ISSN Print: 2394-7500
ISSN Online: 2394-5869
Impact Factor: 8.4
IJAR 2021; 7(12): 213-215
www.allresearchjournal.com
Received: 22-09-2021
Accepted: 07-11-2021

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Studies on determination of dosage mortality response of various EPN isolates against fall armyworm, *Spodoptera frugiperda*, J. E. Smith

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Abstract
The results on dosage mortality response of various indigenous EPN isolates *viz*., *Heterorhabditis spp*. (PKV-1), *Heterorhabditis spp*. (CICR Brown), *Steinernema bicornutum*. (CICR-White) and *Heterorhabditis spp*. (PKV-Guava) against the all-instar larvae *S. frugiperda* (excluding 1st instar) revealed that in case of *Heterorhabditis spp*. (PKV1) LC50 for 2nd instar larvae of S. *frugiperda* was calculated 6.65, for 3rd instar 4.79, for 5th instar 5.39, for 5th instar 8.31 and for 6th instar it was calculated as 6.44. While in case of *Heterorhabditis spp.* (CICR- Brown) the lowest LC50 value 2.29 calculated for the 3rd instar larvae, then 2.72 for 5th instar larvae 4.127 for 2nd instar, 4.98 for 6th in star and highest LC50 calculated for 4th instar larvae. While in case of Isolate *Heterorhabditis spp.* (PKV-Guava) the lowest LC50 value calculated as 5.86 in 5th instar larvae, 6.76 in case of 4th instar, 7.31 in 3rd instar and 7.52 in 2nd instar and EPN isolate *Steinernema bicornutum* has recorded the lowest LC50 calculated 22.23 for 4th instar larvae, 23.14 for 2nd instar, 26. 4 for 5th instar, 28.08 for 3rd in star and 58.78 for 6th instar.

Keywords: Dosage, mortality, lc50, *heterorhabditis*, *steinernema*, fall armyworm, isolates

Introduction
The entomopathogenic nematode in the families of Steinernematidae and Heterohabditidae are potential virulent agents because of their symbiotic association with bacteria *Xenorhabdus spp*. and *Photorhabdus spp*. respectively (Kaya *et al*. 2006) [7]. Both entomopathogenic nematode and their associate bacterial symbionts are non-pathogenic to warm-blooded vertebrates, animals and human (Boemare, *et al.*, 1996) [1]. Biological control of pests using entomopathogenic nematodes is an ideal alternative, is economical, and has long term control, without risk to non-target organisms. The EPNs are potential agents as they serve as vectors of bacteria, achieve a quick kill of target insect pests, have a broad host range, highly virulent, possess chemoreceptor, can be cultured easily *in vitro*, have a numerical but no functional response, are safe to vertebrates, plants and non-targets, have been exempted from registration in USA, are easily applied using standard application equipment, are compatible with many chemical pesticides, and are amenable to genetic selection (Kaya and Gaugler,1993) [8]. This research would help to determine in what situations entomopathogenic nematodes can be used and provide some insight into their effectiveness in various circumstances. The fall armyworm, *Spodoptera frugiperda* (J. E. Smith, 1797) [11] (Lepidoptera: Noctuidae) is native to the tropical region of the western hemisphere from the United States to Argentina considered as a most important pest of corn in Brazil, after making its way to Africa in 2016, it now appears to have found its home in India. First infestation of this pest in India is found on maize crop in Karnataka July 2018. The voracious pest, known to devastate a one-acre field in a week, could endanger the agricultural output of India. The insect "has not only invaded the maize crop in Maharashtra, the area adjoining the borders of Odisha and Chhattisgarh, West Bengal and Gujarat but also sorghum and other millet crops in Telangana and the northern part of Karnataka (Chormule *et al.*, 2019) [2].

Materials and Method
Stock solution of various concentrations *viz*., 5, 10, 15, 20, 25, 30 and 50 IJ/ 20µl of different isolates were prepared and evaluated against all instars *S. frugiperda* (excluding 1st instar).

The infective juvenile (IJ) count was taken by counting the number of IJs per 20µl of EPN isolates. For this purpose, 20µl of suspension was measured using a micro pipette and poured on a sterilized glass slide and count was taken with help of stereo binocular microscope. Filter paper impregnation method was used by exposing host insects to nematode impregnated filter paper at the bottom of rearing tray. EPN juveniles at the rate of 0 (control), 5,10,15,20,25,30 and 50 Infective juveniles in 20µl for each dose were distributed over the filter paper and individual larvae of respective instars were put in nematode inoculated cell of insect rearing trays. Mortality was checked 24 and 48 hours after inoculation.

The LC50 values were calculated by testing of several doses against various larval instars of *Spodoptera frugiperda*. LC50 was calculated by Probit analysis by following procedure defined by Finney (1952) [3].

The mortality data obtained from the experiment was subjected to Probit analysis with the help of online software available on Hissar Agricultural University, Hissar. The result obtained depicts that LC50 values for isolates *Heterorhabditis Spp*. (PKV-1), *Heterorhabditis Spp.* CICR-Br), *Heterorhabditis Spp*. (PKV-Guava), and *Steinernema bicornutum* (CICR- White).

Expected Probit Y are then taken from calculated form. The graph with the help of my fitting line and tabulated. Working Probit Y are taken from the table given by Finney on the basis of corrected % mortality value again standard expected P bit Y. Weighing coefficient (M) are calculated from the table by observing expected Probit Y again. Standard control mortality and the product of weighing coefficient with number of test insects in column. NW that is number of insects taken for experiment and weighting coefficients are calculated from each concentration sum of all NW. For each concentration the product NW, NWX, NWY, NWX2, NWXY, NWY2 were calculated and inserted in the column given in the table. Sum of the value of column with different value like square value are calculated bx, y, b, A, B, C, Exx, Eny, Eyy were calculated.

Chi-square was calculated by applying the formula to know whether the data is homogeneous or heterogeneous. If the value is greater than the table value at (n -2) degree of freedom from 0.0 5 probability that the data is said to be heterogeneous. The Chi-square value should always be positive. For heterogeneous Chi- square value calculated table value should be lesser than the table value the data is known as homogeneous. All these values for different instar's were calculated for the all four isolates *viz. Heterorhabditis spp.* (PKV-1, CICR-Br and PKV-Guava) and *Steinernema bicornutum* (CICR-White).

Results and Discussion

Determination of Dosage Mortality Response of various EPN Isolates against Fall Armyworm, *Spodoptera frugiperda*

The data from table 1 reveals that in case of *Heterorhabditis spp*. (PKV-1) LC50 for 2nd instar larvae of S. *frugiperda* was calculated 6.65, for 3rd instar 4.79, for 5th instar 5.39, for Vth instar 8.31 and for 6th instar it was calculated as 6.44. While in case of *Heterorhabditis spp.* (CICR-Brown) the lowest LC50 value 2.29 calculated for the 3rd instar larvae, then 2.72 for 5th instar larvae 4.127 for 2ndinstar, 4.98 for 6th instar and highest LC50 calculated for 4th instar larvae. While in case of Isolate *Heterorhabditis spp.* (PKV-Guava) the lowest LC50 value calculated as 5.86 in 5th instar larvae, 6.76 in case of 4th instar, 7.31 in 3rd instar and 7.52 in 2nd instar and EPN isolate *Steinernema bicornutum* (CICR-White) has recorded the lowest LC50 calculated 22.23 for 4th instar larvae, 23.14 for 2nd instar, 26. 4 for 5th instar, 28.08 for 3rd instar and 58.78 for 6th instar. The chi-square test showed homogeneity of the test population in all bioassays which indicate good fit of the observed and expected response.

Table 1: Determination of Dosage Mortality Response of various EPN Isolates against Fall Armyworm, *Spodoptera frugiperda*

Isolates	LC50 Against *S. frugiperda*				
	2nd instar	3nd Instar	4th Instar	5th instar	6th Instar
Heterorhabditis spp. (PKV-1)	6.65	4.79	5.39	8.31	6.44
Heterorhabditis spp. (CICR-Br)	4.127	2.29	6.05	2.72	4.98
Steinernema bicornutum	23.14	28.08	22.23	26.4	58.78
Heterorhabditis spp. (PKV-Guava)	7.52	7.31	6.76	5.86	10.26

LC50 for the *Heterorhabditis* spp. isolates was low as compared to the *Steinernema* spp. isolate. LC50 for the *Heterorhabditis spp*. isolate (PKV-1) was recorded 6.65,4.79,5.39,8.31 and 6.44 IJ/larva for 2nd, 3rd, 4th, 5th, and 6th instar larva with lowest LC50 for III instar larvae and highest for 5th instar larvae, respectively. Similarly, LC50 for the *Heterorhabditis spp*. isolates (CICR-Br) and *Heterorhabditis spp*. (PKV-Guava) were 4.12, 2.29, 6.05,2.72 and 4.98 and 7.52, 7.31, 6.76, 5.86 and 10.26 for the larval instars 2nd, 3rd, 4th, 5th, and 6th of *Spodoptera frugiperda*. The isolate *Heterorhabditis spp.* (CICR-Br) has recorded lower LC50 values as compared to *Heterorhabditis spp.* (PKV-Guava) and (PKV-1). However, lowest LC50 was recorded for isolate *Heterorhabditis spp*. (CICR-Br) for 3rd and 5th instar i.e., 2.29 and

Whereas, isolate to *Heterorhabditis spp.* (PKV-Guava) has recorded lowest LC50 value for Vth instar and highest for 6th instar. The LC50 values recorded for isolate *Steinernema bicornutum* (CICR-White) were quite higher than the *Heterorhabditis spp*. isolates and found 23.14, 28.08, 22.23, 26.4 and 58.78 for 2nd, 3rd, 4th, 5th, and 6th instars. Lowest LC50 for *Steinernema* spp. was recorded for 4th instar and highest for 6th instar respectively.

Garcia *et al.*, (2008) [4] reported 280IJs of *Steinernema spp.* were required to kill 100 percent 3rd instar larva of fall armyworm and 400IJs for H*eterorhabditis indica*. Kalia *et al.*, (2014) [6] also reported that LC50 against *Helicoverpa armigera* was 54.68IJ/larva, *Spodoptera litura* was 85IJ/larva and *Galleria mellonella* was 16.88IJs/larva for *Steinernema thermophilum* along with ovicidal virulence up to 84 percent. Kumar *et al.*, (2015) [9] also reported virulence of *Heterorhabditis spp.* with LC50 16.1IJ/larva. These results are in confirmation with the present studies undertaken against *Spodoptera frugiperda* with 3 isolates of *Heterorhabditis spp.* and 01 isolate of *Steinernema spp*. The investigations carried out by Radhakrishnan *et.al.* (2018) [10] confirms that the LC50 of *Heterorhabditis indica* (16.39IJs/larva) and *Steinernema glasserie* (18.03 IJs /larva) was low in laboratory conditions whereas it is higher in glass house condition against *Agrotis ipsilon.* The bioassays conducted by Thorat *et al.*, (2018) [12] with 3 isolates of *Heterorhabditis spp.* from Pravranagar area of Maharashtra

has resulted that 50IJ per ml was sufficient for 40.8% and 62.1% mortality of *Galleria mellonella* at 3 and 7DAT. The treatment with concentration 100IJ/ml was found most effective which recorded 43% mortality which causes 43% mortality of Early shoot borer and 40% mortality for armyworm after 3DAT. These findings are in line with the present research carried out against *Spodoptera frugiperda.*

Conclusion

EPN isolate *Steinernema bicornutum* has recorded the lowest LC50 calculated 22.23 for 4th instar larvae, 23.14 for 2nd instar, 26. 4 for 5th instar, 28.08 for 3rd instar and 58.78 for 6th instar and found to be most virulent isolate of EPN.

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