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Dosage-mortality response of gram pod borer, *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) to different geographical isolates of *Helicoverpa armigera* nucleopolyhedrosis virus

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Abstract
The relative toxicity of eight geographical isolates of *Helicoverpa armigera* nucleopolyhedrosis virus (HaNPV) to gram pod borer, *Helicoverpa armigera* (Hubner) was assessed in the laboratory. The median lethal concentration (LC_{50}) values of different HaNPV isolates ranged from 4.3 x 10^6 to 1.9 x 10^7 PIBs/ml. Among the eight isolates, the Gulbarga (Karnataka) isolate was highly toxic, with the LC_{50} value of 4.3 x 10^6 PIBs/ml, while the Dhule (Maharashtra) isolate was least toxic (1.9 x 10^7 PIBs/ml). The median lethal time (LT_{50}) for the eight isolates ranged between 117.54 to 127.69 hrs and 128.56 to 154.61 hrs at higher (1.3 x 10^9 PIBs/ml) and lower (2.6 x 10^8 PIBs/ml) concentrations, respectively. Among the tested isolates, Gulbarga isolate was faster in causing mortality of the test population with the LT_{50} value of 117.54 hrs and 128.56 hrs at higher and lower concentrations, respectively. Maximum time for causing mortality was noticed in Dhule isolate (127.69 hrs and 154.61 hrs). Results suggested a direct relationship between concentration of NPV and time taken (LT_{50}) to cause mortality in *H. armigera*. Assessed HaNPV isolates showed considerable variability in their virulence and time taken to cause mortality. Therefore, it is critical to carefully choose suitable virulent commercial formulations of HaNPV for management of *H. armigera* in field.

Keywords: *Helicoverpa armigera*, Ha NPV isolates, LC_{50}, LT_{50}, virulence

Introduction
India is the largest producer, importer and consumer of pulses, accounting for 35 per cent of the total area under pulses and 25 per cent of the total production in the world [1]. In India, during 2016-17 pulses were cultivated in 3.96 million ha, with a total production of 4.89 million tonnes and a productivity of 913 kg per ha [2]. Gram pod borer, *H. armigera* (Hubner) is considered as one of the major biotic constraints in the production of pulses. This species is a polyphagous pest and causes significant losses in pigeonpea, chickpea, green gram, black gram and other pulses. The larvae feed on the developing grains by keeping their head inside the pods and rest of the body outside [3]. The estimated loss due to this pest is around $350 million annually in pigeonpea and $2billion in various other crops in the semiarid tropics [4]. Synthetic chemical insecticides have been widely used for timely and efficient management of pod borer on pigeonpea and other pulses. Indiscriminate applications of insecticides have led to various hazards like development of insecticidal resistance, pest resurgence, environmental pollution and negative impacts on non-target organism [5]. Hence, increased emphasis is being given for utilizing safer alternatives that are also efficient in controlling the pest. Entomo pathogens belonging to baculovirus group, *i.e.*, the nuclearpolyhedrosis virus (NPV) offers immense scope for eco-friendly suppression of *H. armigera* [6]. The major advantage is that these viruses are host specific and as the virus particles are occluded in proteinaceous crystals called 'occlusion bodies', they can be formulated in any carrier material [5]. Different geographical isolates of entomopathogenic NPV are known to exhibit different levels of virulence against the target pests [7, 8]. Therefore, identification and selection of the most virulent isolate is a prerequisite for the production of commercial formulations of NPV. Through this article we demonstrate variation in virulence among different geographical isolates of *Helicoverpa armigera* Nucleopolyhedrosis virus against gram pod borer, *H. armigera* and suggest the suitable isolates for commercial-scale utilization.

Materials and Methods

Multiplication and determination of concentration of virus isolates

The rearing of *Helicoverpa armigera* and multiplication of *Helicoverpa armgera* nucleopolyhedrosis virus (HaNPV) was done as per the reviewed methodology [9, 10]. As the virus is an obligate pathogen, the gram pod borer, *H. armigera* was reared on chickpea based semi-synthetic diet. Eight isolates of Ha NPV belonging to different regions of India were procured from different public and private institutions. The Gulbarga, Chandapura, Rajanukunte isolates were from Karnataka state, Akola and Dhule isolates were from Maharashtra, Udaipur isolate was from Rajasthan, Hosur isolate was from Tamil Nadu and Hyderabad isolate was from Telangana State. These isolates were further multiplied for two generations to get the pure culture. For this, the semi-synthetic diet cube of 0.5 g was inoculated with 10 µl of virus suspension of strength 6.5 x 10^9 PIBs/ml. Later, the fourth instar larvae of *H. armigera* were allowed to feed on the inoculated diet for 24 hrs and then transferred onto the healthy diet. Mortality of the larvae was noticed seven or eight days after feeding on a virus inoculated diet. Dead larvae were collected in small quantity of distilled water and then allowed to putrify for three days. The content was homogenized in equal volume of distilled water by using a blender. The resulting slurry was filtered with a double layer muslin cloth. More water was added to the filtrate and centrifuged at 500 rpm for 1 minute. The supernatant was collected and the pellet was discarded. The supernatant was centrifuged further at 2500 rpm for 5 minutes. The pellet was collected and supernatant was discarded. The resulting pellet was suspended in distilled water and the suspension was stored in a deep freezer. All the equipments and laboratory was regularly cleaned with 0.1 per cent sodium hypochlorite solution. Precautions were taken to avoid the cross contamination of the isolates. The virus could be seen as bright refractive irregular shaped crystals, called as occlusion bodies (OBs). The concentration of virus suspension was determined by using an improved Neubauer hem cytometer under phase contrast light microscope, at 400X magnification and explained in terms of number of polyhedral inclusion bodies (PIBs) per ml of solution (PIBs/ml)

Procedure adopted for the bioassay

The bioassay was conducted by following the diet surface contamination method [11]. Initially bracketing was done to get the highest dose which could cause more than 90 per cent mortality of the test population. The highest dose was serially diluted to get six different test concentrations *viz.* 1.3 x 10^9, 2.6 x 10^8, 5.2 x 10^7, 1.04 x 10^7, 2.1 x 10^6 and 0.42 x 10^6 PIBs/ml. The bioassay was carried out at these concentrations along with untreated control. The experiment was conducted using multilocular trays. A diet cube of 0.25 g was placed in each locule and 10 µl of NPV suspension (concentration wise) was applied on diet cube by using a micropipette. The second instar larvae of *H. armigera* were pre-starved for 8 hrs and later transferred individually onto the treated diet cubes. Fifteen larvae were used per concentration per replication and three replications were maintained for every concentration. The larvae were allowed to feed for 24 hrs. Later, larvae were transferred to the untreated diet. The data was recorded on the number of dead larvae at 24 hrs interval, upto 168 hrs. The mortality data was corrected depending upon the mortality in the control, by using Abbott's formula. The LT_{50} was determined for 1.3 x 10^9 and 2.6 x 10^8 PIBs/ml concentrations. Median lethal concentration and median lethal time was determined through probit analysis by using the statistical package for social sciences (SPSS version 23) software. The efficacy of different geographical isolates of HaNPV against *H. armigera* were compared based on the LC_{50} and LT_{50} values.

Results and Discussions

Dose-mortality response

The dose-mortality response of second instar larvae of *H. armigera* to different Ha NPV isolates are as detailed in Table 1. The Chi-square (χ^2) values were statistically non-significant for all the isolates, suggesting non-heterogeneous response of the test population of *H. armigera* to all the isolates.

The LC_{50} values of different HaNPV isolates ranged from 4.3 x 10^6 to 1.9 x 10^7 PIBs/ml. Among the isolates assessed, Gulbarga isolate recorded lowest LC_{50} value of 4.3 x 10^6 PIBs/ml, followed by Hosur isolate (4.8 x 10^6 PIBs/ml) > Chandapura isolate (9.0 x 10^6 PIBs/ml) > Rajanukunte isolate (1.2 x 10^7 PIBs/ml) > Hyderabad isolate = Akola isolate (1.3 x 10^7 PIBs/ml) > Udaipur isolate (1.5 x 10^7 PIBs/ml) > Dhule isolate (1.9 x 10^7 PIBs/ml) in that decreasing order of toxicity. The Dhule isolate was least toxic. Akola and Hyderabad isolates were similar with respect to their toxicity levels.

Similar range of LC_{50} values (5.1 x 10^7 to 7.2 x 10^9 PIBs/ml) have been reported previously for different geographical isolates of HaNPV *viz*., Junagadh, Surat and Anand from Gujrat and Patancheru from Andhra Pradesh in India [8]. The LC_{50} values of different commercial formulations of HaNPV ranged between 3.12 x 10^4 and 11.42 x 10^6 PIBs/ml [11]. These findings clearly suggest that the different isolates of HaNPV vary greatly in their levels of virulence against *H. armigera*.

Time-mortality response

To know the time required by HaNPV to cause mortality in *H. armigera*, the time-mortality response (Table 2) of second instar larvae of *H. armigera* was determined at higher dose (1.3 x 10^9 PIBs/ml) and lower dose (2.6 x 10^8 PIBs/ml) for all the Ha NPV isolates. The Chi-square (χ^2) values were found to be statistically on par for all the isolates, indicating non-heterogeneous response of the test population of *H. armigera* to all the Ha NPV isolates tested.

At higher dose (1.3 x 10^9 PIBs/ml), the LT_{50} values of different isolates ranged between 117.54 and 127.69 hrs. Among the isolates, Gulbarga isolate registered the lowest LT_{50} value (117.54 hrs) thus causing mortality in shorter period, followed by Hosur (119.94 hrs), Chandapura (120.09 hrs), Rajanukunte (121.99 hrs), Hyderabad (124.59 hrs), Akola (125.44 hrs), Udaipur (127.45 hrs) and Dhule (127.69 hrs) isolates.

At lower dose (2.6 x 10^8 PIBs/ml), the LT_{50} values ranged between 128.56 and 154.61 hrs. Lowest LT_{50} value was noticed in case of Gulbarga isolate (128.56 hrs), followed by Hosur (133.21 hrs), Chandapura (137.68 hrs), Rajanukunte (140.04 hrs), Hyderabad (147.44 hrs), Akola (149.24 hrs), Udaipur (150.15 hrs) and Dhule (154.61 hrs) isolates. Dhule isolate was found to be slowest in killing the test population as it registered the highest LT_{50} values.

These results clearly suggested that the time required to cause mortality depends on the dose of HaNPV. The average LT_{50} at higher dose across the isolates was 123.09 hrs and at the lower dose it was 142.61 hrs. At the higher dose, the larvae were killed 19.53 hrs earlier than the time taken at lower dose.

Such variations in LT_{50} values depending on isolates and the dose have been reported previously on *H. armigera*, wherein the LT_{50} values of different geographical isolates of HaNPV ranged between 109.9 and 118.7 hrs (Parbhani, Mumbai, Rahuri, Ooty, Coimbatore, Negamum and Hyderabad) [7], 120.26 to 143.10 hrs (Junagadh, Surat, Anand and Patancheru) [8], 134.4 to 175.2 (Udhyewala, Chennai and Samba) [12] depending on the dose of HaNPV.

Table 1: Dosage-mortality response of II instar larvae of *H. armigera* to different geographical isolates of Ha NPV

Isolate	LC_{50} (PIBs/ml)	Fiducial limits (PIBs/ml)		Regression equation	Slope±S.E.	Heterogeneity*	
		Lower	Upper			χ^2	d.f. (n-2)
Dhule	1.9×10^7	9.9×10^6	3.7×10^7	Y=-4.33+0.59X	0.59±0.075	1.26	4
Udaipur	1.5×10^7	7.7×10^6	2.8×10^7	Y=-4.34+0.60X	0.60±0.075	3.73	4
Akola	1.3×10^7	5.9×10^6	2.8×10^7	Y=-3.54+0.50X	0.50±0.071	5.25	4
Hyderabad	1.3×10^7	7.0×10^6	2.4×10^7	Y=-4.49+0.63X	0.63±0.077	5.94	4
Rajanukunte	1.2×10^7	5.7×10^6	2.4×10^7	Y=-3.91+0.55X	0.55±0.074	0.16	4
Chandapura	9.0×10^6	4.5×10^6	1.7×10^7	Y=-4.22+0.61X	0.61±0.077	0.44	4
Hosur	4.8×10^6	1.7×10^6	1.0×10^7	Y=-3.20+0.48X	0.48±0.073	0.21	4
Gulbarga	4.3×10^6	1.4×10^6	9.8×10^6	Y=-3.04+0.46X	0.46±0.072	0.24	4

*Non-significant among isoates at $p<0.05$; S.E.: standard error, d. f.: degrees of freedom

Table 2: Time-mortality response of II instar larvae of *H. armigera* to different geographical isolates of Ha NPV

Isolate	Dose (PIBs/ml)	LT_{50} (hrs)	Fiducial limits (hrs)		Regression equation	Slope± S.E.	Heterogeneity*	
			Lower	Upper			χ^2	d. f. (n-2)
Dhule	1.3×10^9	127.69	119.95	136.71	Y=-14.54+6.91X	6.91±0.838	3.34	5
	2.6×10^8	154.61	141.33	176.45	Y=-11.37+5.19X	5.19±0.802	5.13	5
Udaipur	1.3×10^9	127.45	118.71	138.04	Y=-12.26+5.82X	5.82±0.722	4.92	5
	2.6×10^8	150.15	141.34	163.01	Y=-16.96+7.79X	7.79±1.146	1.34	5
Akola	1.3×10^9	125.44	117.24	134.95	Y=-13.02+6.21X	6.21±0.760	2.39	5
	2.6×10^8	149.24	140.34	161.84	Y=-16.95+7.80X	7.80±1.140	2.26	5
Hyderabad	1.3×10^9	124.59	116.74	133.47	Y=-13.74+6.56X	6.56±0.791	1.37	5
	2.6×10^8	147.44	137.81	161.21	Y=-14.82+6.83X	6.83±0.986	2.57	5
Rajanukunte	1.3×10^9	121.99	114.17	130.87	Y=-13.31+6.38X	6.38±0.747	4.69	5
	2.6×10^8	140.04	131.37	151.42	Y=-14.89+6.94X	6.94±0.944	5.12	5
Chandapura	1.3×10^9	120.09	111.20	130.60	Y=-10.81+5.20X	5.20±0.631	5.81	5
	2.6×10^8	137.68	129.50	147.91	Y=-15.49+7.24X	7.24±0.961	2.26	5
Hosur	1.3×10^9	119.94	112.86	127.62	Y=-15.09+7.26X	7.26±0.829	3.13	5
	2.6×10^8	133.21	124.67	143.70	Y=-13.66+6.43X	6.43±0.839	5.26	5
Gulbarga	1.3×10^9	117.54	109.50	126.61	Y=-12.04+5.82X	5.82±0.680	6.39	5
	2.6×10^8	128.56	122.31	135.27	Y=-20.39+9.66X	9.66±1.163	5.23	5

* Non-significant among isoates at $p<0.05$; S.E.: standard error, d. f.: degree of freedom

Conclusion

The present study showed that the different Ha NPV isolates varied in their virulence to cause mortality of test population of *H. armigera*. This calls for a rigorous screening and testing of all possible isolates of Ha NPV before the production of Ha NPV on a commercial scale. Among the isolates studied, the Gulbarga isolate recorded lowest LC_{50} values and LT 50 values and hence this isolate may be explored in commercial formulations.

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