Original Research Paper

Biocidal Efficacy of a New and Native Species of Entomopathogenic Nematode against Gram Pod Borer, *Helicoverpa armigera* (Hubner)

Abstract

Efficacy of new and native species of entomopathogenic nematode (EPN),*Heterorhabditis casmirica* SKUAST-K 104 was evaluated against gram pod borer,*Helicoverpa armigera*in laboratory conditions. Larval mortality was directly proportional to size of nematode inoculum level as well as time period but inversely proportional to larval size. *H. casmirica* SKUAST-K 104 applied @ 50, 100, 150 and 200 IJs per 2nd instar larva resulted in pest mortality by0.00, 8.33, 16.66, and 25.00 per cent, respectively at 24 hoursand they were statistically significant ($p \leq 0.05$)from each other. At 200 IJs inoculum level, 8.33, 16.66, 25.0, 41.66 and 50 per cent mortality of 5th instar larva was recorded at 24, 48, 72, 96 and 120 hours post inoculation, respectively.LC_{50} values was directly proportional to the size of larva but inversely proportional to size of nematode inoculum level. On the other hand, LT_{50} values was directly proportional to the size of larva but inversely proportional to size of nematode inoculum level.LC_{50}values calculated at 24 hours for 2nd, 3rd, 4th and 5thinstar larvae was256.88, 277.24, 326.25 and 384.25, respectively, whereas at 120 hours it was 126.11, 160.22, 184.36 and 219.14, respectively. Similarly, LT_{50}values calculated at inoculum level of 50 IJs per 2nd, 3rd, 4th and 5th instar larvae were 105.0 , 113, 122 and 131 hours, respectively but at highest inoculum level of 200 IJs, it was 75, 89, 94 and 100 hours, respectively.Nematode multiplication ratewithin the host cadaver was directly proportional to the size of the host. Minimum and maximum number of IJs/ larva was 2.72 x $10^5$and 1.03 x 10^5, obtained from 2nd and 5th instar larva, respectively.

Keywords: *Entomopathogenic nematodes, Heterorhabditis, Helicoverpa armigera, inoculum,* LC_{50}, LT_{50},*reproductive potential.*

1. INTRODUCTION

Helicoverpa armigera (Hübner), (Lepidoptera: Noctuidae) commonly known as the gram pod borer, poses a substantial threat to global agriculture and horticulture due to its notable characteristics such as high mobility, polyphagy, and facultative diapause as pupae, leading to a rapid turnover in generations [1]. This pest has exhibited its voracious appetite by feeding on 182 plant species from 47 families in the Indian subcontinent alone, causing significant economic losses estimated up to Rs. 1,000 crores in crops like cotton, pigeonpea, chickpea, groundnut, sorghum, pearl millet, and tomato [2]. The damage inflicted by *H. armigera* larvae includes the consumption of chickpea plant leaves and young seedlings. During pod formation, larvae penetrate the developing grain, creating holes in the pod and cause substantial agricultural damage. The predominant method of controlling *H. armigera* involves the use of pesticides, however, the development of resistance to commonly used insecticides has led to outbreaks of this pest [3]. Consequently, there is a pressing need for an alternative, eco-friendly, and economically viable pest management approach for chickpea growers.Entomopathogenic nematodes (EPNs), specifically those belonging to the families Steinernematidae and Heterorhabditidae, emerge as promising candidates for pest control. These nematodes are generalist pathogens, targeting insect pests from various orders. Their pathogenicity is facilitated by symbiotic bacteria *viz*., *Photorhabdus*or*Xenorhabdus*, which are introduced into insect pests by infective juveniles (IJs), the only free-living stage found in soil [4,5]. IJsenter into the insect body through natural openings or by rupturing the cuticle, releasing bacteria that produce toxins and hydrolytic exo-enzymes, leading to the host's death within 48 hours [6,7,8,9].EPNs offer advantages such as ease of mass production, formulation, and application [8,10]. They are also compatible with many conventional insecticides at low doses and short-term exposure, making them a globally exploited beneficial microorganism against foliar and soil-dwelling insect pests [11,12].

Keeping in view the advantages of EPNs in crop insect pest control, the present study was undertaken to evaluate its efficacy against *H. armigera* under laboratory conditions.

2. MATERIALS AND METHODS

2.1.Collection of Gram pod borer, *Helicoverpa armigera*

Larvae of *Helicoverpa armigera* were collected from unsprayed experimental plots of chickpea cultivated at the Faculty of Agriculture, Wadura, Sopore campus of Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-K). The collection aimed to assess the bioefficacy of a locally isolated entomopathogenic nematode, *Heterorhabditis casmirica* SKUAST-K 104.

2.2. Preparation of nematode culture

H. casmirica SKUAST-K 104, a new nematode strain, recently isolated and identified from Anantnag district of Jammu and Kashmir, India [13] was obtained from the laboratory of Division of Entomology, Faculty of Agriculture, SKUAST-K. *H. casmirica*.The nematodestrain was cultured using larvae of the Greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae). Ten 5th instar larvae of *G. mellonella* were placed in 20 cm diameter petri dishes lined with filter paper, each inoculated with approximately 1 x 10^3IJscontained in 0.5 ml of sterilized distilled water.The petri dishes were placed in BOD incubator at 19 ± 2^oC. After 2-3 days, dead larvae were transferred to a modified White trap [14]. IJs emerging from *G. mellonella* larvae were harvested in a clean beaker till the production declined. After one hour, supernatant was discarded and the process of re-suspending IJs in sterilized distilled water and decanting was repeated three times till a clean nematode suspension is obtained.IJs were surface sterilized with 0.1% sodium hypochlorite [15] and washed with H_2O.The resulting suspension was then re-suspended in distilled water at a concentration of approximately 1 x 10^3IJs/ml and stored in 250 ml tissue culture flasks in a BOD incubator maintained at 10 ± 1 °C.

2.3. Efficacy of nematode against gram pod borer,*Helicoverpa armigera*

In the present study, bioassays were conducted to assess the efficacy of nematode, *H. casmirica* SKUAST-K 104@ 50, 100, 150 and 200 IJs against 2nd, 3rd, 4th and 5th larval instars of *H. armigera*. Bioassays were carried out in six-well plates, lined with Whatman filter paper No. 1.Each well of a single plate was evenly sprayed with one of the above mentioned single concentration of IJs suspended in 350µl of distilled water.A surface sterilized larva of *H. armigera*approximately equal in size and weight was placed in each well. Such eight six-well plates were prepared, two for each concentration of IJs (n =12). Moreover, two six well plate wherein larva inoculated with distilled water only was included as control. Each plate was covered with their respective lid, labeled, kept in plastic bags to conserve moisture and incubated in BOD at 20 ± 2^o C temperature. The experiment was observed at five specific time intervals: 24, 48, 72, 96, and 120 hours, for recording of larval mortality.

2.4. Reproductive potential of nematode within insect cadaver

To record the reproductive potential of EPN strain, White traps were observed daily under a stereoscopic microscope for the emergence of IJs from the cadaver of *H.armigera*.IJs emerged from a single cadaver were collected in a beaker on daily basis till the emergence stopped. IJs were stored in BOD at 15±1 ºC and the number of IJs produced per cadaver was determinedby dilution counts [12].

2.5. Statistical analysis

Larval mortality was subjected to probit analysis using SPSS software. LC_{50} (Lethal concentration 50) and LT_{50} (Lethal time 50) values were calculated at 95% confidence limit.

3. RESULTS AND DISCUSSION

3.1. Nematode susceptibility to*Helicoverpa armigera*

Larvae of *H. armigera*exhibited susceptibility to the test nematode, *H. casmirica* SKUAST-K 104.However, notable variations were observed in nematode pathogenicity, encompassing both virulence (lethality) and efficacy (time to lethality). The duration required by the nematode to cause larval mortality increased proportionally with larval size. Lower nematode inoculum levels necessitated more time for larval mortality, but with the increase in inoculum mortality time decreased.In case of 2nd instar larva, *H. casmirica* SKUAST-K 104 applied @ 50, 100, 150 and 200 IJs perlarva resulted in pest mortality by0.00, 8.33, 16.66, and 25.00 per cent, respectively at 24 hours, which increased to 25.0, 41.66, 50.0 and 58.33 per cent, respectively at 72 hours and 50.0, 66.66, 75 and 83.33 per cent, respectively at 120 hours post inoculation and they were statistically significant ($p \leq 0.05$)from each other within each time period and at each inoculum level(Table 1).Similar trends were observed for 3rd, 4th and 5th instar larvae. Though, in case of 5th instar larvae, no mortality was observed upto 72 hours post inoculation interval with the treatmentof *H. casmirica* SKUAST-K 104 applied @ 50 IJs/ larva but at 96 and 120 hours larval mortality was recorded 8.33 and 25.0 per cent respectively. At 200 IJs inoculum level, 8.33, 16.66, 25.0, 41.66 and 50 per cent mortality of 5th instar larva was recorded at 24, 48, 72, 96 and 120 hours post inoculation, respectively and they were significantly different ($p \leq 0.05$) from each other.Hence, in the present study, time required by the nematode to cause larval mortality increased with the increase in larval size. At lower nematode inoculum levels more time was consumed for larval mortality and vice-versa. Our findings confirm the report of several other workers that nematode concentration was directly proportional to rate of insect mortality[11, 16, 17]

Table 1. Efficacy of *Heterorhabditis casmirica* SKUAST-K 104against different larval instarsof gram pod borer, *Helicoverpa armigera* under laboratory conditions.

IJs/larva	2nd Instar larvae						3rd Instar larvae					
	% mortality hours after treatment						% mortality hours after treatment					
	24	48	72	96	120	Mean	24	48	72	96	120	Mean
50	0.00** (4.05)*	8.33 (10.00)	25.00 (30.01)	41.66 (40.52)	50.00 (45.57)	**24.99 (26.03)**	0.00 (4.05)	8.33 (10.00)	16.66 (24.09)	33.33 (35.00)	41.66 (40.52)	**19.99 (22.73)**
100	8.33 (10.00)	25.00 (30.01)	41.66 (40.52)	58.33 (49.42)	66.66 (53.95)	**39.99 (36.78)**	0.00 (4.05)	16.66 (24.09)	25.00 (30.01)	41.66 (40.52)	50.00 (45.57)	**26.66 (28.85)**
150	16.66 (24.09)	33.33 (35.00)	50.00 (45.57)	66.66 (53.95)	75.00 (60.03)	**48.33 (43.72)**	8.33 (10.00)	16.66 (24.09)	33.33 (35.00)	50.00 (45.57)	58.33 (49.42)	**33.33 (32.81)**
200	25.00 (30.01)	41.66 (40.52)	58.33 (49.42)	75.00 (60.03)	83.33 (70.08)	**56.66 (50.01)**	16.66 (24.09)	25.00 (30.01)	41.66 (40.52)	58.33 (49.42)	75.00 (60.03)	**43.34 (40.81)**
Control	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	8.33 (10.00)	8.33 (10.00)	**3.33 (6.43)**	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	8.33 (10.00)	8.33 (10.00)	**3.33 (6.43)**
Mean	9.99 (14.44)	21.66 (23.91)	34.99 (33.91)	49.99 (42.78)	56.66 (47.92)	**34.65 (32.59)**	4.99 (9.24)	11.66 (18.44)	23.33 (26.73)	38.33 (36.10)	46.66 (41.10)	**24.99 (26.32)**
IJs/larva	**4th Instar larvae**						**5th Instar larvae**					
	% mortality hours after treatment						**% mortality hours after treatment**					
	24	**48**	**72**	**96**	**120**	**Mean**	**24**	**48**	**72**	**96**	**120**	**Mean**
50	0.00 (4.05)	0.00 (4.05)	8.33 (10.00)	16.66 (24.09)	33.33 (35.00)	**11.66 (15.43)**	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	8.33 (10.00)	25.00 (30.01)	**6.66 (10.43)**
100	0.00 (4.05)	8.33 (10.00)	16.66 (24.09)	25.00 (30.01)	41.66 (40.52)	**18.33 (21.73)**	0.00 (4.05)	0.00 (4.05)	8.33 (10.00)	16.66 (24.09)	33.33 (35.00)	**11.66 (15.43)**
150	0.00 (4.05)	16.66 (24.09)	25.00 (30.01)	33.33 (35.00)	50.00 (45.57)	**24.99 (27.74)**	0.00 (4.05)	8.33 (10.00)	16.66 (24.09)	33.33 (35.00)	41.66 (40.52)	**19.99 (22.73)**
200	8.33 (10.00)	25.00 (30.01)	33.33 (35.00)	50.00 (45.57)	66.66 (53.95)	**36.66 (34.90)**	8.33 (10.00)	16.66 (24.09)	25.00 (30.01)	41.66 (40.52)	50.00 (45.57)	**28.33 (30.03)**
Control	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	8.33 (10.00)	8.33 (10.00)	**3.33 (6.43)**	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	8.33 (10.00)	8.33 (10.00)	**3.33 (6.43)**
Mean	1.66 (4.43)	9.99 (14.44)	16.66 (20.63)	26.66 (28.93)	39.99 (37.00)	**18.99 (21.08)**	1.66 (5.24)	4.99 (9.24)	9.99 (14.44)	21.66 (23.92)	31.66 (32.22)	**13.99 (17.01)**
CD(p≤0.05)	Treatments (T) = **0.652**, Time (Ti) =**0.623**, Instar (I) =**0.566** Treatments*Time (T*Ti) =**0.141**, Treatments*Instar (T*I) =**0.126** Time*Instar (Ti*I) = **0.639** Treatments*Time*Instar (T*Ti*I) =**0.283** *Figures in parentheses are arc sine transformed values ** Each figure is mean of mean of 12 replications											

3.2. Median lethal concentration

Lethal concentration 50 (LC_{50}) values were inversely proportional to time period but directly proportional to larval size. At 24 hours, calculated LC_{50}value for 2nd instar larva of *H. armigera* was 256.88 which decreased to 185.76 and 126.11 at 72 and 120 hours, respectively (Table 2). For 3rd, 4th and 5th instar larvae, LC_{50}values were 277.24, 326.25 and 384.25, respectively at 24 hours, 231.85, 268.23 and 298.21, respectively at 72 hours and 160.22, 184.36 and 219 .14, respectively at 120 hours. Thus, IJs required to kill 50 per cent population of *H. armigera* have inverse relationship with the time period but directly proportional to size of larva.Thus, LC_{50} values calculated in the present experiment showed that IJs of *H. casmirica* SKUAST-K 104 have an inverse relationship with the time period but directly proportional to larval size[12, 18, 19, 20].

Table 2.Median lethal concentration (LC_{50}) (IJs/ larva) of *Heterorhabditis casmirica* SKUAST-K 104 against different larval instars of gram pod borer, *Helicoverpa armigera* at different time intervals.

Helicoverpa armigera	**Lethal concentration (LC_{50}) (*IJs/ larva)**				
	Post nematode inoculation interval (hours)				
	24	**48**	**72**	**96**	**120**
2nd Instar	256.88	225.41	185.76	148.22	126.11
3rd Instar	277.24	248.32	231.85	194.38	160.22
4th Instar	326.25	295.14	268.23	237.59	184.36
5th Instar	384.25	326.21	298.21	251.26	219 .14

*IJs = Infective Juveniles

3.3. Median lethal time

Calculated Lethal time 50 (LT_{50}) was directly proportional to the size of larva but inversely proportional to size of nematode inoculum level. At inoculum level of 50 IJs per larva, time required to kill 50 per cent2nd instar larva of *H. armigera* was 105.0 hours, which increased to 113, 122 and 131 hours for 3rd, 4th and 5th instar larvae, respectively (Table 3). Similarly at 100 IJs, 93.0, 105, 112 and 122 hours were required to kill 2nd, 3rd, 4th and 5th instar larvae, respectively. At the highest nematode inoculum level used in the experiment *i.e.* @ 200 IJs per larva, LT_{50}values for 2nd, 3rd, 4th and 5th instar larvae were 75, 89, 94 and 100, respectively. The results demonstrate a clear inverse relationship between the concentration of IJs and the time required to attain 50 per cent mortality to different instar larvae of*H. armigera* but on the other hand direct relationship between the larval size and time period.These results are in good agreement with the findings of other workers who evaluated native isolates of *Steinernema* and *Heterorhabditis* against different insect pests[12, 18, 19, 20].

Table 3.Median lethal time (LT_{50}) of *Heterorhabditis casmirica* SKUAST-K 104 against differentlarval instars of gram pod borer, *Helicoverpa armigera* at different nematode concentrations.

Helicoverpa armigera	Lethal Time (LT_{50}) (hours)			
	Number of nematodes (*IJs/ larva)			
	50	100	150	200
2nd Instar	105.00	93.00	84.00	75.00
3rd Instar	113.00	105.00	94.00	89.00
4th Instar	122.00	112.00	104.00	94.00
5th Instar	131.00	122.00	109.00	100.00

*IJs = Infective Juveniles

3.4. Nematode reproductive potential

Multiplication rate of *H. casmirica* SKUAST-K 104 within the host cadaver was directly proportional to the size of the host. Nematode multiplied profusely in large sized larvae as compared to smaller size.On an average, maximum production of IJs per larva was recorded from 5th instar larva (2.72 x 10^5), followed by 4th (2.38 x 10^5), 3rd (1.82 x 10^5) and 2nd instar larva (1.03 x 10^5) (Fig. 1).Thus, with the increase in larval size of *H. armigera*, the multiplication rate of nematode also increased.

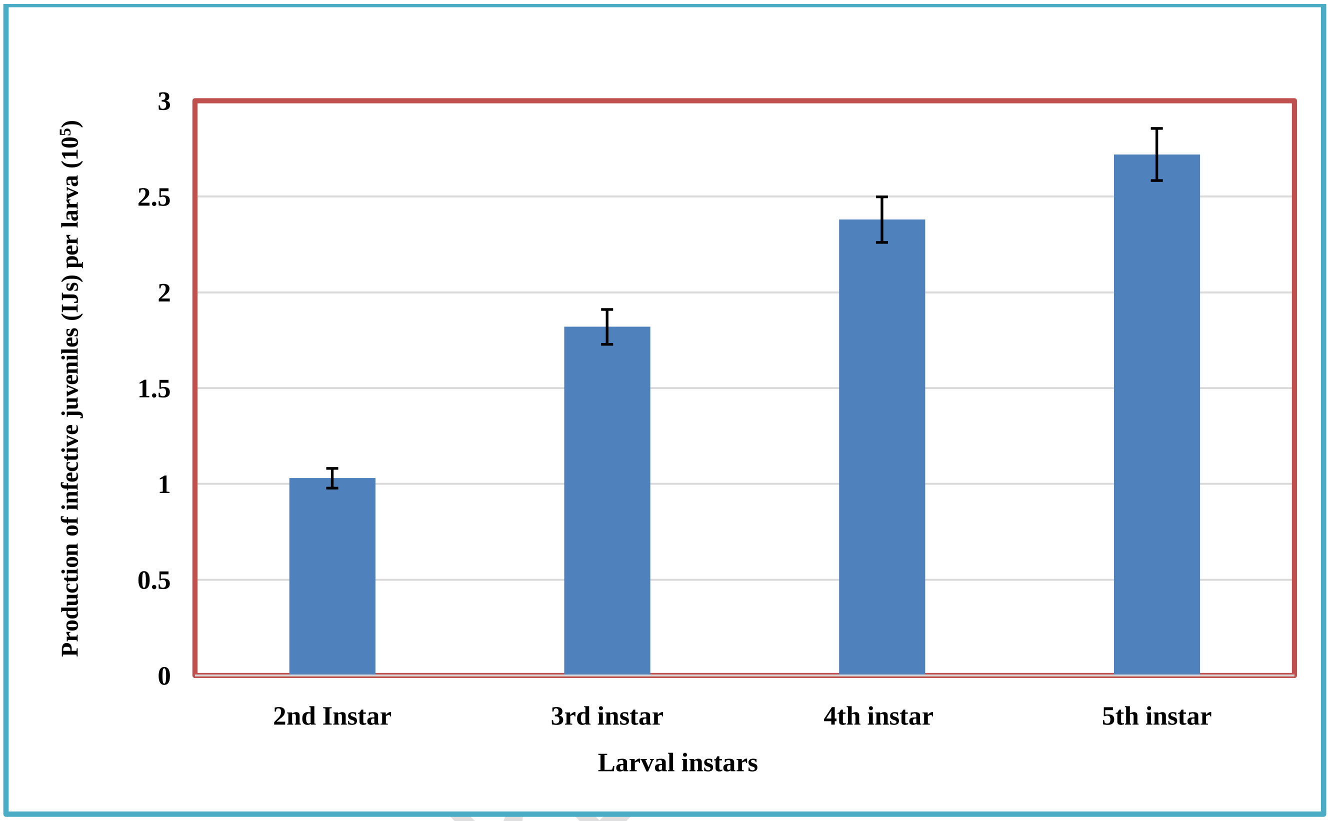

Fig. 1.Population of infective juveniles (IJs) of *Heterorhabiditscasmirica* SKUAST-K104obtained from different larval instars of Gram pod borer, *Helicoverpa armigera.*

Hence, it can be suggested that more nutrients are available for nematodes in large sized larva which became conducive for their growth and development, ultimately resulted in high multiplication rate and producing more number of progenies. Our findings support the work of many other researchers who assessed the reproductive potential of *Steinernema* and *Heterorhabditis* in insect larvae of varying sizeand observed differences in progeny production[11, 21].However, other factors may also be responsible such as type of nematode isolates, species, type of bacterial symbiont carried by the nematode, hostsusceptibility, nematode invasion rates and other abiotic conditions[22, 23, 24, 25, 26, 27, 28, 29].

4. CONCLUSION

Under laboratory conditions, the native nematode strain,*H. casmirica* SKUAST-K 104 demonstrated high efficacy in terms of causing mortality to gram pod borer, *H. armigera*and multiplying within its body. However, on the basis of our preliminary results, the nematode performance needs to be evaluated for its efficacy under field conditionsbefore its final recommendation to include it as one of the components in integrated management programme of *H.armigera*.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

COMPETING INTERESTS

We, all the authors declare that no competing interests exist.

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