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EFFECTIVE INOCULATION METHOD AND OPTIMUM CONCENTRATION OF *ORYCTES* VIRUS FOR BIOLOGICAL CONTROL OF COCONUT BEETLE *(Oryctes rhinoceros)* ADULTS

S.P. Jayawardena*

Department of Plant Sciences, Faculty of Agriculture, Rajarata University of Sri Lanka, Anuradhapura, Sri Lanka.

[1]Crop Protection Division, Coconut Research Institute, Lunuwila, Sri Lanka.

**E-mail: sithuminijayawardena@yahoo.com*

ABSTRACT: The study was carried out to determine the effective inoculation method and optimum concentration of local *Oryctes* virus (*OrV*) for successful infection of *Oryctes rhinoceros* (L.) adults in laboratory which released to the field to spread the viral disease among healthy larvae and beetles for their biological control. 0.1ml of 10^4 ppm of viral suspension was introduced orally to one set of beetles and another set of beetles were allowed to swimming in the suspension for 10 minutes. Beetles were dissected at different intervals to determine the period taken for infection. Five concentrations (10^1 ppm to 10^5 ppm) of virus suspensions were introduced to adult beetles orally to find the lethal concentration (LC_{50}) and lethal time (LT_{50}). Percentage of infected beetles was significantly different ($P<0.001$) among the two methods of inoculation and untreated control. After 21 days of inoculation, oral introduction method, swim method and control recorded 88.8%, 44.4% and 11.1% of *OrV* infection, respectively indicating the most effective inoculation method as oral introduction. Cumulative per cent mortality of *O. rhinoceros* adults with 10^1 ppm, 10^2 ppm, 10^3 ppm, 10^4 ppm and 10^5 ppm concentrations were recorded as 7.3%, 25.1%, 33.3%, 81.4% and 100%, respectively. LC_{50} was $10^{2.7}$ ppm and LT_{50} for 10^4 ppm and 105ppm concentrations were 23 days and 12 days, respectively. Best concentration to oral inoculation of *O. rhinoceros* with *OrV* for field release was selected as 10^4 ppm.

Keywords: *Oryctes rhinoceros, Oryctes virus, lethal dose, lethal time, coconut.*

The black beetle (*Oryctes rhinoceros* L.) is a major pest occurring in all coconut growing areas of Sri Lanka and other countries. Palms with 50% of frond damage corresponded to leaf area reduction of 13% and decrease in nut yields by 23% as compared to normal palms. Attack in young seedlings may out rightly kill them. It may also provide entry points for lethal secondary attacks by the red weevils or by pathogens.

A virus disease of black beetle was discovered by Huger (4) in Malaysia and this pathogen has recently been placed under its own virus category *Oryctes.* Adult and larval stages of black beetle are susceptible to diseases. Midgut epithelial cells of virus infected beetles are the main sites of virus replication. Many gut cells die from the infection and gut swells with milky content. The adults after taking up infection become lethargic, short lived (25 days of 70) and reduced the egg laying capacity in 90% infection (Zelazny, 6). Infected beetles act as a flying virus vectors which ingested the virus to healthy larvae and beetles by feeding, excreta and at breeding sites (Zelazny, 5). Five per cent of the grubs and 12% of the adults had natural virus infection. But that natural transmission enables it to persist in nature and reduce the beetle population. Therefore beetles which were inoculated with the virus under laboratory condition released to field as biological control method.

Different methods have been used to inoculate the virus for infecting beetles. Most frequently used method is letting beetles to swim in a suspension of virus. Feeding beetles with a virus suspension prepared from beetle gut is another method. The experiment was conducted to find

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out the most suitable inoculation method, lethal dosage and lethal time of virus inoculum and optimum concentration for biological control of black beetle.

MATERIALS AND METHODS

The present study was conducted at the laboratory of Crop Protection Division, Coconut Research Institute (CRI), Lunuwila, Sri Lanka and the experimental period was six months.

Collection of beetles

Beetles were collected from pheromone traps installed in coconut plantations which haven't been released with *Oryctes* virus infected beetles. They were kept separately in plastic bottles filled with sterilized coir dust and ripen banana pieces were given as feeding materials.

Identification of virus infection

Beetles were dissected (Fig.1) to determine the virus infection by observing the midgut symptoms. Virus infected beetles has swollen, whitish midgut with white mucous fluid (Zelazny, 6). Further confirmations of virus infection were done by staining the midgut contents using Giemsa staining method. The gut contents of healthy beetles usually contain no cells, except for few small pieces of tissues (Fig. 2) while infected guts contain many mid gut epithelial cells (Fig. 3) which were stain in pink colour (Gorick, 3).

Preparation of inoculums

Midguts of virus infected beetles, stored in the refrigerator, were used for the preparation of virus inoculum. One gram of infected guts was ground and added to 100ml of 5% sucrose solution to prepare a 10^4ppm virus inoculum.

Experiment 1: Identification of effective inoculation method

Experiment consisted of two treatments and one control trial with three replicates. A group of 432 beetles of approximately same age was separated by observing the amount of hairs remaining on the two posterior ventral segments of the abdomen that worn out with age (Cumber, 2). One group of 108 beetles were infected with *Oryctes* virus by placing 0.1ml of 10^4ppm virus inoculum on beetles' mouth using a pasture pipette and allowed to suck up. Another group of 108 beetles were allowed to swim in 100ml of 10^4ppm virus suspension for 10 minutes. The third group of 108 beetles was kept uninfected as control

Data collection and analysis

Six beetles were randomly selected from each replicate of the treatments and control at 2,5,8,11,14 and 21 day intervals for dissection. The number of beetles infected with virus was determined. The invariate analysis of variance for mean percentage infection was performed at 5% probability level by using SAS (1995).

Experiment 2: Identification of optimum concentration of inoculum

A group of 180 beetles of approximately the same age was collected by observing the amount of hair remaining on the two posterior ventral segments of the abdomen (Cumber, 2) and they were randomly assigned in to 6 groups of 30 beetles. Five concentrations of 10^1ppm, 10^2ppm, 10^3ppm, 10^4ppm and 10^5ppm of virus inoculum were prepared by mixing 5% sucrose solution and infected guts (Table 1)

Table 1: Weight of guts and amount of 5% sucrose solution used to prepare each concentration.

Concentration	Weight of guts	Volume of 5% sucrose solution
10^1ppm	1 mg	100 ml
10^2ppm	10 mg	100 ml
10^3ppm	100 mg	100 ml
10^4ppm	1000 mg	100 ml
10^5ppm	10000 mg	100 ml

Experiment had 5 treatments which were represent each concentration and uninfected control. Thirty beetles per concentration were inoculated by placing 0.1ml of virus suspension on beetles' mouth and allowed to suck up. Thirty beetles were kept uninfected as control and they were fed with 5% sucrose solution only.

Data collection and analysis

Number of dead beetles due to virus infection in each concentration was counted at 24 hour intervals during the experimental period of 38 days.

Fig. 1: Dissection procedure for virus infected beetles.

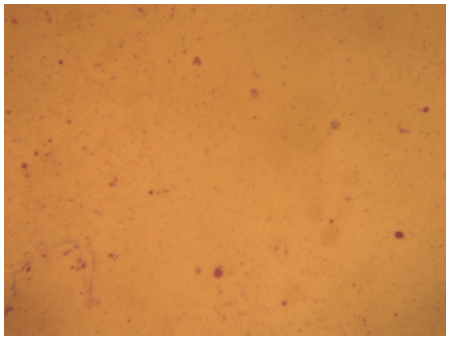

Fig. 2: Gut content of healthy beetle after staining.

Cumulative percentage mortality of treatments was corrected by using Abbott's formula. Transform the concentration to log concentration and corrected percentage mortality to probit mortality and graphs were plotted to determine the LC_{50} value and LT_{50} value. Data were analysed by using the CATMOD procedure.

RESULTS AND DISCUSSION

Oryctes rhinoceros adults were infected by both oral and swim method of inoculation. The percentage of infected beetles increased up to 11th day of inoculation at a higher rate and thereafter at a low rate (Table 2) in both oral and swim method of inoculation. After getting entry, *OrV* reaches the nuclei of midgut epithelial cells of adults where it replicates. In the four hour post infection period, virus absorption in to the plasma membrane and uptake in cytoplasmic vesicles occurs. In seven to 12 hours post infection, viral replication in the clear area of the hyper trophied nucleus occurs, and finally at 16 hours or more virus release from plasma membrane take place and it takes at least more than 2 days to show infection symptoms (Crawford and Sheehan, 1). Results of this study also agree with above observations and it took 5

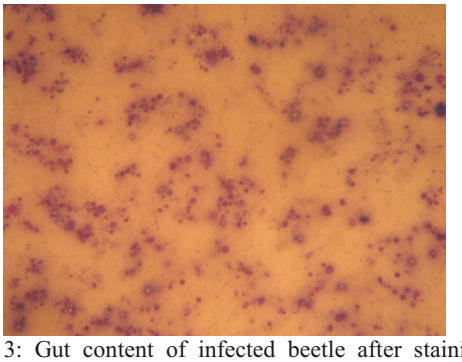

Fig. 3: Gut content of infected beetle after staining.

Table 2 : Mean percentage OrV infection with respect to time in two inoculations methods and control.

Treatment	Mean % infection SE									
	5 days		8 days		11 days		14 days		21 days	
Oral	27.7	0.054^{A}	61.1	0.054^{A}	83.3	0.054^{A}	83.3	0.054^{A}	88.8	0.054^{A}
Swim	5.5	0.054^{B}	16.6	0.054^{B}	38.8	0.054^{B}	44.4	0.054^{B}	44.4	0.054^{B}
Control	5.5	0.054^{B}	11.1	0.054^{B}	16.6	0.054^{C}	11.1	0.054^{C}	11.1	0.054^{C}

*SE–Standard Error. Means denoted by same letters are non-significant.

to 11 days for *Oryctes rhinoceros* adults to show the viral symptoms.

All concentrations of virus inoculum tested caused mortality in black beetle adults. The percentage mortality increased with the increasing concentration of *OrV* inoculum. There was no significant difference in cumulative percentage mortality in concentrations of 10^1ppm to 10^3ppm. They were significantly lower ($P < 0.004$) than the mortality recorded in concentration of 10^4ppm and 10^5ppm. Cumulative percentage mortality between 10^4ppm and 10^5ppm were Also significantly different ($P < 0.02$). At 10^5ppm concentration all beetles were dead (Table 3).

Table 3: Cumulative percentage mortality of *O. rhinoceros* beetles at different concentrations of virus inoculum.

Concentration	Cumulative percentage Mortality ± SE
10^1 ppm	7.3 ± 0.27[a]
10^2 ppm	25.1 ± 0.83[a]
10^3 ppm	33.3 ± 0.46[a]
10^4 ppm	81.4 ± 0.40[b]
10^5 ppm	100.0 ± 0.39[c]

* SE – Standard Error.

Means denoted by same letters are non significant

Concentrated preparations of cell culture fluid were tested as 10^0 ppm, 10^1 ppm, 10^2 ppm and 10^3ppm dilutions in 10% sucrose (Zelazny *et al*., 7). Percentage infection by cell culture fluids of 10^0 ppm, 10^1 ppm, 10^2 ppm and 10^3 ppm were recorded as 10%, 35%, 47% and 63%, respectively. Zelazny *et al*. (7) used the virus inoculum derived from *Heteronychus arator* cell culture. In present study, virus inoculum was derived from guts of infected beetles. By comparing the results of both research, it can be revealed that the cell culture fluid gave higher rate of infection than the virus inoculum prepared by guts of infected beetles in present study.

LC_{50} is the concentration of virus inoculum required to kill 50% of the beetle population. In present study the LC_{50} of the local *OrV* inoculums was $10^{2.7}$ppm (Fig. 4). Concentration that kills 90% of pest population known as LC_{90} and it is defined mainly for microbial insecticides. Concentration of *OrV* inoculums that kills 90% of *Oryctes rhinoceros* population was $10^{4.1}$ppm (Fig. 4).

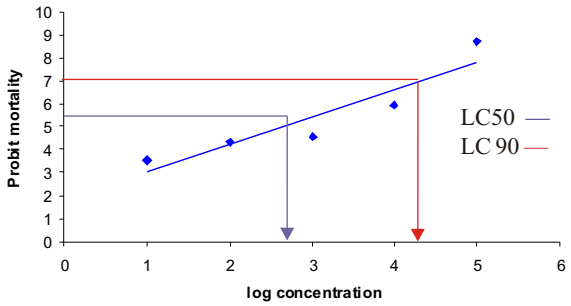

Fig. 4: Changing of probit mortality with log concentration.

LT_{50} is the time required to kill 50% of a pest population. Fifty per cent mortality was not achieved in 10^1ppm, 10^2ppm and 10^3ppm concentration during the 38 days of experimental period. The concentrations of 10^4ppm and 10^5ppm recorded LT_{50} as 23 days and 12 days, respectively (Fig. 5).

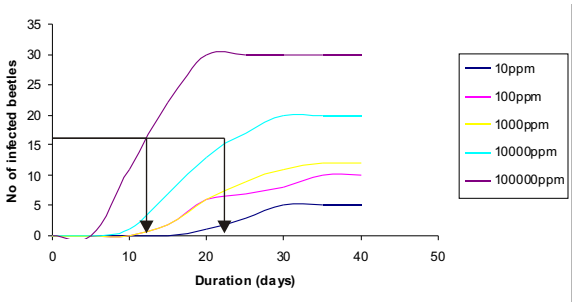

Fig. 5: No. of death beetles with respect to time in five virus inoculums.

The objective of releasing laboratory infected beetles of *OrV* to coconut fields is spreading the disease among healthy beetles and larvae. Therefore, those beetles should act as

successful flying virus vectors that effectively spread the disease. Activities of those beetles as effective virus vectors are mainly determined by the inoculation method and concentration of virus inoculum ingested by them in the laboratory. The infected beetles should be able to remain in the field for a reasonably long period to infect the field population. Although the concentration of 10^5ppm gave 100% mortality it kills 50% of the beetle population in 12 days. Virus inoculum of 10^4ppm kills less number of beetles (81.4%) but it takes 23 days to kill 50% of the beetle population. Therefore oral inoculation of *Oryctes rhinoceros* adults with 10^4ppm *OrV* inoculum could be taken as effective concentration to infect *Oryctes rhinoceros* in laboratory.

CONCLUSION

Oral feeding method is the best inoculation method of *OrV* suspension to infect *Oryctes rhinoceros* adults in laboratory conditions. After inoculation of *OrV* suspension to *Oryctes rhinoceros* adults it takes 5 to 11 days to show viral symptoms. Lethal concentration (LC_{50}) was $10^{2.7}$ppm. Lethal time (LT_{50}) for 10^4ppm and 10^5ppm virus inoculum was 23 days and 12 days, respectively. Virus inoculum of 10^4ppm is the effective concentration for infecting *Oryctes rhinoceros* adults in laboratory condition for field release to control black beetle biologically.

REFERENCES

1. Crawford, A. M. and Sheehan, C. (1984). An *Oryctes rhinoceros* bacculovirus inoculum derived from tissue culture. *J. Econ. Entomol.,* **77**: 1610 – 1611.
2. Cumber, R. A. (1957). Ecological studies of the rhinoceros beetles *Oryctes rhinoceros* (L.) in Western Samoa. *Technical Paper*. 107 – 32.
3. Gorick, B.D. (1980). Release and establishment of the bacculovirus disease of *Oryctes rhinoceros* (L.) in Papua New Guinea. *Bull. Entomol.Res.,* **70**: 445 – 453.
4. Huger, A. (1966). A virus disease of the Indian rhinosero beetle *Oryctes rhinoceros*. *J. Invertib. Pathol.,* **8***:* 38 – 51.
5. Zelazny, B. (1976). Transmissions of baculovirus in population of *Oryctes rhinoceros. J. Invertib. Pathol.,* **27***:* 221 – 227.
6. Zelazny, B. (1978). Methods of inoculating and diagnosing the Bacculovirus disease of *Oryctes rhinoceros*. *FAO Plant Prot. Bull*., **26**: 163-168.
7. Zelazny, B.; Alfiler, A.R.; and Crawford, A.M. (1987). Preparation of a bacculovirus inoculum for use by coconut farmers to control Rhinoceros beetle. *FAO Plant Prot. Bull.,* **35**: 37-42.