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Bio-efficacy of entomopathogenic nematode *Heterorhabditis indica* against *Helicoverpa* *armigera* (Hubner)

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Abstract

The investigations on bio-efficacy of entomopathogenic nematode, *Heterorhabditis indica* against *Spodoptera litura* (Fabricius) was carried out at Biocontrol Research Laboratory, Department of Entomology, Junagadh Agricultural University, Junagadh during 2014-15. Bio-efficacy of *H. indica* against *H. armigera* revealed that *H. indica* caused cent per cent larval mortality of 3rd, 4th and 5th instar larvae of *H. armigera* at inoculum levels of 20, 40, 60, 80 and 100 IJs/larva after exposure time of 120 hrs. Third instar larvae were most susceptible to *H. indica* than fourth and fifth instar larva.

Keywords: Entomopathogenic nematode *Heterorhabditis indica*, *Helicoverpa armigera* (Hubner)

Introduction

Among the different insect pests infesting this crop in Saurashtra region of Gujarat state, *Helicoverpa armigera* (Hubner) are considered as a key foliar insect pests infesting this crop in Saurashtra region of Gujarat state.

To overcome these insect pest problems, chemical insecticides are largely in use. Their indiscriminate use has posed several risks and ill effects such as environmental pollution, ecological imbalance, resistance in insects, pests resurgence, destruction of beneficial insects and natural enemies of pests, increased the level of pesticide residues in soil, water, fodder, as well as food crop, health hazards etc. In view of these, today's need is to promote the ecologically and environmentally compatible bio control methods in crop protection as alone as well as a major input in overall Integrated Pest Management (IPM) practices/packages. Several bio agents including arthropod parasites, predators and microbes (bacteria, fungi, virus, protozoa and entomophilic nematodes) are now proved very useful in sustainable and safe agriculture.

Entomo pathogenic nematodes (EPNs) especially members of genus *Steinernema* (27 species) and *Heterorhabditis* (8 valid species) are innovative bio agents for plant protection scientists of India. These EPNs are having symbiotic bacteria (Genus *Steinernema* – *Xenorhabdus* spp. and Genus *Heterorhabditis* – *Photorhabdus* spp.) which are gram negative, facultative anaerobic rods belonging to enterobacteriaceae having dimorphism nature. These mutually associated bacteria cause quick mortality of target insects having wide host range among class Insecta. They are also found safe to non - target organisms and compatible with many pesticides. Symbiont also produces antifungal and antibacterial metabolites like Xenorhabdin, Xenocoumacins, Xenoxodus, Nematophines (3' indol ethyl 3' methyl-2' oxo) and soluble proteinoous compounds which make EPN a broad spectrum bio agents for biological suppression of agricultural pests (Vyas, 2000) [8].

EPNs are naturally found in soil and are extra ordinarily lethal to many important soil insect pests and safe to plants and animals (Smart, 1995) [7]. Due to this high degree of safety compared to chemicals, Application of EPN does not require special safety equipments and reduces time. Also they have no residues, avoid ground water contamination, general environmental pollution and are safe to pollinators and arthropod parasites.

In general many biological agents require days to weeks to kill the target, but EPN juveniles (IJs) working with their symbiotic bacteria, kills target insect within 24-72 hrs. Extreme conditions like temperature and moisture will affect moderately to the immature stage of EPN, many EPN species and strains are better adapted to wide range of extreme environmental conditions with long persistence in soil.

In India, *Steinernema* (nr. riobrave) was first time reported from Gujarat state (Ganguly *et al.*, 2002) [2]. Besides these few more species were discovered during last decade in India and many scientists have taken keen interest in entomopathogenic nematodes as an arsenal for soil insect pests in the country.

Entomopathogenic nematode families, *Steinernematidae* and *Heterorhabditidae* have been proved more useful against insect pests. EPNs are now emerged as second most valuable bio insecticide besides *Bacillus thuringiensis* for the effective suppression of insect pests in western countries during last three decades. In India, scientists have tested imported EPN cultures against few important insect pests during last three decades proving them very useful. EPN DD – 136 strain (*S. carpocapsae*) against *S. litura* (Narayan and Gopalkrishna, 1987) [4] DD – 136 strain (*S. glaseri*) against *H. consanguinea* (Vyas and Yadav 1992) [9] and *H. armigera* (Patel and Vyas, 1995) [5].

Materials and Methods

Laboratory Rearing of *H. armigera*

In order to develop the initial culture of *H. armigera* (Plate V), large number of full grown larvae were collected from groundnut fields of Junagadh Agricultural University campus, Junagadh. The field collected larvae were reared individually in laboratory for further multiplication. Fresh groundnut leaves were provided as food for larvae and the foods were changed every day in the morning till pupation. Healthy pupae were selected and allowed to emerge as adults. The male and female adults were transferred in oviposition cages in ratio of 3:1 and provided fresh tender leaves bouquet of their host plants or plastic strips for laying eggs by adults. Adults were fed with 10% honey solution. The larvae hatch out from eggs were transferred individually in plastic vials and further reared on fresh, clean host plant leaves. All the containers used for insect rearing were chemically cleaned to avoid cross infection.

Methodology

Bio-efficacy of *H. indica* against 3rd, 4th and 5th instar larvae of *H. armigera* was carried out individually in petri dishes. Different doses (20, 40, 60, 80 and 100 IJs/ larva) of the *H. indica* were prepared in sterile distilled water following serial dilutions. Groundnut leaves were provided as food. EPN suspension of each dilution was poured using micro pipette into respective petri dishes, simultaneously sterile distilled water was applied in control treatment.

After 10 minute, laboratory reared all the instars of *H. armigera* were released individually in each petridish. The tray with petri dishes was kept at 27± 2° C for 5 days in BOD incubator. Fresh groundnut leaves were provided daily to *H. armigera*.

Observation recorded

The dead larvae was recorded at 24 h intervals up to 120 h. *H. indica* induced mortality in the larvae was confirmed by observing under microscope for presence of EPN.

Analysis of data

The data analysis was carried out by appropriate statistical method in Microsoft excel software.

Results and Discussion

The present study was framed with an aim to assess the bio-efficacy of *H. indica* against 3rd, 4th and 5th instar larva of *H. armigera* in Biocontrol Research Laboratory. The result observed on bio-efficacy is presented and discussed here.

Third instar larva

After 24 hrs

The results demonstrated in Table 1 and exemplified in Fig. 1 discovered that the *H. indica* caused 80% larval mortality at

100 IJs/larva. At 80, 60, 40 and 20 IJs/larva, 66.69%, 53.34%, 46.66% and 33.17% larval mortality, respectively was observed. The mortality decreased with lower dose of inoculum level. In control set, no larval mortality was recorded.

After 48 hrs

The data on bio-efficacy of *H. indica* against *H. armigera* is summarized in Table 1 and depicted in Fig. 1. The experimental result indicated that the higher dose (100 IJs/larva) caused cent per cent larval mortality and it was at par with treatments of 80 IJs/larva, which caused 93.17% larval mortality. Whereas, treatments of 60, 40 and 20 IJs/larva caused 80%, 66.69% and 60.00% larval mortality, respectively. In control set, no larval mortality was recorded.

After 72 hrs

The results (Table 1 and Fig. 1) on mortality of 3rd instar larva of *H. armigera* at 72 hrs after the application of *H. indica* revealed that the higher doses (100, 80 and 60 IJs/larva) caused cent per cent larval mortality and it was at par with treatments of 40 IJs/larva which caused 93.17% mortality followed by treatments of 20 IJs/larva, which caused 80% larval mortality. In control set, no larval mortality was recorded.

After 96 hrs

The data presented in Table 1 and illustrated in Fig. 1 discovered that the all the doses (100, 80, 60, 40 and 20 IJs/larva) caused cent per cent larval mortality of *H. armigera*. In control set, no larval mortality was recorded.

After 120 hrs

The results demonstrated in Table 1 and exemplified in Fig. 1 discovered that the mortality of 3rd instar larvae of *H. armigera* reached up to cent per cent at all the doses (100, 80, 60, 40 and 20 IJs/larva) after 120 hrs exposure time. In control set, no larval mortality was recorded.

Fourth instar larva

After 24 hrs

The result on bio-efficacy of *H. indica* against 4th instar larva of *H. armigera* were precisely framed in Table 2 and portrayed in Fig. 2. It indicated that the higher dose (100 IJs/larva) caused 66.74% larval mortality followed by treatments of 80, 60, 40 and 20 IJs/larva, which caused 46.65%, 40%, 33.31% and 19.98% larval mortality, respectively. In control set, no larval mortality was recorded.

After 48 hrs

The data summarized in Table 2 and illustrated in Fig. 2 discovered that the 86.63% larval mortality was obtained at 100 IJs/larva of *H. armigera* at 48 hrs after the application of *H. indica*. While, the remaining doses (80, 60, 40 and 20 IJs/larva) caused 80%, 73.37%, 60% and 46.66% larval mortality, respectively. In control set, no larval mortality was recorded.

After 72 hrs

The results (Table 2 & Fig. 2) on mortality of 4th instar larva of *H. armigera* at 72 hrs after the application of *H. indica* revealed that the higher doses (100 and 80 IJs/larva) caused cent per cent larval mortality and it was at par with treatments of 60 IJs/larva, which caused 93.17% mortality. Treatments of 40 and 20 IJs/larva caused 86.13% and 73.37% larval mortality, respectively. In control set, no larval mortality was recorded.

After 96 hrs

At 96 hrs of application of *H. indica*, the mortality of 4th instar larva of *H. armigera* reached up to cent per cent at inoculum level of 100, 80, 60 and 40 IJs/larva. In case of 20 IJs/larva, 92.51% larval mortality was obtained. In control set, no larval mortality was recorded.

After 120 hrs

The results demonstrated in Table 2 and exemplified in Fig. 2 discovered that the all the doses (100, 80, 60, 40 and 20 IJs/larva) caused cent per cent larval mortality of 4th instar larva of *H. armigera* at 120 hrs after the application of *H. indica*. In control set, no larval mortality was recorded.

Fifth instar larva**After 24 hrs**

The data presented in Table 3 and represented in Fig. 3. discovered that the higher dose (100 IJs/larva) caused 53.37% larval mortality of 5th instar larva of *H. armigera* at 24 hrs after the application of *H. indica*. The remaining doses of 80, 60, 40 and 20 IJs/larva caused 40%, 33.33%, 26.63% and 13.85% larval mortality, respectively. In control set, no larval mortality was recorded.

After 48 hrs

At 100 IJs/larva of *H. indica*, 80% larval mortality (Table 3 and Fig. 3) was obtained at 24 hrs after the application. In case of treatments of 80, 60, 40 and 20 IJs/larva, 73.37%, 66.69%, 53.41% and 40% larval mortality is obtained, respectively. In control set, no larval mortality was recorded.

After 72 hrs

The data on bio-efficacy of *H. indica* against *H. armigera* is summarized in Table 3 and depicted in Fig. 3. The experimental result indicated that the higher dose (100 IJs/larva) caused cent per cent larval mortality followed by treatments of 80, 60, 40 and 20 IJs/larva, which caused

92.51%, 86.29%, 80% and 66.74% larval mortality, respectively. In control set, no larval mortality was recorded.

After 96 hrs

The results (Table 3 & Fig. 3) on mortality of 5th instar larva of *H. armigera* at 96 hrs after the application of *H. indica* revealed that the higher doses (100, 80, 60 and 40 IJs/larva) caused cent per cent larval mortality followed by treatments of 20 IJs/larva, which caused 86.32% larval mortality. In control set, no larval mortality was recorded.

After 120 hrs

The data presented in Table 3 and illustrated in Fig. 3 discovered that the all the doses (100, 80, 60, 40 and 20 IJs/larva) of *H. indica* caused cent per cent larval mortality of 5th instar larva of *H. armigera* at 120 hrs after the application. In control set, no larval mortality was recorded.

It is concluded from the present study that the bio-efficacy of *H. indica* against *H. armigera* is increased with rise in inoculum level and exposure time. Highest mortality was observed in third instar larva than fourth and fifth instar larva.

It is accomplished from the present study that the third instar larva of *H. armigera* are more sensitive to *H. indica* than fourth and fifth instar larva and it support the work carried out by Divya *et al.* (2010) [1], who reported that the all the stages of larvae of *H. armigera* were highly susceptible to *H. indica* however, the degree of susceptibility differed according to instars, dose and periods of exposure. The result also showed that early larval instars were more susceptible to 300 IJs of *H. indica*/larva than 4th and 5th larval instars when exposed for 24 h.

Present findings are in agreement with the results of Rishi Pal and Prasad (2012) [6], who reported that the *H. indica* causing 73.3 to 100% larval mortality at 48 hrs of exposure.

Our research pertinent with findings of Glazer and Novan (1990) [3], who reported that insect mortality was directly correlated with doses of *S. feltiae*. They reported cent per cent killing of larvae at 200 IJs after 48 hrs exposure.

Table 1: Bio-efficacy of *H. indica* against third instar larva of *H. armigera*

Treatment No.	Dose (IJs / larva)	Percent mortality of third instar larva after				
		24 hrs.	48 hrs.	72 hrs.	96 hrs.	120 hrs.
T1	20	35.17* (33.17)	50.77 (60.00)	63.44 (80.00)	82.73 (100)	82.73 (100)
T2	40	43.08 (46.66)	54.75 (66.69)	74.85 (93.17)	82.73 (100)	82.73 (100)
T3	60	46.91 (53.34)	63.44 (80.00)	82.73 (100)	82.73 (100)	82.73 (100)
T4	80	54.75 (66.69)	74.85 (93.17)	82.73 (100)	82.73 (100)	82.73 (100)
T5	100	63.44 (80.00)	82.73 (100)	82.73 (100)	82.73 (100)	82.73 (100)
T6	Control	7.40 (0)	7.40 (0)	7.40 (0)	7.40 (0)	7.40 (0)
S. Em. ±		1.45	1.76	1.72	0.57	0.57
C. V. %		6.02	5.51	4.54	1.41	1.41
C.D. at 5%		4.47	5.45	5.30	1.76	1.76

*Arc sin transformed values Figures in parenthesis are retransformed values.

Table 2: Bio-efficacy of *H. indica* against fourth instar larva of *H. armigera*

Treatment No.	Dose (IJs / larva)	Percent mortality of fourth instar larva after				
		24 hrs.	48 hrs.	72 hrs.	96 hrs.	120 hrs.
T1	20	26.55 (19.98)*	43.08 (46.66)	58.93 (73.37)	74.12 (92.51)	82.73 (100)
T2	40	35.25 (33.31)	50.77 (60.00)	68.14 (86.13)	82.73 (100)	82.73 (100)
T3	60	39.23 (40.00)	58.93 (73.37)	74.85 (93.17)	82.73 (100)	82.73 (100)
T4	80	43.08 (46.65)	63.44 (80.00)	82.73 (100)	82.73 (100)	82.73 (100)
T5	100	54.78 (66.74)	68.55 (86.63)	82.73 (100)	82.73 (100)	82.73 (100)
T6	Control	7.40 (0)	7.40 (0)	7.40 (0)	7.40 (0)	7.40 (0)
S. Em. ±		1.24	1.54	1.77	0.89	0.57
C. V. %		6.26	5.48	4.92	2.26	1.68
C.D. at 5%		3.83	4.74	5.47	2.75	1.41

*Arc sin transformed values Figures in parenthesis are retransformed values.

Table 3: Bio-efficacy of *H. indica* against fifth instar larva of *H. armigera*

Treatment No.	Dose (IJs / larva)	Percent mortality of fifth instar larva after				
		24 hrs.	48 hrs.	72 hrs.	96 hrs.	120 hrs.
T1	20	21.85 (13.85)*	39.23 (40.00)	54.78 (66.74)	68.29 (86.32)	82.73 (100)
T2	40	31.07 (26.63)	46.95 (53.41)	63.44 (80.00)	82.73 (100)	82.73 (100)
T3	60	35.26 (33.33)	54.75 (66.69)	68.26 (86.29)	82.73 (100)	82.73 (100)
T4	80	39.23 (40.00)	58.93 (73.37)	74.12 (92.51)	82.73 (100)	82.73 (100)
T5	100	46.93 (53.37)	63.44 (80.00)	82.73 (100)	82.73 (100)	82.73 (100)
T6	Control	7.40 (0)	7.40 (0)	7.40 (0)	7.40 (0)	7.40 (0)
S. Em. ±		1.18	1.54	1.42	0.83	0.57
C. V. %		6.79	5.94	4.23	2.13	1.41
C.D. at 5%		3.65	4.76	4.40	2.56	1.76

*Arc sin transformed values Figures in parenthesis are retransformed values.

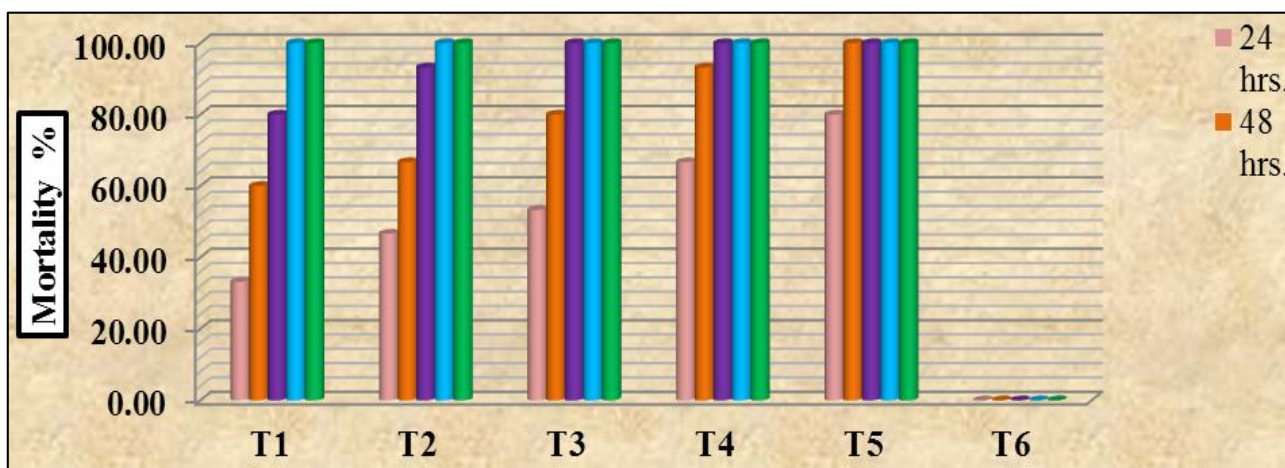


Fig 1: Bio-efficacy of *H. indica* against third instar larva of *H. armigera*

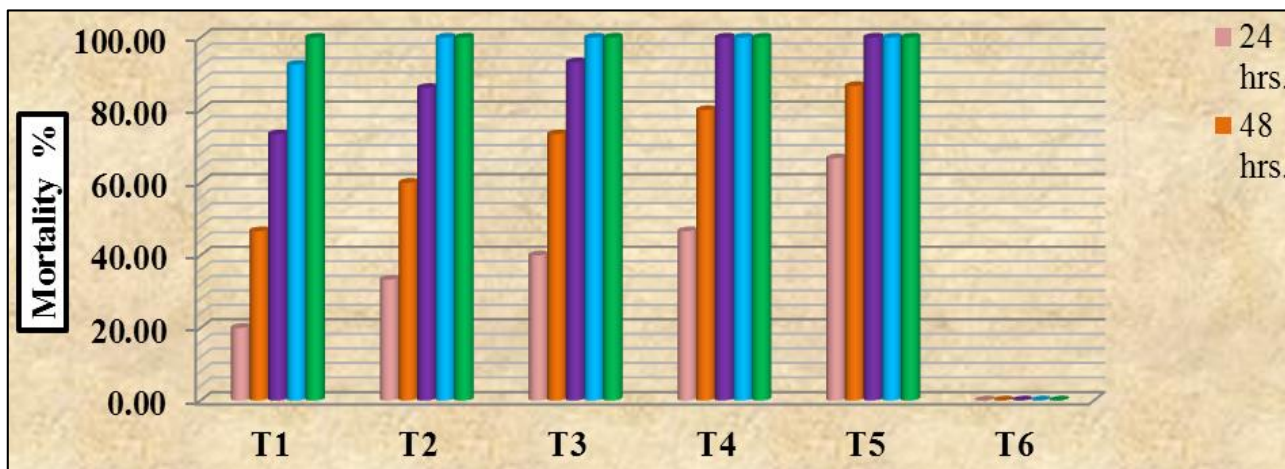


Fig 2: Bio-efficacy of *H. indica* against fourth instar larva of *H. armigera*

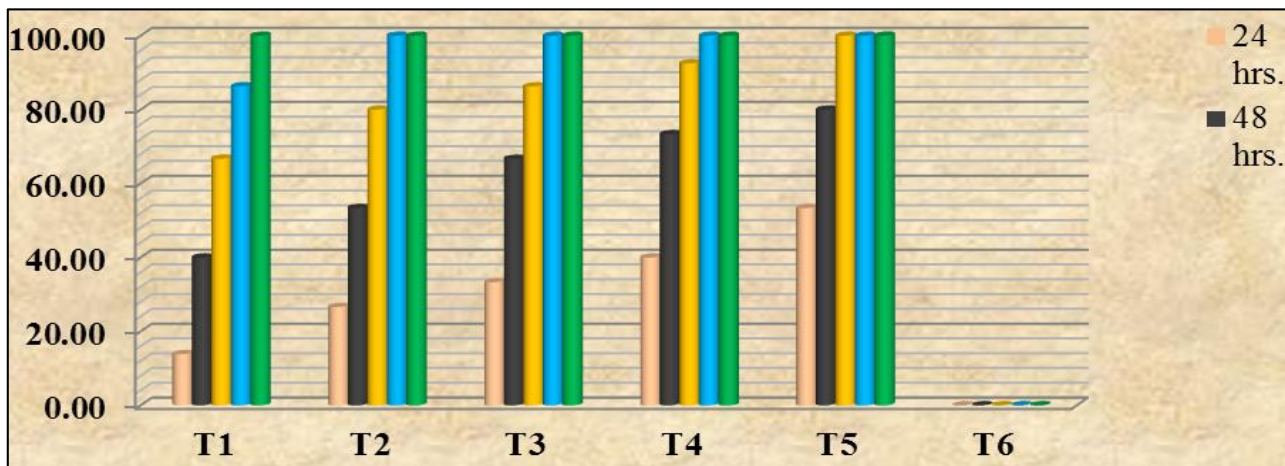
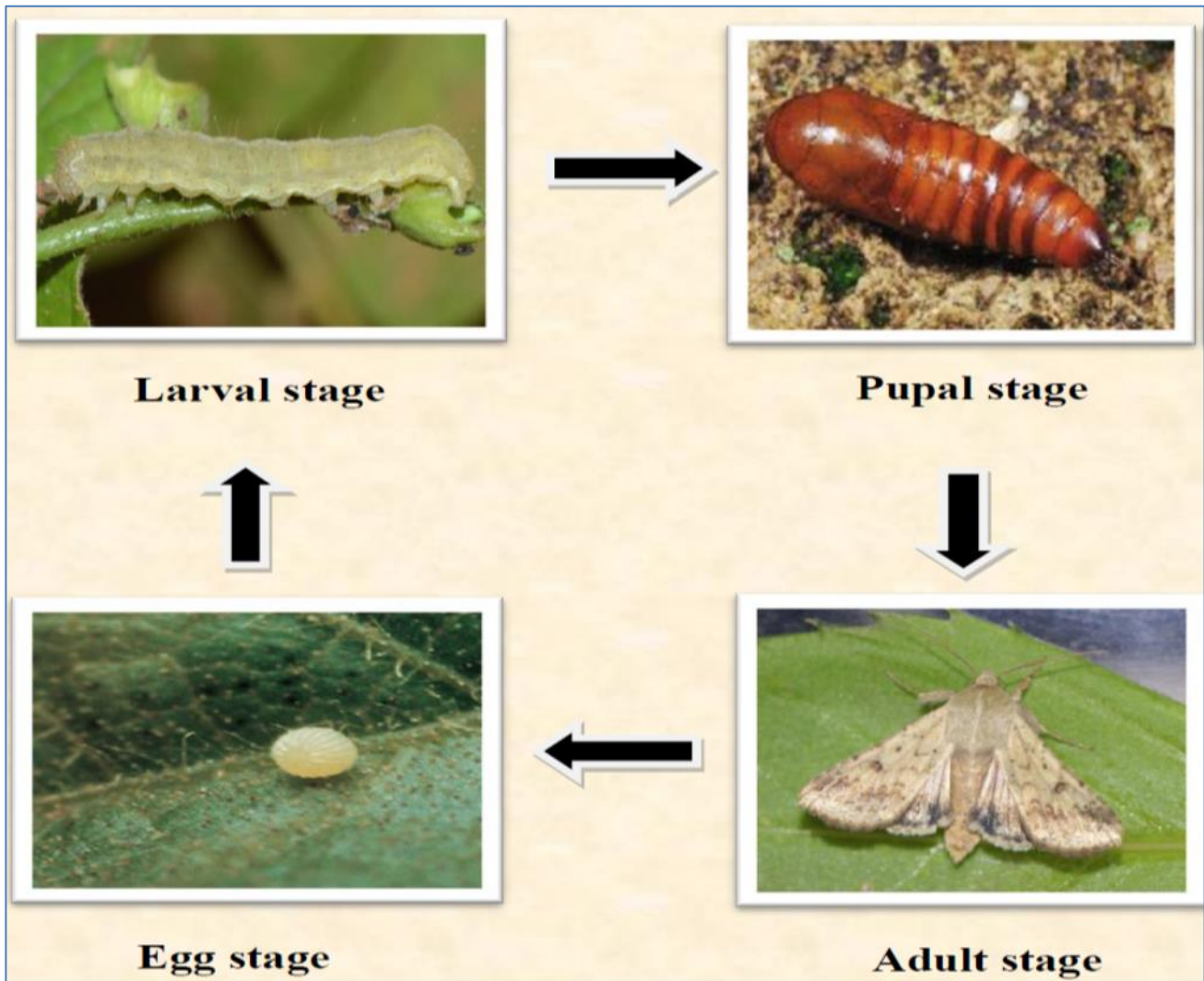


Fig 3: Bio-efficacy of *H. indica* against fifth instar larva of *H. armigera*



Different developmental stages of *H. armigera*

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