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Characterization of granulosis viruses of sugarcane early shoot borer, *Chilo infuscatellus* (Snell.) and internode borer, *Chilo sacchariphagus indicus* (Kapur)

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A study was undertaken to characterize the granulosis viruses (GVs) of early shoot borer, *Chilo infuscatellus* (Snell.) (Crambidae: Lepidoptera) and internode borer, *Chilo sacchariphagus indicus* (Kapur) (Lepidoptera: Crambidae) in sugarcane. Scanning electron photomicrographs revealed ovo-cylindrical occlusion bodies (OBs) of GVs in early shoot and internode borers with an average size of 425.03 and 230.21 nm, 387.64 and 208.68 nm in length and breadth respectively. Transmission electron photomicrographs also showed ovo-cylindrical OBs embedded with a rod-shaped virion. The average length and breadth of the virion in the OB was 271.0×52.6 nm, 257.0×50.2 nm for early shoot and internode borer GVs respectively. Toxicity studies with the respective GVs revealed lethal concentration values of 4.38, 4.61, 6.89 OBs/mm^2 and 1.85, 135.43, 8045.27 OBs/mm^2 to second, third and fourth larval instars of *Chilo infuscatellus* granulosis virus (ChinGV) and *Chilo sacchariphagus indicus* granulosis virus (ChsaGV) respectively.

INSECT pests are the key constraint in sugarcane production as they damage all stages of the crop. Early shoot borer, *Chilo infuscatellus* Snellen and internode borer, *Chilo sacchariphagus indicus* (Kapur) are the most economic pests of sugarcane in India and cause 33%, 35% in yield and 3%, 3.07% in sugar recovery respectively[1]. *C. infuscatellus* is commonly known as shoot borer in the North Indian sugarcane belt and as early shoot borer in peninsular India[2]. High usage of chemical insecticides in its management has led to changes in the biology and reproductive potential of the pest and development of insecticide resistance[3]. The microbial biopesticide, granulosis virus (GV) belonging to the Baculoviridae family is an effective alternative for managing sugarcane borers. Natural occurrences and high pathogenicity of *Chilo infuscatellus* granulosis virus (ChinGV) and *Chilo sacchariphagus indicus* granulosis virus (ChsaGV) to all the larval instars, post-larval stages under laboratory and field conditions have been reported by several workers[4–6]. Laboratory studies proved that early instars of internode borer were more susceptible to GV than late instars. First and second instar larvae were found to be highly susceptible to ChinGV and mortality ranged from 61.9% to 100% (ref. 5). Detailed information is not available on the characterization of GVs infecting sugarcane borers. Thus in the present study, we characterize the GVs for the development of green biopesticide formulation to manage sugarcane borers.

Larvae of shoot and internode borers were collected from sugarcane fields of Coimbatore and its surroundings in Tamil Nadu, South India. The insects were reared on sugarcane shoot bits or internode bits. The infected larvae became restless and fed voraciously in the early stage of infection. They appeared robust with pale integument. As the infection progressed, the larvae became lethargic and ceased feeding. During rearing, larvae showing characteristic symptoms of infection with GVs were isolated and reared separately. The skin of the moribund larvae turned white, except in the intersegmental zone. Dead larvae were collected and put in individual sterile vials. Cadavers were labelled individually and used for extraction of GVs. Occlusion bodies (OBs) of GVs were extracted from the virosed larvae according to the standard procedure[7] and stored at 4°C till further use. The viruses were confirmed by the presence of OBs in the discharged

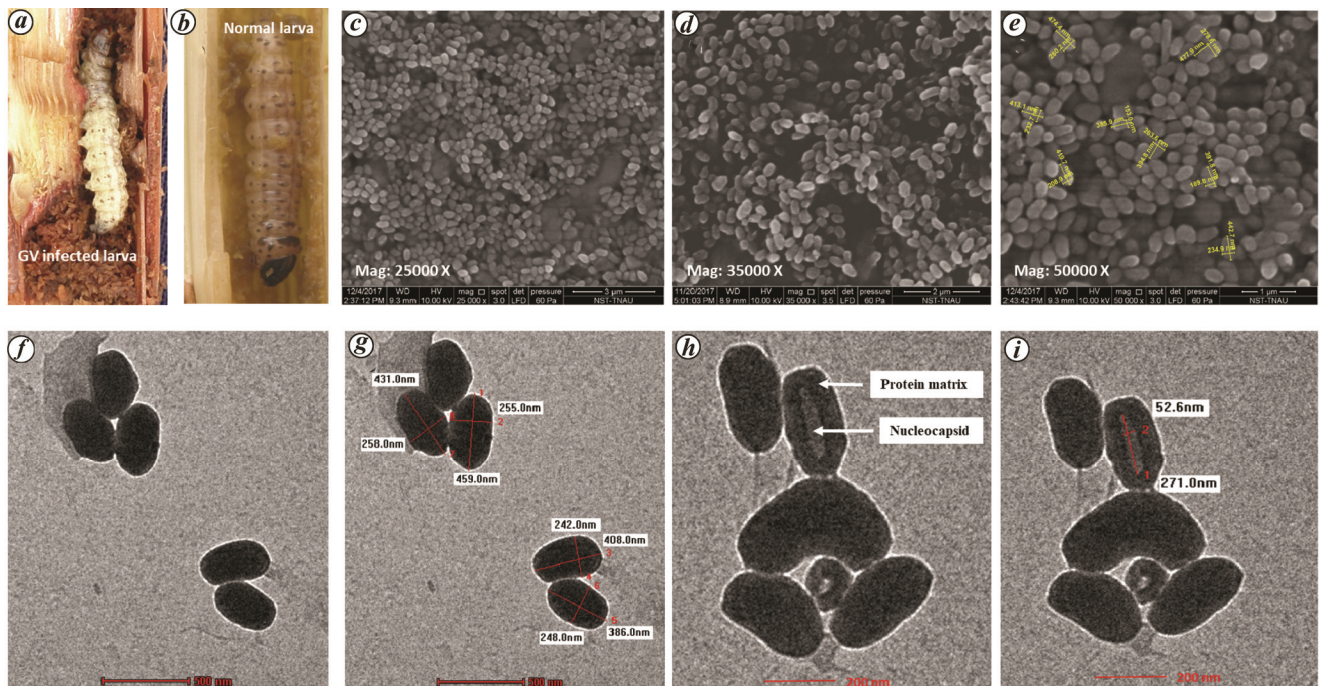*For correspondence. (e-mail: sivakumarg.nbaii@gmail.com)

Figure 1. *a*, Granulosis virus (GV)-infected larva of *Chilo infuscatellus*. *b*, Normal larva of *C. infuscatellus*. *c*–*e*, SEM of ovoid-shaped occlusion bodies (OBs) of *C. infuscatellus* GV at magnification (*c*) 25,000×, (*d*) 35,000× and (*e*) 50,000×. *f*, *g*, TEM of ovo-cylindrical OBs at magnification 500 nm. *h*, *i*, TEM of OBs with a singly embedded, rod-shaped nucleocapsid virion.

body fluids from the individual infected larvae under phase contrast microscopy (400×; Olympus BX 41 from Japan).

Morphological characterization of the extracted OBs of GVs was done using the standard method[8], and described by scanning electron microscopy (SEM) and transmission electron microscopy (TEM) available at the Tamil Nadu Agricultural University, Coimbatore. Further, the size and shape of OBs and nucleocapsid virion were measured from the electron photomicrographs.

Larval cultures of both borers were maintained on sugarcane shoot bits in the laboratory (with a mean temperature of 27°C, 60% relative humidity (RH) and with a photoperiod of 14 : 10 L : D). Bioassays were conducted with three larval instars (II, III and IV) of shoot and internode borers according to the procedure described by Subramanian *et al.*[7]. Around 40 larvae per dose or treatment and starting with a concentration of 2.0×10^9, 2.0×10^8 and 2.0×10^7 OBs ml^{-1} as the highest dose tested for IV, III and II instars respectively, with a control (40 larvae fed by an equal quantity of distilled water). Each larva was fed with 1 μl of OBs, dispensed through a Hamilton glass syringe, applied to individual larva per os holding the head capsule. As the larva swallowed up the liquid, it was transferred to the sugarcane shoot/internode bits placed in filter paper-lined plastic boxes (200 ml capacity). The shoot/internode bits were changed manually on alternate days to provide fresh feed. Each dose was repeated four times and each replication had 10 larvae. The mortality of larvae was observed and recorded daily up to nine days of post treatment. Bioassays were carried out thrice and the pooled larval mortality data obtained were subjected to probit analysis using the standard POLO software[9] to calculate the lethal concentration values (LC_{50}) of different larval instars of the test insect.

Discharged larval body fluid of infected larvae was observed under a phase-contrast light microscope. It showed a large number of ovo-cylindrical virus particle entities similar to the OBs of GVs. SEM photomicrographs revealed the ovo-cylindrical OBs of GVs in both early shoot and internode borers with an average size of about 425.03×230.21 nm and 387.64×208.68 nm in length and breadth respectively (Figures 1 and 2). TEM studies also revealed the size of the singly embedded, rod-shaped nucleocapsid virion protected by a tough proteinaceous matrix within the OBs in early shoot borer (271.0 and 52.6 nm in length and breadth respectively) (Figure 1) and internode borer (257.0 and 50.2 nm in length and breadth respectively) (Figure 2). The presence of these nucleocapsid virion structures in GVs is in confirmation with the results of Kathleen *et al.*[10] in *Plodia interpunctella*. Comparing the average size of the GVs in both borers, the ovo-cylindrical OBs and singly embedded, rod-shaped nucleocapsid virion within the OBs were larger in the GVs of early shoot borer and relatively smaller in case of the internode borer. The results are in accordance with the findings of Sciocco-Cap *et al.*[11] in *Epinotia aporema* GV, where the OBs were reported as ovoid-shaped and of similar size (466 nm) with single, rod-shaped virion (226 nm). Likewise, ovoid-shaped GVs
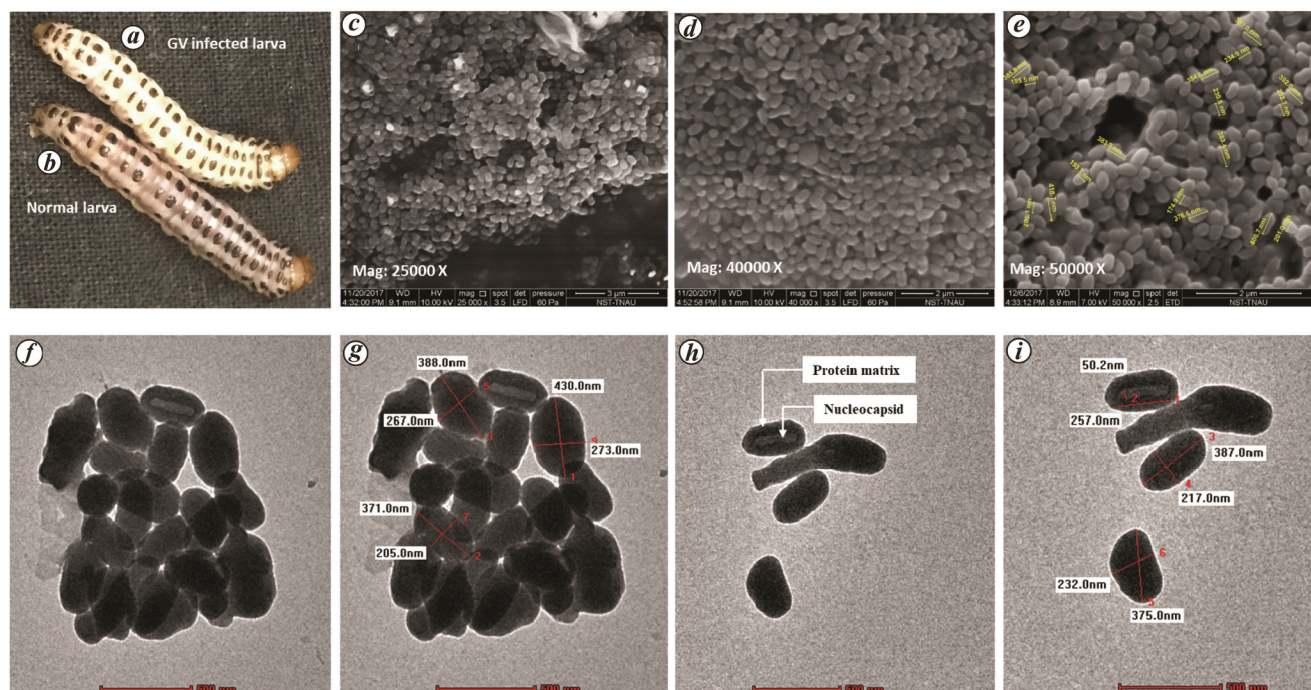
Figure 2. *a*, GV-infected larva of *Chilo sacchariphagus indicus*. *b*, Normal larva of *C. sacchariphagus indicus*. *c–e*, SEM of ovo-cylindrical OBs of *C. sacchariphagus indicus* GV at magnification (*c*) 25,000×, (*d*) 35,000× and (*e*) 50,000×. *f*, *g*, TEM of ovoid-shaped OBs at magnification 500 nm. *h*, *i*, TEM of OBs with a singly embedded, rod-shaped nucleocapsid virion.

Table 1. Bioassay of ChinGV and ChsaGV against sugarcane early shoot borer and internode borer

Granulovirus	Larval instar	LC_{50} ($\times 10^1$ OBs/larva)	Lower ($\times 10^1$ OBs/larva)	Upper ($\times 10^1$ OBs/larva)	Slope	χ^2*	Degree of freedom
			95% confidence limit				
ChinGV	II	4.38	2.23	8.20	1.82	4.21	3
	III	4.61	2.14	9.56	2.06	3.90	3
	IV	6.89	3.36	15.82	1.58	7.02	3
ChsaGV	II	1.85	0.94	4.68	1.64	6.90	3
	III	135.43	2.953	614.66	2.10	5.83	3
	IV	8,045.27	275.557	31,833	1.92	4.65	3

*In each case χ^2 value from the goodness-of-fit test was less than the tabular value ($P = 0.05$), indicating that the data fit the probit model.

have been reported in *Achaea janata*[12], *Spodoptera frugiperda*[13] and *Diatraea saccharalis*[14].

Bioassay studies revealed instar-dependent larval mortality pattern. Larval mortality was observed in second, third and fourth instars of *C. infuscatellus* and *C. sacchariphagus indicus*. The assessed LC_{50} values for the second, third and fourth instars of ChinGV larva were 4.38, 4.61 and 6.89 OBs/larva respectively. In the case of ChsaGV, the LC_{50} values were 1.85, 135.43 and 8045.27 OBs/larva for the second, third and fourth larval instars respectively (Table 1). Our findings are in accordance with those reported in the literature for *E. aporema* (LC_{50} to fourth instar larvae was 4000 granules per larva)[11], *S. frugiperda* (LC_{50} to second instar larvae was 4.5×10^5 OBs/ml)[13] and *D. saccharalis* (LD_{50} to third instar larvae was 42.30 OBs/larva)[14].

This study presented results of the characterization of GVs of sugarcane early shoot borer and internode borer. Electron microscopy studies revealed that the OBs of both GVs appeared as ovo-cylindrical in shape and each OB was embedded with a rod-shaped virion protected by a tough proteinaceous matrix. LC_{50} values of 4.38, 4.61, 6.89 OBs larva, 1.85, 135.43, 8045.27 OBs larva were recorded against the second, third and fourth larval instars of ChinGV and ChsaGV respectively.

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Evaluation of probability distribution functions applied to tree diameter in a mixed uneven Kiker (*Robinia pseudoacacia*) stand of Kashmir Himalaya, India

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Probability distribution is of significance to predict tree distribution and estimate productivity in different ages as well as thinning out in forest stands to ensure optimized and stable stands. Statistical probability distributions, viz. lognormal, Weibull, exponential and gamma were used to fit tree-diameter data generated from the Manasbal forest stand of Kashmir Himalaya, India containing a heterogeneous population of trees with the objective to determine the best probability distribution of tree diameter. To estimate the parameters of the fitted distributions, the method of maximum likelihood was used. The various distributions were evaluated using different goodness-of-fit tests, viz. Kolmogorov–Smirnov, Cramer–von Mises and Anderson–Darling statistics, and the best distribution pertaining to the forest stand was ascertained. Lognormal distribution fitted the data well and could be used in modelling, planning and scheduling the forest stand in the study region.

THE genus *Robinia* is noteworthy for its numerous uses. Black locust (*Robinia pseudoacacia*) is part of the Leguminosae family and is known locally as 'Kiker', in Jammu and Kashmir, India. It is a rapidly growing species, making it a primary source of biomass fuel in the region. In many native forests where it occurs, *R. pseudoacacia* dominates early forest regeneration[1]. It is a spiny, deciduous tree of medium size, reaching a height of 25–30 mt and a diameter of 80–90 cm under favourable conditions. Models of forest simulation have been widely used to predict future stand structure. Tree-diameter distribution plays a vital role in simulation modelling. Different probability distributions are being used extensively to model the diameter of trees in the forest stands. Some of the

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