJs/100µl, 40 IJs/100µl, 30 IJs/100µl, 20 IJs/100µl and 10 IJs/100µl respectively.

Determination of LC_{50} of EPNs against *Galleria mellonella*

The data obtained on percent mortality from experiment were used as input data for probit analysis to calculate LC_{50}. The online software of Hissar Agricultural University, Hissar was used for probit analysis. Concentrations of infective juveniles were considered as log dose concentration at X axis and percent mortality was marked on Y axis for computing LC_{50} value. Calculation of LC_{50} probit analysis by following the procedure of Finney (1952) was conducted. Since galleria larvae showed 100% mortality after 72h LC_{50} was calculated for 48h post inoculation period. Results in Fig 1, Fig 2 and Fig 3 illustrated that, median lethal concentration (LC_{50}) of *Heterorhabditis indica* (CICR-Guava) for 50 percent mortality (LC_{50} values) of *G.mellonella* larvae was 18.391 IJs/100µl. Concerning *Steinernema bicornatum (*CICR-W), LC_{50} value for 50% mortality of larvae on 48h of inoculation was 24.904 IJs/100µl. Median lethal concentration (LC_{50}) of *Steinernema siamkayai* (SAM 4) for 50% mortality of larval population against *G. mellonella* was recorded as 30.413 IJs/100µl. Therefore, here we can clearly observe that Strain *Heterorhabditis indica* (CICR-Guava) showed low LC_{50} doses for all instars, this represents higher pathogenicity of CICR-Guava over *Steinernema* spp.

Pathogenicity of EPN isolates against *Galleria mellonella*

All three isolates were pathogenic to *G. mellonella*. Time period required for mortality of larvae and multiplication of IJs of EPN varied with the strains. Results presented in this chapter revealed that there was direct and linear relationship between dosage of infective juveniles of EPNs and mortality percent of *G. mellonella* by three nematode isolates viz., *Heterorhabditis indica* (CICR-Guava), Steinernema bicornatum (CICR-W) and Steinernema siamkayai (SAM 4). The effectiveness of nematodes increased with increase in dose, which was confirmed by the findings of Istkhar *et al*., (2015) [5] who reported that insect mortality was high and low at higher and lower nematode concentrations respectively. This research resulted that, larval mortality percent of *G. mellonella* increased with rise in dose of IJs and exposure time. *H. indica* caused 95% and 100% mortality at 30 and 100IJs dose, respectively after 72hrs of inoculation and it was approximate to the results of Istkhar *et al*. (2015) [5] who reported 100% mortality at 25, and 100IJs doses respectively, after 60h of inoculation. In the present study *Heterorhabditis indica* caused 100% mortality at minimum dose of 40IJs/larva, whereas *Steinernema bicornatum* caused 100% mortality at minimum dose of 80ijs/100µl after 72h of inoculation, which was in line with the findings of Istkhar *et al*., (2015) [5] who reported that *Heterorhabditis indica* caused significantly higher mortality of *G. mellonella* at lower IJs level than Steinernema carpocapsae. There he concluded that *Steinernema* spp. recorded 100% mortality at 72 h in 100IJ/larva and that in *Heterorhabditis spp*. was at 48h after

inoculation. Similarly S.Prabhu *et al*. (2008) [14] reported 80% and 42% of mortality in filter paper assay within 24 h by *H. indica* and *Steinernema sp*. respectively. Therefore, mortality was directly proportional to EPN exposure time and dose concentration. The present study revealed that, *Heterorhabditis indica* was superior over *Steinernema spp* in pathogenicity against *G. mellonella larvae*. The variability in nematode virulence observed might be due to difference in the host preference by nematode species.

LC_{50} of EPN isolates against *G. mellonella*

The present study revealed that all the three strains of EPN were found to be effective and could cause 50 per cent mortality below 30 IJs/larva under laboratory conditions. Amongst all the isolates of EPNs, *H. indica*: (CICR-Guava) was highly effective and has lower LC_{50} value against *G. mellonella*, followed by isolate *S. bicornatum* (CICR-W) and *S. siamkayai*: (SAM 4), which was in line with Istkhar *et al*, (2015) [5], who calculated LC_{50} values in number of IJ/larva at three different time intervals viz., 24, 48 and 72 h, through probit analysis which decreased with time increment. He concluded that *Heterorhabditis spp* registered lowest LC_{50} of 95.19 and119.22 IJs/larva and *Steinernema spp.* recorded highest LC_{50} of 211.89 and 357.51 IJs/larva for 24h post inoculation period. Similar findings were made by Banu *et al*. (2007) [2] where LC_{50} value for 3rd instar larva were 2.43 IJ/larva and 3.69 IJ/larva for *S. glaseri* and *H. indica* respectively. Pupa recorded the highest LC_{50} value of 91.46 and 84.67 IJ/pupa for *Steinernema glaseri* and *Heterorhabditis indica* respectively. Similar findings were made by Radhakrishnan and Shanmugam (2017), who reported that, *H. indica* registered lowest LC_{50} of 6.81 IJs/larva and *S. glaseri* recorded highest LC_{50} 8.45IJs/larva.

Reproductive potential of EPN isolates on *Galleria mellonella*

A precise knowledge about reproduction and recovery of the EPN is considered important in determining the time and dose of subsequent application in field. Reproduction and recycling of EPN strain in host insect plays a key role in their persistence in soil, infectivity and overall effectiveness in pest control. The observation data on reproduction suggested that in the following treatment inoculation, all the tested EPN isolates were able to infect and propagate within the body of the host insect viz., *Galleria mellonella*. The highest recovery of 2,70,810IJs was recorded at the concentration of 100IJs/100µl in case of *Heterorhabditis indica* and followed by 2,38,470IJs and 2,08,260IJs emergence from *Steinernema siamkayai* and *Steinernema bicornatum* respectively, here both the *Steinernema spp.* exhibited less reproductive potential than *Heterorhabditis spp.*, which was in line with Ali *et al*. (2018) who concluded that there was greater emergence of IJ from the Heterorhabditids species than those from the Sterinernamaditis species.

Table 1: Pathogenicity of EPN isolate *Heterorhabditis indica* (CICR-Guava) against *Galleria mellonella*.

Sr. No.	Treatment Concentration	Larval Mortality of *G. mellonella* larvae		
		24 Hrs	48 Hrs	72 Hrs
1	10 IJs/100µl	15.00 (22.777)	41.67 (40.182)	81.67 (64.668)
2	20 IJs/100µl	18.33 (25.295)	51.67 (45.948)	85.00 (67.377)
3	30 IJs/100µl	23.33 (28.843)	56.67 (48.816)	95.00 (79.528)
4	40 IJs/100µl	25.00 (29.913)	61.67 (51.902)	100.00 (90)
5	60 IJs/100µl	26.67 (31.058)	65.00 (53.74)	100.00 (90)
6	80 IJs/100µl	35.00 (36.223)	68.33 (55.83)	100.00 (90)
7	100 IJs/100µl	36.67 (37.243)	73.33 (59.031)	100.00 (90)
8	Control (Distilled Water)	0.00 (00)	0.00 (00)	5.00 (10.448)
	F Test	Sig	Sig	Sig
	C.D. @ 5%	3.658	7.234	8.742
	S.E (m)±	1.21	2.392	2.891

(Figures in the bracket are arc sine transformation)

Table 2: Pathogenicity of EPN isolate *Steinernema bicornatum* (CICR-W) against *Galleria mellonella*.

Sr. No.	Treatment Concentration	Larval Mortality of *G. mellonella* larvae		
		24 Hrs	48 Hrs	72 Hrs
1	10 IJs/100µl	13.33 (21.33)	41.67 (40.182)	80.00 (63.409)
2	20 IJs/100µl	18.33 (25.295)	45.00 (42.104)	85.00 (67.377)
3	30 IJs/100µl	20.00 (26.554)	53.33 (46.894)	88.33 (70.086)
4	40 IJs/100µl	23.33 (28.843)	56.67 (48.846)	91.67 (73.374)
5	60 IJs/100µl	25.00 (29.913)	60.00 (50.748)	96.67 (81.365)
6	80 IJs/100µl	26.67	65.00	100.00

		(31.058)	(53.74)	(90)
7	100IJs/100µl	31.67 (34.217)	71.67 (57.836)	100.00 (90)
8	Control (Distilled Water)	0.00 (00)	1.67 (4.305)	5.00 (10.448)
	F Test	Sig	Sig	Sig
	C.D. @ 5%	3.523	6.225	8.243
	S.E (m) ±	1.165	2.059	2.726

(Figures in the bracket are arc sine transformation)

Table 3: Pathogenicity of EPN isolate *Steinernema siamkayai* (SAM 4) against *Galleria mellonella*.

Sr. No.	Treatment Concentration	Larval Mortality of *G. mellonella* larvae		
		24 Hrs	48 Hrs	72 Hrs
1	10 IJs/100µl	10.00 (18.428)	40.00 (39.195)	80.00 (63.524)
2	20 IJs/100µl	15.00 (22.586)	43.33 (41.117)	88.33 (70.665)
3	30 IJs/100µl	18.33 (24.99)	51.67 (45.938)	90.00 (71.924)
4	40 IJs/100µl	21.67 (27.51)	53.33 (46.934)	91.67 (73.374)
5	60 IJs/100µl	23.33 (28.654)	56.67 (48.826)	95.00 (77.048)
6	80 IJs/100µl	26.67 (30.983)	61.67 (51.755)	98.33 (85.683)
7	100IJs/100µl	30.00 (33.147)	66.67 (54.727)	100.00 (90)
8	Control (Distilled Water)	0.00 (00)	1.67 (4.305)	3.33 (8.611)
	F Test	Sig	Sig	Sig
	C.D. @ 5%	7.108	7.735	8.862
	S.E (m) ±	2.351	2.558	2.931

(Figures in the bracket are arc sine transformation)

Table 4: Multiplication of *Heterorhabditis indica* (CICR-Guava) *Steinernema bicornatum* (CICR-W) *Steinernema siamkayai* (SAM 4) on *Galleria mellonella*.

Sr. no.	Treatment concentration	Number of infective juveniles emerged from single larvae $\times 10^2$		
		H. indica	***S. bicornatum***	***S. siamkayai***
1	10IJs/100µl	1886.5 (43.44)	1213.8 (34.854)	1714.8 (41.422)
2	20IJs/100µl	2045.9 (45.241)	1355.1 (36.824)	1822.9 (42.706)
3	30IJs/100µl	2209.3 (47.013)	1582.6 (39.794)	1928.2 (43.922)
4	40IJs/100µl	2366.8 (48.659)	1792.5 (42.35)	2051.2 (45.3)
5	60IJs/100µl	2487.7 (49.886)	1883.4 (43.409)	2143.6 (46.31)
6	80IJs/100µl	2603.5 (51.034)	1975.8 (44.46)	2266.6 (47.619)
7	100IJs/100µl	2708.1 (52.048)	2082.6 (45.646)	2384.7 (48.844)
	F Test	sig**	sig**	sig**
	C.D. @5%	0.804	0.565	0.437
	SE(m) ±	0.263	0.185	0.143

(Figures in the bracket are square root transformation)

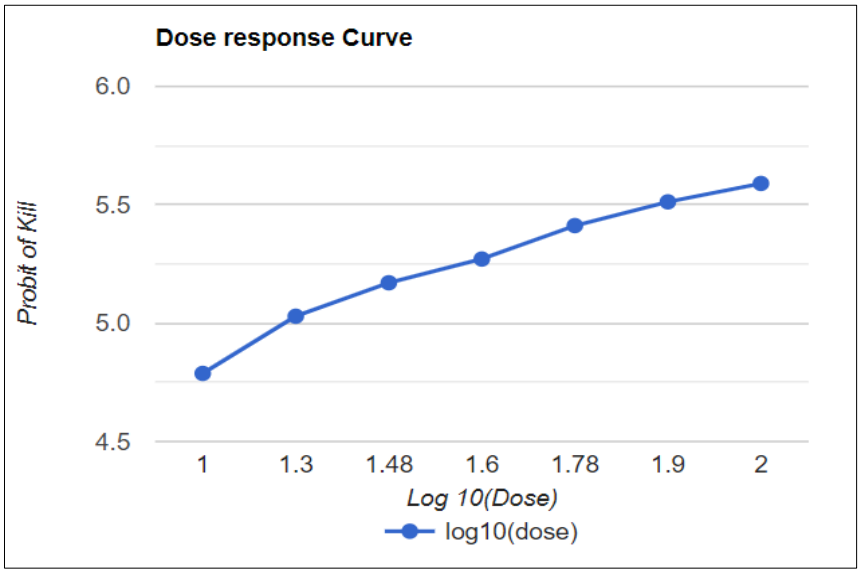

Fig 1: Working probit regression line for CICR Guava against *G. mellonella* larvae.

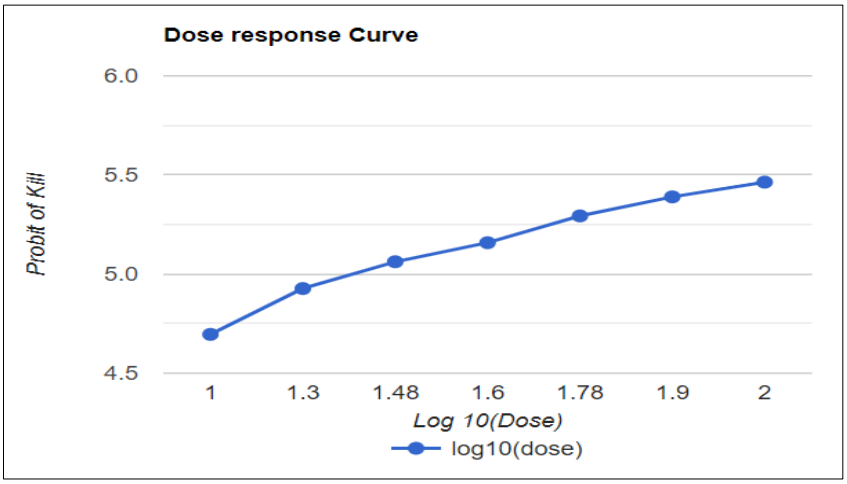

Fig 2: Working probit regression line for CICR W against *G. mellonella* larvae.

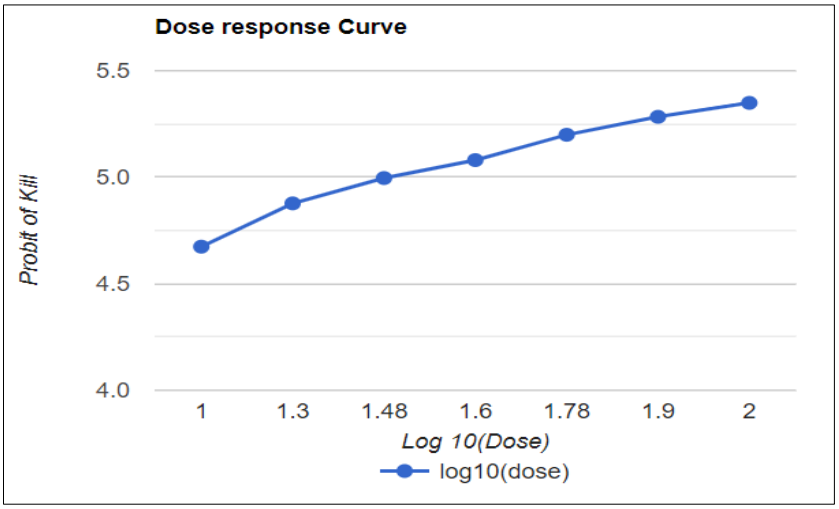

Fig 3: Working probit regression line for SAM 4 against *G. mellonella* larvae.

Conclusion

From the studies conducted it can be concluded that biocontrol potential in concern with pathogenicity, LC_{50} and reproductive potential of EPNs *Heterorhabditis indica* is highest in comparison of *Steinernema bicornatum* and *Steinernema siamkayai*.

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