PROPAGATION OF *Oryctes* NUDIVIRUS FOR THE MANAGEMENT OF RHINOCEROS BEETLE

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721

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Rhinoceros beetle, *Oryctes rhinoceros* (Coleoptera: Scarabaeidae) (*Figure 1*) is a major pest of the oil palm, causing 25% of crop losses two years after infestation (Norman *et al.*, 1997). The *Oryctes* nudivirus (OrNV) (*Figure 2*) is a biological control agent of the rhinoceros beetle (Huger, 1966). The OrNV was discovered in Malaysia and introduced into many coconut and oil palm producing countries to control the rhinoceros beetle (Bedford, 2014). In Malaysia, four types of OrNV

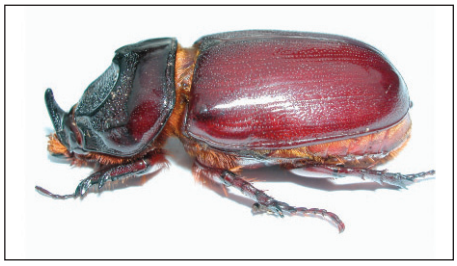

Figure 1. Rhinoceros beetle, Oryctes rhinoceros *adult.*

were identified where Type B was the most virulent compared to Types A, C and D, causing 65% mortality on larvae and 86.7% on adults (*Figure 3*) (Ramle *et al.*, 2005). Introduction of OrNV Type B to oil palm plantations has reduced the population of rhinoceros beetle and palm damage (Ramle *et al.*, 2011).

INSECT CELL CULTURE

The insect cell line DSIR-HA-1179 (*Figure 4*) was developed from the black beetle, *Heteronychus arator* (Coleoptera: Scarabaeidae) and used to propagate high purity OrNV (Crawford, 1982). The cells were propagated in a 25 cm^2 flask containing 5 ml of fresh prepared PS100 medium. The flasks were then placed inside an air tight container and stored in an incubator at 27°C for 15 days. The cell production reached up to 6.0 x 10^5 cells/ml two weeks after incubation (*Figure 5*).

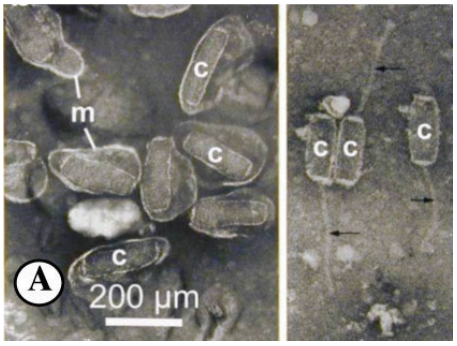

Photo by Huger *et al.* (2005)

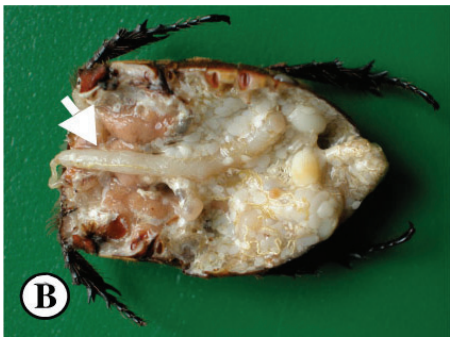

Figure 2. A) Morphological characteristics of Oryctes *nudivirus, B) symptom of infected adult having swollen gut, full with milky content (arrow).*

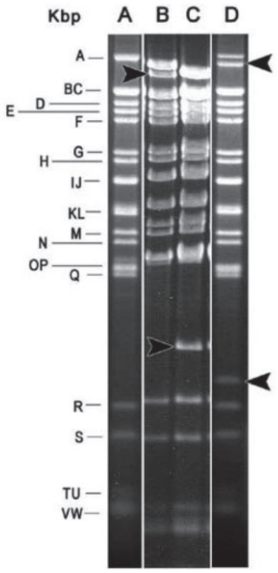

Figure 3. DNA profiles of four types of Oryctes *nudivirus in Malaysia.*

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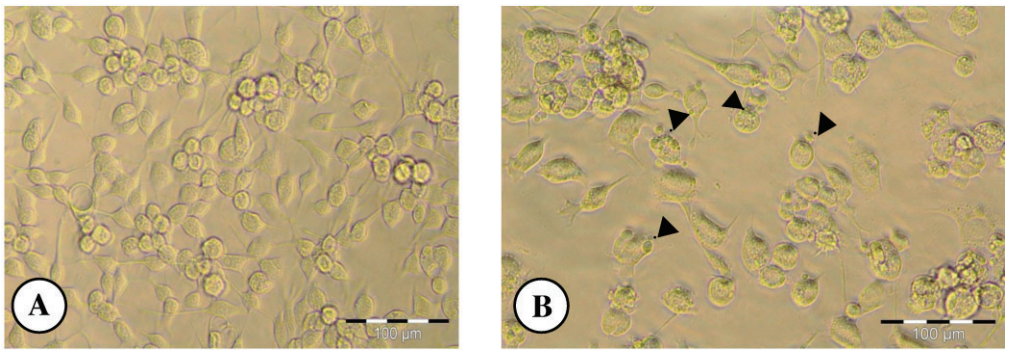

Figure 4. Morphological characteristics of insect cell line DSIR HA-1179. A) Healthy cells and B) nudivirus infected cells with cytophatic effect (arrows).

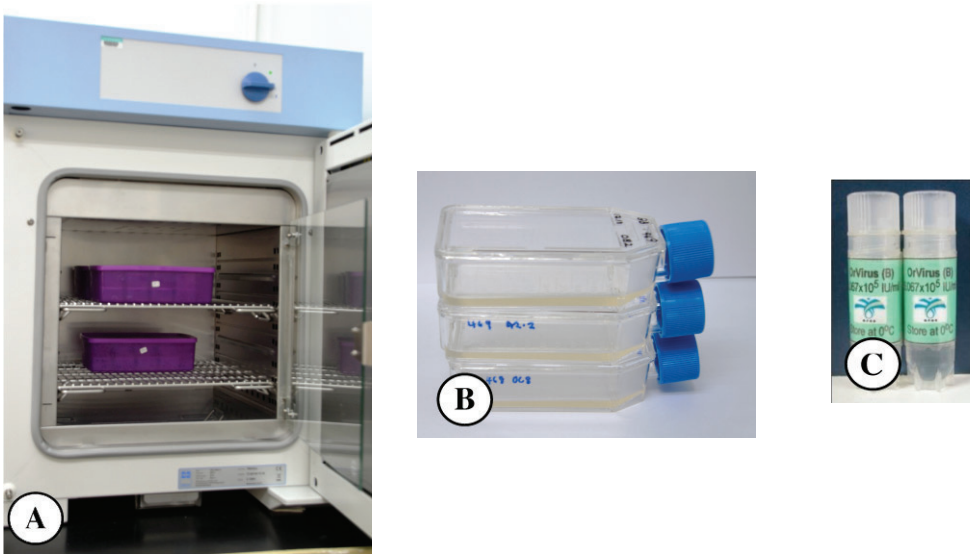

Figure 5. Some apparatus used for production of Oryctes *nudivirus.*
A) Incubator, B) 25 cm² cell culture flasks and C) microtubes containing formulated Oryctes *nudivirus suspensions.*

PRODUCTION OF NUDIVIRUS

1. Preparation of nudivirus suspension from nudivirus infected gut tissues using filtration techniques, via 0.45 µm then 0.22 µm membrane filter.
2. Adding virus suspension into a flask with insect cells between 60% to 70% confluent.
3. Incubate at 27°C for 15 days. Infected cells will show morphological changes or cytophatic effect.
4. Harvesting of nudivirus from the cell culture using centrifugation method.
5. Formulated nudivirus suspension in a 2.0 ml sterile microtube and store at 4°C.
6. Conduct product quality analysis to identify type of nudivirus and determine the concentration of virus by Tissue Culture Infectious Dose 50% ($TCID_{50}$) analysis.

INTRODUCTION OF NUDIVIRUS IN THE FIELD

Three phases are involved (Ramle *et al.,* 2003) as follows:

Phase 1: Pre-release Site Assessment

1. Identification of existing type of nudivirus.
2. Determination of nudivirus infection on immatures and adults *Oryctes.*

3. Assessment of palm damage.
4. Collection of young healthy adults for nudivirus inoculation and dissemination.

Phase 2: Time of Release

1. Inoculation of young adults and field release.

Phase 3: Post-release Monitoring and Impact Assessment

1. Determination of adult beetle population by trapping method.
2. Determination of virus infection on immatures and adults *Oryctes*.
3. Assessment of palm damage.
4. Determination on the spread of released virus.

BENEFITS

The cell culture could be used to propagate a specific OrNV which has high infectivity against identified beetle populations. Furthermore, it can be used to monitor the quality of individual OrNV prior to field release.

COST

Estimated cost to produce 50 ml of *Oryctes* nudivirus suspension in 2015 is RM 1000 (subject to change). This volume is sufficient for inoculating 1250 rhinoceros beetle adults. The cost includes training on introduction of nudivirus in the field using capture, inoculate and release technique.

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