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MA Patil
Department of Agricultural Entomology, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahmednagar, Maharashtra, India

PR Palande
Department of Agricultural Entomology, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahmednagar, Maharashtra, India

SR Kulkarni
Department of Agricultural Entomology, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahmednagar, Maharashtra, India

ST Aghav
Department of Agricultural Entomology, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahmednagar, Maharashtra, India

Corresponding Author:
MA Patil
Department of Agricultural Entomology, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahmednagar, Maharashtra, India

Bioefficacy study of *Heterorhabditis indica* (Poinar) against *Helicoverpa armigera* (Hubner) in laboratory

MA Patil, PR Palande, SR Kulkarni and ST Aghav

Abstract
The present investigations on "Bioefficacy of *Heterorhabditis indica* (Poinar) against *Helicoverpa armigera* (Hubner)" was carried out during the year 2018-19 at Research Laboratory, Department of Agricultural Entomology, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri (M.S.). The bioefficacy of different doses of *H. indica* was studied against 3rd, 4th and 5th larval instars of *H. armigera*. Larvae were exposed to 0 (control), 25, 50, 75, 100, 150, 200 infective juveniles (IJs) per ml per petri dish and for various time periods (24 hr, 48 hr and 72 hr). It was observed that among all doses, the dose with 200 IJs/ml brought quicker 100 per cent mortality as compared with other lower doses which brought slower mortality. It was also observed that the third instar showed mortality ranged from 26.67 to 63.33 per cent 24 HAE. 48 and 72 HAE mortality ranged from 43.33 to 90 per cent and 80 to 100 per cent, respectively. In case of 4thinstar larvae 24 HAE larval mortality ranged from 23.33 to 60.00 per cent, 48 HAE 40.00 to 86.67 per cent and 72 HAE 63.33 to 100 per cent. In case of 5th instar mortality range recorded was 20.00 to 56.67 per cent for 24 HAE, in case of 48 HAE it was 33.33 to 90.00 per cent and 72 HAE range was from 46.66 to 100 per cent. Thus it was concluded that mortality percentage increase with increase in time of exposure. The LC_{50}values for 3rd, 4th and 5th instar *H. armigera* larvae when exposed to 72 hr period were 12.09, 21.88 and 32.91 IJs of *H. indica* per ml of water, respectively, whereas 3rd, 4th and 5th instar larvae exposed for 24 hr period showed LC_{50}values 99.99, 118.92 and 140.07 IJs per ml of water, respectively. The LC50 values were less when *H. armigera* larvae were exposed for 72 hr as compared to 24 hr exposure period which meant 3rd instar *H. armigera* larvae exposed for 72 hr were more susceptible as it required less number of IJs/ml to bring about 50 per cent mortality than 4th and 5th instar larval stages.

Keywords: *H. indica*, bioefficacy, mortality, instar, *helicoverpa*

Introduction
Chickpea (*Cicer arietinum* L.) is an important pulse crop in our country. Chickpea seed contains about 18-20 per cent proteins (Malunga *et al.* 2014) [13]. Average productivity of chickpea in Maharashtra during last five years was 771 kg /ha (Pulses in India Retrospect & Prospects). In India during *rabi* season of 2017-18, chickpea was cultivated on about 106 lakh ha area, with a production of more than 111 lakh tonne and productivity of 1056 kg/ha, (Anonymous 2018) [1].

The low yield of chickpea is attributed mainly to the regular outbreaks of pod borer, *H. armigera* (Hubner) which is considered to be one of the major pests of chickpea crop. It is extremely polyphagous and one of the major pests of chickpea, cotton in almost all of the chickpea and cotton growing areas in India causing quantitative and qualitative losses. It has been reported to feed on 181 cultivated and uncultivated species (Manjunath *et al*. 1989) [12]. In chickpea the damage is characterized by feeding activity on flower buds, flowers and pods. It begins their feeding at the seedling stage and feeds on the leaves by scrapping green tissue and pods and later infests on the buds, flowers and developing pods until the crop maturity. The typical symptom shows circular bore holes on gram pods plugged by the head of a larva.

H. armigera has been controlled by various pesticides but this pest has developed resistance to many groups of insecticides particularly synthetic pyrethroids (Castle *et al.* 1996) [2]. The problem of insecticide resistance as well as indiscriminate or injudicious use of pesticides has resulted in residues in the food chain, pesticide resistance, and pest resurgence, in addition to causing harm to non-targeted beneficial organisms and the environment (Patil *et al*. 2017) [15]. "A modern endeavor is to bring insect pests under natural control. The 'natural control' of

insects will be most effective if all possible agencies and factors are utilized; and among these agencies nemas are by no means negligible" (Cobb 1927) [3]. So, alternative control measures for this insect pest include the use of entomopathogenic nematodes and other biocontrol agents and to incorporate it into the Integrated Pest Management programme against this pest. *H. indica* is particularly having wide host range and have potential to be used as biocontrol agent against *H. armigera*. Taking in view the severity of damage/losses caused by most harmful pests like *H. armigera* on different crops, feasibility of different doses of *H. indica* against *H. armigera* was evaluated under laboratory condition.

Materials and Methods

Nematode sources

A laboratory trials were conducted to compare the pathogenicity of different concentrations of entomopathogenic nematode *H. indica* against 3rd, 4th and 5th instar larvae when each exposed for 24, 48 and 72 hours. Filter paper bioassay method was used for lab study. *H. indica* culture was obtained from Patron Organics Pvt. Ltd., Khandelwal Bio Fertilizes and K. N. Biosciences.

Test insects

Early instar larvae of *H. armigera* were collected from chickpea field and reared in the laboratory on a chickpea based semisynthetic diet as described by Dang *et al.* (1970) [4] and modified by Nagarkatti and Satyaprakash (1974) [14]. Rearing environment of 27 ±2°C, 60±5% relative humidity was maintained until they reached the life stages to be tested. All the adult moths were offered 10% honey solution fortified with multivitamins during oviposion period.

Larval mortality bioassay

The bioefficacy of *H. indica* was tested at seven concentrations including 0, 25, 50, 75, 100, 150, 200 infective juvenile (IJs) against 3rd, 4th and 5th instar larvae of *H. armigera*. Individual 3rd, 4thand 5thinstar larvae were placed in each petri plate. Nematode suspensions were prepared in double distilled water. 1 ml suspension containing 25, 50, 75, 100, 150 and 200 IJs individually incorporated onto the filter paper before releasing larvae of *H. armigera*. Distilled water alone served as a control. Ten replicates per concentration were used and each treatment was replicated thrice. Incubation was performed at a constant temperature of 27 ± 2ºC and 60 ± 5 % RH. Mortality was observed at 24 hr interval up to 72 hr of IJs inoculation. Corrected mortality was calculated by arc sin transformation. Median lethal concentration (LC_{50}) was calculated at 24, 48 and 72 hr.

Statistical analysis

The per cent mortality was calculated by the formula,

$$\text{Per cent mortality} = \frac{\text{No. of dead larvae}}{\text{Total number treated larave}} \times 100$$

The data were transformed into $\sqrt{(x+0.5)}$ before statistical analysis as necessary and two way factorial analysis of variance (ANOVA) was carried out. The data on per cent mortality was subjected to Probit analysis, (Finney, 1971) [8] to calculate LC_{50}. The LC_{50} was considered significantly different only in case of non-overlapping fiducial limits at 95% confidence level.

Results

At all the concentrations of *H. indica* the third instar larvae of *H. armigera* were reported to be susceptible However, the degree of susceptibility of insect larvae to nematode infection varied according to exposure period in a concentration dependent manner. There exist a positive correlation between the tested concentrations of *H. indica* infective juveniles and time of larval mortality.

Different doses of EPN (*H. indica*) formulations were evaluated against 3rd instar larvae of *H. armigera* under laboratory conditions. Data pertaining to larval mortality of has been presented in Table 1 and graphically depicted in Fig. 1. Table 1 reveals that the larval mortality was ranged from 26.67 to 63.30 per cent, 43.33 to 90.00 per cent and 80.00 to 100.00 per cent at 24, 48 and 72 HAE, respectively at different doses of IJs ranging from 25 to 200 IJs/ ml.

Data pertaining to larval mortality (4th instar) is presented in Table 2 depicted in Fig 2. It rveals that the larval mortality was ranged from 23.33 to 60.00 per cent, 40.00 to 86.67 per cent and 63.33 to 100.00 per cent at 24, 48 and 72 HAE, respectively. The treatment with *H. indica* formulation I @ 200 IJs/ml was found to be significantly superior over rest of the treatments in which highest larval *H. indica* formulation I @ 100 IJs/ml in which more than 50 per cent larval mortality was observed.

Data pertaining to larval mortality (5th instar) has been presented in Table 3 and depicted in Fig 3. It reveals that the larval mortality was ranged from 20.00 to 56.67 per cent, 33.33 to 90.00 per cent and 46.66 to 100.00 per cent at 24 HAE, 48 HAE and 72 HAE, respectively at different EPN (*H. indica*) doses ranging from 25 to 200 IJs/ ml. The results showed that all the treatments were significantly superior over untreated control.

Table 1: Bioefficacy study of diiferent doses EPN based formulations against 3rd instar larvae of *H. armigera* under laboratory condition

Tr. No.	Treatment	Dose IJs/ml	Larval mortality (%)		
			24 HAE**	48 HAE	72 HAE
T_1	*H. indica* formulation I	200	63.30 (52.73)*	90.00 (71.57)	100.00 (90.00)
T_2	*H. indica* formulation I	100	56.67 (48.83)	76.67 (61.12)	100.00 (90.00)
T_3	*H. indica* formulation II	150	56.67 (48.83)	86.67 (68.56)	100.00 (90.00)
T_4	*H. indica* formulation II	75	43.3 (41.17)	70.00 (56.79)	93.33 (75.03)
T_5	*H. indica* formulation III	50	33.33 (35.26)	56.67 (48.83)	86.67 (68.58)
T_6	*H. indica* formulation III	25	26.67 (31.09)	43.33 (41.17)	80.00 (63.43)
T_7	Untreated control	0	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	SE(±)		3.78	5.04	2.81
	CD @ 5 %		11.46	15.29	8.55

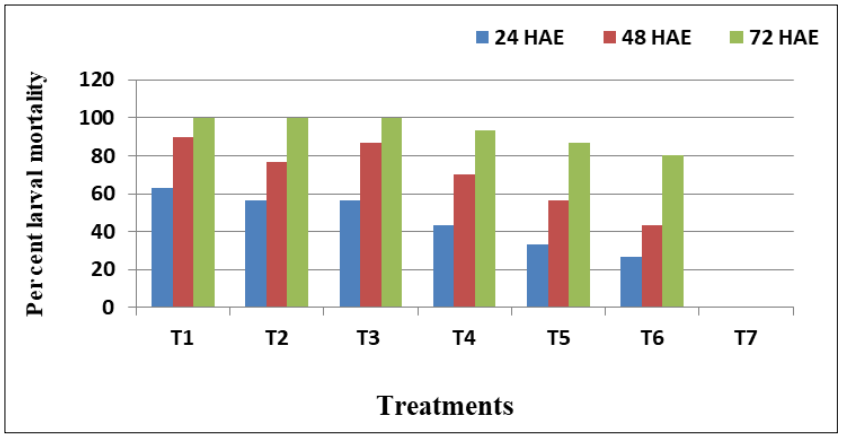
Table 2: Bioefficacy study of different doses EPN based formulations against 4th instar larvae of *H. armigera* under laboratory condition

Tr. No.	Treatment	Conc. (IJs/ml)	Larval mortality (%)		
			24 HAE**	48 HAE	72 HAE
T_1	*H. indica* formulation I	200	60.00 (50.77)*	86.67 (68.59)	100.00 (90.00)
T_2	*H. indica* formulation I	100	53.33 (46.09)	80.00 (63.43)	90.00 (71.57)
T_3	*H. indica* formulation II	150	56.67 (48.83)	83.33 (65.90)	96.67 (78.46)
T_4	*H. indica* formulation II	75	36.60 (37.27)	56.67 (48.83)	83.33 (65.90)
T_5	*H. indica* formulation III	50	26.67 (31.09)	43.33 (41.17)	66.67 (54.73)
T_6	*H. indica* formulation III	25	23.33 (28.88)	40.00 (39.23)	63.33 (52.54)
T_7	Untreated control	0	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	SE (±)		2.82	2.52	2.52
	CD @ 5per cent		8.54	7.64	7.64

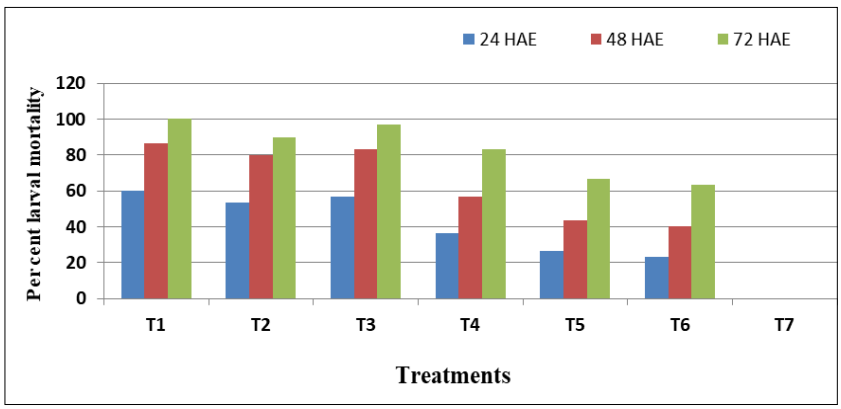
Table 3: Bioefficacy study of EPN based products against 5th instar larvae of *H. armigera* under laboratory condition

Tr. No.	Treatments	Conc. IJs/ml	Larval mortality (%)		
			24 HAE**	48 HAE	72 HAE
T_1	*H. indica* formulation I	200	56.67 (48.83)*	90.00 (71.57)	100.00 (90.00)
T_2	*H. indica* formulation I	100	50.00 (45)	76.67 (61.12)	96.67 (79.49)
T_3	*H. indica* formulation II	150	53.33 (46.91)	86.67 (68.59)	100.00 (90.00)
T_4	*H. indica* formulation II	75	26.67 (31.09)	43.30 (41.15)	70.00 (56.79)
T_5	*H. indica* formulation III	50	26.66 (31.09)	36.67 (37.27)	63.33 (52.73)
T_6	*H. indica* formulation III	25	20.00 (26.57)	33.33 (35.26)	46.66 (48.08)
T_7	Untreated control	0	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	SE (±)		3.33	2.81	2.18
	CD @ 5 %		10.11	8.55	6.62

* Figures in parenthesis are arc sin transformed values ** HAE- Hours after exposure



A



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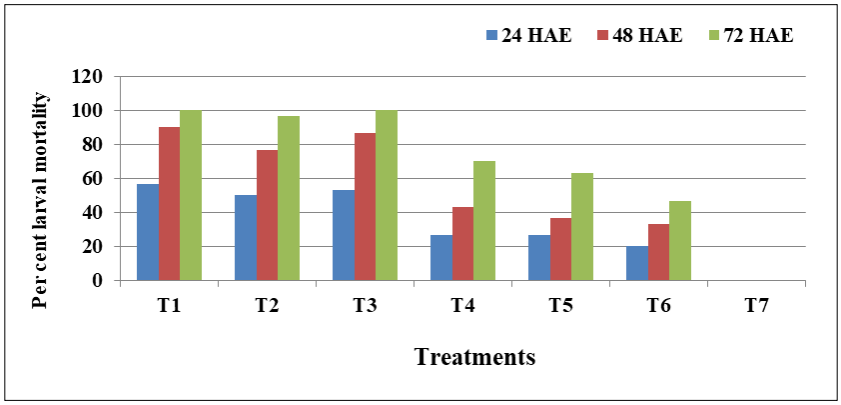

C

tr. no	Conc. (IJs/ml)	Tr. No	Conc. (IJs/ml)
T_1	0	T_5	100
T_2	25	T_6	150
T_3	50	T_7	200
T_4	75		

Fig 1: The percentage mortality of *H. armigera* larvae following to different concentrations of infective juveniles (IJs) of *H. indica* exposed for different time periods (24 hr, 48hr, 72hr) under laboratory conditions. A. Third instar B. Fourth instar C. Fifth instar.

Table 4: Toxicity of EPN (*H. indica*) formulation against larval instars of *H. armigera* at various time periods of exposure (LC_{50} values)

Sr. No.	Time of exposure (HAE)	LC_{50} IJs/larva	95% Fiducial limit		Slope ± SE	Chi square value	Df	Pc
			Lower	Upper				
3rd instar larvae								
1	24	99.99	81.20	128.01	1.13± 0.50	1.25	4	0.87
2	48	33.01	20.50	52.57	3.24 ± 0.91	0.206	4	0.87
3	72	12.09	0.80	22.65	2.18± 0.26	0.68	4	0.45
4th instar larvae								
1	24	118.92	97.93	153.27	1.25± 0.04	3.04	4	0.55
2	48	45.60	20.40	66.42	1.68± 0.09	5.34	4	0.25
3	72	21.88	7.47	35.23	1.97± 0.14	2.79	4	0.59
5th instar larvae								
1	24	140.07	80.49	181.50	1.29± 0.66	5.11	3	0.16
2	48	57.02	23.73	88.51	2.10± 0.10	12.4	4	0.01
3	72	32.91	9.90	49.95	2.78± 0.13	5.51	4	0.25

df- degrees of freedom Pc- critical probability slope

The LC_{50} of 3nd instar larvae of *H. armigera* exposed for 24 hr period was 99.99 IJs/larva with 81.20 IJs/larva and 128.01 IJs/larva as upper and lower fiducial limits, while the LC_{50} value was 33.01 IJs/larva with 20.50 IJs/larva and 52.57 IJs/larva as lower and upper fiducial limits respectively when exposed for 48 hr. *H. armigera* larvae when exposed for 72 hr the LC_{50} value was 12.09 IJs/larva with lower and upper fiducial limits of 0.80 IJs/larva and 22.65 IJs/larva, respectively. For the 4th instar larvae in case of 24 hr exposure period LC_{50} value obtained was 118.92 IJs/ larva with 97.93 IJs/ larva as lower and 153.27 IJs/ larva upper fiducial limits. The LC_{50} value at 48 hr exposure period was 45.60 IJs/ larva with 20.40 IJs/ larva as lower fiducial limit and 66.42 IJs/ larva as upper fiducial limit. At 72 hours exposure period 4th instar larvae of *H. armigera* recorded LC_{50} value of 21.88 IJs/ larva with 7.47 IJs/ larva and 35.23 IJs/ larva as lower and upper fiducial limits, respectively. The LC_{50} value for 5th instar larvae of *H. armigera* at 24 hr exposure period was 140.07 IJs/ larva with 80.49 IJs/ larva as lower and 181.50 IJs/ larva as upper fiducial limits and at 48 hr exposure period LC_{50} was 57.02 with 23.73 as lower and 88.51 as upper fiducial limits. Whereas LC_{50} for 5th instar when exposed for 72 hr period was 32.91 IJs/ larva with 9.90 IJs/ larva as lower and 49.95 IJs/ larva as upper fiducial limits. It was found that the lowest LC_{50} value was recorded for the 3rd instar *H. armigera* larvae for all the three exposure periods as compared to the 4th and 5th instar *H. armigera* larvae. The LC_{50} value recorded was lowest for 72 hr exposure period for all the three stages of *H. armigera* indicating that the least number of IJs were required to cause 50 per cent mortality of test larvae as compared to 24 and 48 hr exposure period. Hence, the 3rd instar *H. armigera* exposed for 72 hr period was found to be the most susceptible with the lowest LC_{50} value (12.09 IJs/larva). The highest LC_{50} value was recorded for the 5th instar larvae exposed for 24 hr (140.07 IJs/ larva).

Discussion

The study of bioefficacy of different EPN based formulations containing different concentrations of infective juveniles (IJs) of *H. indica* against different instars of *H. armigera* when exposed for various periods (24, 48 and 72 hr) showed that the efficacy of various EPN based formulation for controlling a particular insect pest may differ significantly depending on concentration of entomopathogenic nematode. Also it was found that laravl stages were highly susceptible to entomopathogenic nematode infection and there was variation in susceptibility between different instars of *H. armigera* to entomopathogenic nematodes. Different doses of EPNs showed variation in larval mortality.

As shown in Table 1, concentration of 25 IJs/ larva at 72 hr

exposure period resulted in 80 per cent larval mortality was recorded. Whereas at higher doses of IJs *i.e.* 200 IJs/ larva, 150 IJs/ larva and 100 IJs/ larva 100 per cent mortality of third instar *H. armigera* larvae was recorded at 72 hr exposure period. Similar results were found in case of late instar like 4th in which mean mortality ranged from 42 to 82 per cent for various formulations whose IJs concentration ranged 25 IJs/ larva to 200 IJs/ larva and 5th instar in which mean mortality ranged from 33 to 82 per cent for concentrations ranged from 25 IJs/ larva to 200 IJs/ larva. Thus results revealed that the rate of infectivity varied among the doses used i. e. higher doses gave higher mortality these findings are in conformity with Maketon *et al.* (2011) who found that mortality generally increased when pathogen density was increased from 50 to 500 IJs per host in every insect host like *Aphis gossypii*, adult *Sitophilus zeamis*, 2nd instar *Bactrocera correcta* and nymphal *Coptotermis gestroi.* Studies by Pavel, Hyrsl (2011) and Divya *et al.* 2010 also say that pathogenicity was correlated with the number of invaded infective juveniles. Table 1, 2 and 3 showed that in case of lower doses like 25 IJs/ml, 50 IJs/ml and 75 IJs/ml, 50% mortality is achieved after 24 or 48 HAE whereas higher doses bring quicker mortality. Similar findings were reported by Prabhu and Sudheer (2008) who indicated that the higher nematode inoculum levels caused higher and faster mortality than lower levels. High virulence of *H. indica* was attributed due to the presence of mural tooth which helps to penetrate the soft joints of the insect. Similarly, Divya *et al*. (2010) also recorded high mortality when exposed for 24 hr as compared to 18 and 12 hr exposure period. As the dose increased, the number of host invading infective juveniles also increased and hence the time taken for fifty per cent mortality of *S. litura* larvae was less when treated with 500 IJs per ml of water as compared to lower doses.

Table 4.4 shows that LC_{50} values in case of 3rd instar larvae at different exposure periods are lower as compared with 3rd and 4th instar. The 3rd and instar larvae are more susceptible as compared with 4th and 5th instar. At 24 HAE for 3rd instar LC_{50} value is 99.99 IJs/larva, for 4th instar LC_{50} is 118.92 and for 5th instar it is 140.07. From the results it was found that 3rd and 4th instars were more vulnerable to the EPN attack than the 5th instar in all the treated doses and for all the exposure periods. The findings are in conformity with Glazer and Navon (1990) [7] who reported that the youngest instars of *H. armigera* were the most susceptible to nematode infection from the genera Steinernema and Heterorhabditis under laboratory conditions. Similar findings were reported by Geden *et al.* (1985) who reported that early instar larvae of *Alphitobius diaperinus* Panzer (Coleoptera: Tenebrionidae) were more susceptible than late instars.

Similarly, studies by (Divya *et al.*, 2010) revealed that the pathogenicity of *H. indica* can be affected by size and age of larva. Also King (1994) reported that 2nd instar *H. armigera* was most susceptible than later instars.

Conclusion

From this research work it was concluded that higher nematode inoculum caused higher and faster mortality than small quantity *i.e.* efficacy of various EPN based formulation for controlling a particular insect pest may differ significantly depending on concentration of EPN IJs it contains. Also early instars were more vulnerable to the EPN attack than the late instars. The 3rd instar *Helicoverpa* larvae were more susceptible than 4th and 5th instar larvae. The LC_{50} value required for early instars was less as compared to older instars. Therefore, instars in all the treated doses and for all the exposure periods. Novaluron 10 EC @ 1.5 ml/lit proved to be the best treatment in field to control *H. armigera* as compare with various EPN doses. Among various EPN doses field applications of *H. indica* @ 60 lakh IJs /plot and 45 lakh IJs /plot managed the *H. armigera* on chickpea effectively. It is also concluded that higher EPN (IJs) dose recorded lower larval population and more larval mortality under field condition. The IJs of EPN *H. indica* were effective against *H. armigera* larvae under field conditions. But it was observed that their activity lowered 14 DAS as effectiveness of EPN (*H. indica*) as a foliar spray to control *H. armigera* in the field decreased. Hence, the EPN (*H. indica*) as a foliar spray on chickpea crop against the *H. armigera* larvae has scope for further improvement.

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