# Use of *Oryctes* Virus in the Management of Black beetle (*Oryctes rhinoceros)* in Coconut Plantations

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## ABSTRACT

In Sri Lanka, previous attempts to use *Oryctes* virus (*Or*V) infected grubs for biological control of coconut black beetle *Oryctes rhinoceros* had a limited success. Collection of a large number of beetles is now possible with the availability of the aggregation pheromone of *O. rhinoceros* for inoculation by *Or*V and release them back in the field. In this study, the effective dose of the local isolate of *Or*V for inoculating adult beetles and the impact of releasing infected beetles in reducing damage levels in the coconut palms were determined. Adult beetles were inoculated with virus solutions of $10^1$, $10^2$, $10^3$, $10^4$and $10^5$ ppm orally and the mortality rate over time was monitored to determine effective dose. All the beetles fed with $10^5$ ppm were killed in 21 days after inoculation. The doses, $10^1$, $10^2$, $10^3$ ppm gave only <50% mortality of beetles while $10^4$ ppm gave 65% mortality. The field experiment was conducted in two sites with an extent of 30 ac and having plenty of breeding sites. The beetles inoculated with $10^5$ppm dose were released at the rate of 10 beetles per acre at 6-month intervals for 3 years. The incidence of *Or*V increased from a level of 14% prior to release to 50% after 3 years. The mean damage levels in randomly-selected palms showed a significant negative relationship between percentage damage on the spear and the first leaves and the time after releasing *Or*V. The results confirmed that *Or*V is a prospective biological control agent of *O. rhinoceros,* and it is suggested to use this agent in integrated management programme of *O. rhinoceros.*

**Keywords:** Biological control, Coconut, Damage levels, *Oryctes rhinoceros*, *Oryctes* Virus,

## INTRODUCTION

Black beetle, *Oryctes rhinoceros* Linnaeus (Coleoptera:Scarabeidae) is an injurious foliar pest of coconut plantations worldwide. The adult beetle burrows into the bud region of the palm resulting in loss of leaf area and breaking of spear leaf when leaves unfold and occasional destruction of developing inflorescence. It has been reported that 50% of frond damage leads to 13% reduction in leaf area and 23% decrease in nut yield (Singh and Rethinam, 2004). Although, the attack on mature palms are not much economically important in Sri Lanka, the damage to seedlings and young palms is important as it causes growth retardation and even death of the seedlings (Suwandharathne and Kumara, 2007). A diagnostic survey conducted by the Coconut Research Institute reported that in Sri Lanka, black beetle is considered as one of the most troublesome pests in coconut plantations (Peiris *et al.,* 2006).

An integrated pest management package has been recommended for black beetle, which includes cultural methods (field sanitation and destruction of breeding sites), mechanical method (extraction of beetles using a metal hook), chemical methods (application of repellants or insecticides) and trapping of beetles by the aggregation pheromone (Suwandharathne and Kumara, 2007). However, the coconut growers do not pay much attention to these methods and only few methods are practised to manage the pest, probably due to various difficulties encountered by them. Except the adult stage, all stages of the beetle development are spent in the breeding medium such as decaying coconut logs, cow dung, coir dust and organic manure heaps and decaying vegetable debris. Most of these breeding grounds are left without destruction due to shortage of labour and inaccessibility to them. Therefore, the use of biological control agents; Green Muscardine Fungus, *Metarahizium anisopliae* and especially the *Oryctes* virusare potential means to overcome this problem.

*Baculovirus oryctes* (Syn: *Rhabidino virus oryctes*) was discovered by Huger in 1966 (David, 1975) and later classified to a new group called *Oryctes* virus (Evans and Shapiro, 1997). Natural occurrence of this virus has been reported from the Philippines, Indonesia (Zelazny, 1977) Kerala state in India (Zelazny, 1981; Mohan *et al.*, 1983) and Sri Lanka (Kangaratnam *et al.*, 1984). Ever since *Oryctes* Virus (*Or*V) was discovered, it has been widely introduced to South Pacific Islands (Marschall, 1970; Bedford, 1976, 1980; Gorick, 1980; Caltagirone, 1981; Marschall and Ioane, 1982) Mauritius (Monty, 1982) and Maldives (Zelazny *et al.*, 1990). It has been ranked a land mark in a classical biological control (Caltagirone, 1981). Release of *Or*V-infected beetles has been found to be the most effective method to introduce the virus disease into the black beetle populations as they act as mobile virus reservoirs. For many weeks, infected beetles fly around and defecates large quantities of virus to their habitats. Most frequently, the virus is transmitted directly to other adults by oral contact with fecal matter during mating or by co-occupation of the same habitat (Huger, 2005).

Due to the difficulty in collecting live beetles for infecting and release, use of infected adult beetles has not been attempted in Sri Lanka. However, release of infected larvae into breeding grounds has been practised, but with limited success (Kanagaratnam *et al.*, 1984). Experiences from other countries indicate that if the *Or*V disease is remained at its natural level, and it does not control the beetle population sufficiently. Hence, the need of periodical release of infected beetles has been suggested (Marschall and Ioane, 1982). A strain of *Or*V is also found locally, and it has been confirmed as more similar to the Philippines type by the Institute of Virology, UK (Kanagaratnam *et al.*, 1984).

With the availability of the aggregation pheromone (ethyl 4-methyloctanate), it is now possible to trap a large number of beetles. Therefore, this study was conducted with the objective of determining the lethal dosage of *Or*V to adult beetles and the effect of releasing *Or*V- infected beetles in reducing black beetle damage on palms.

## MATERIALS AND METHODS

The laboratory experiments were conducted at CRI during 2008-2009 and a field study was conducted at two adjoining villages, namely, Sagaragama (20 ac) and Niramesiya (10 ac) in North-Western Province during 2008 to 2012.

### *Identification of Virus*

Several methods could be used.

a. Visual symptoms: Swollen mid-gut with milky content due to rapid virus reproduction in the mid-gut epitheliumm (Zelazny, 1978; Huger, 2005).

b. White colour excreta on the rectum of the infected beetle is visible after third day of infection and onwards. This symptom is used for rapid detection without killing beetles (Singh and Rethinam, 2004).

c. For conformation of the virus, staining method could be used. Mid-gut content of the infected beetle should be dissected and smear of the gut content should be stained by 3% Giemesa staining for 45-60 minutes. The infected mid-gut fluid and its epithelial tissues show pink coloured enlarged nucleus with vacuoles under light microscope (Singh and Rethinam, 2004).

***Virus inoculum***

The local isolate of *Or*V was isolated from the infected adult beetles in a natural population. The infected beetles were identified by examining the white colour excreta on the abdominal tip. They were dissected in the laboratory to collect the mid guts. If the infection is present, the mid-gut appears swollen with milky content inside (Plate 1) (Zelazny, 1978). To confirm the presence of *Or*V, a smear of the gut contents was stained in 3% Giemsa stain. These mid guts were collected in plastic vials and stored in refrigerator at 0°C for future use.

***Collection of beetles***

Pheromone-baited traps were installed in the field, and the trapped beetles were collected periodically. The beetles showing external symptoms of virus infection were discarded and the active beetles were kept separately in plastic bottles with coir dust and ripened banana pieces for the experiments.

***Determination of the lethal dosage of OrV***

A group of 180 beetles of approximately the same age was collected and randomly assigned into 6 groups of 30 beetles. The age of the beetles was determined by observing the amount of hairs remaining on the two posterior ventral segments (Cumber, 1957). Five concentrations of $10^1$, $10^2$, $10^3$, $10^4$ and $10^5$ ppm of virus inoculum were prepared by mixing 100 ml of 5% sucrose solution in 1, 10, 100, 1000 and 10,000 mg of infected guts respectively.

Each group of 30 beetles was inoculated with each concentration of virus solution by oral feeding. A 0.1 ml of virus suspension was placed on each beetle's mouth and allowed to imbibe using a pasteur pipette. Thirty beetles were fed with the same amount of 5% sugar solution and kept as the control. The beetles were kept separately in plastic bottles with coir dust and ripened banana. They were checked daily and the number of dead beetles due to virus infection was recorded during the experimental period of 38 days. The death of beetles due to virus infection was determined by the presence of white excreta on the rectum and further confirmed by staining by 3% Giemsa stain (Gorick, 1980).

Plate 1 Progression of dissection of infected beetles and the beetles showing the milky mid gut

***Determination of the effect of releasing OrV infected beetles on damage levels***

The experiment was conducted in a 30 ac area. The sites consisted of ample number of breeding grounds as many villagers were self-employed in rafter and coir processing industries. The nature of the coconut stand was mixed and coconut was grown as a home-garden crop with minimum management.

Before releasing *Or*V infected beetles, the natural incidence of the virus in the beetle population was assessed by trapping beetles by installing 2 and 3 pheromone-baited traps in Niramisiya and Sagaragama respectively for one week. The trapped beetles were dissected and the presence or absence of virus infection was ascertained by staining method as described above.

A virus solution of $10^5$ ppm was prepared by macerating 100g of infected guts using a sterilised mortar and pestle and adding 100 ml of 5% sucrose solution. The healthy beetles collected in pheromone-baited traps installed elsewhere were infected with 1-2 µl of inoculum solution by oral feeding with the aid of a pasteur pipette.

The infected beetles were released at the rate of 10 beetles per acre at six-month intervals into coir dust heaps in the area up to 3 years. Before each release, a sample of beetles equivalent to 10% of the released number were kept in the laboratory to confirm the infection.

Before the first release, a total of 150 palms randomly selected at Sagaragama and 75 palms at Niramesiya were marked to assess the black beetle damage. In each palm, the total number of leaves and the number of black beetle-damaged leaves having geometric cuts or damage on the spear (bud) leaf were recorded. The bud leaf of each palm was marked at each observation for ease of subsequent assessments. Thereafter, assessments were taken at 4-5 month intervals, and at each observation, the presence or absence of damage on the bud leaf and the first opened leaf was recorded.

Every year after the first release, the incidence of *Or*V in the population was determined by collecting beetles from each 5 pheromone-baited traps installed in the two areas. They were dissected and mid gut was stained to confirm the infection.

***Data analysis***

To find the most suitable *Or*V concentration, the total number of dead beetles due to virus infection in each concentration was taken and the cumulative percentage mortality of each treatment was corrected by using Abbott's formula. The percentage mortality and the concentration of the virus were transformed to probit mortality and log concentration respectively and graphs were plotted to determine $LC_{50}$ (concentration killing 50% of the population) and $LC_{90}$ values (concentration killing 90% of the population). The data analysis was done by using CATMOD procedure.

The quadratic regression analysis ($Y=\alpha+\beta_1 T-\beta_2 T^2$, where Y is the percentage of the damage of different leaf types, T is time and $\alpha$ , $\beta_1$ and $\beta_2$ was constants) were performed to determine the relationship between releasing *Or*V infected beetles in reducing damage levels over the time using Minitab 15.

## RESULTS AND DISCUSSION

***Determination of the lethal dosage of Or*V**

All the concentrations of virus inoculum tested caused mortality of black beetle adults. The cumulative percentage of mortality of beetles increased with the increase of *Or*V concentration (Table 1). There was no significant difference in cumulative percentage mortalities of 7.3, 25.1 and 33.3 among the concentrations of $10^1$, $10^2$ and $10^3$ ppm respectively and they were significantly lower ($P< 0.004$) than the mortalities recorded in concentrations $10^4$ ppm and $10^5$ ppm.

The cumulative percentage mortalities between $10^4$ ppm and $10^5$ ppm were significantly different ($P < 0.02$), and at $10^5$ ppm, all beetles were dead (Table 1). The calculated $LC_{50}$ of this study for local *Or*V inoculum was $10^{2.7}$ ppm while $LC_{90}$ was $10^{3.7}$ ppm (Figure 1). A 100% mortality was not achieved in all the doses except the $10^5$ ppm dose, during the experimental period. At $10^5$ ppm, all the beetles were killed by the 20th day (Figure 2).

Table 1 Cumulative percentage mortality of *O. rhinoceros* beetles at different concentrations of virus inoculum

| Concentration (ppm) | Cumulative percent mortality ± SE |
|---|---|
| $10^1$ | $7.3 \pm 0.27^A$ |
| $10^2$ | $25.1 \pm 0.83^A$ |
| $10^3$ | $33.3 \pm 0.46^A$ |
| $10^4$ | $81.4 \pm 0.40^B$ |
| $10^5$ | $100.0 \pm 0.39^C$ |

Note: The means followed by same letter are not significant at 5%.

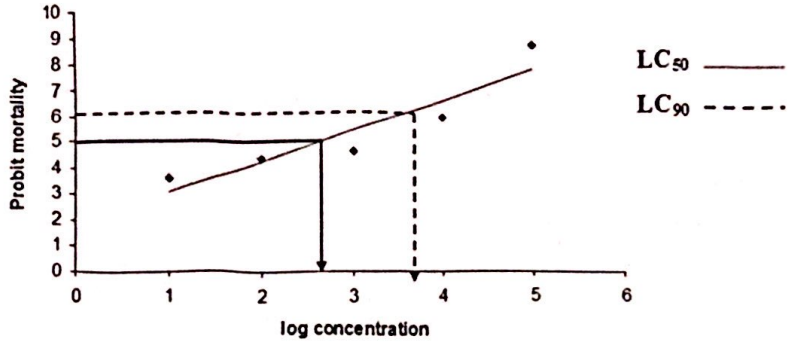


Figure 1 Probit mortality against log concentrations of *Or*V

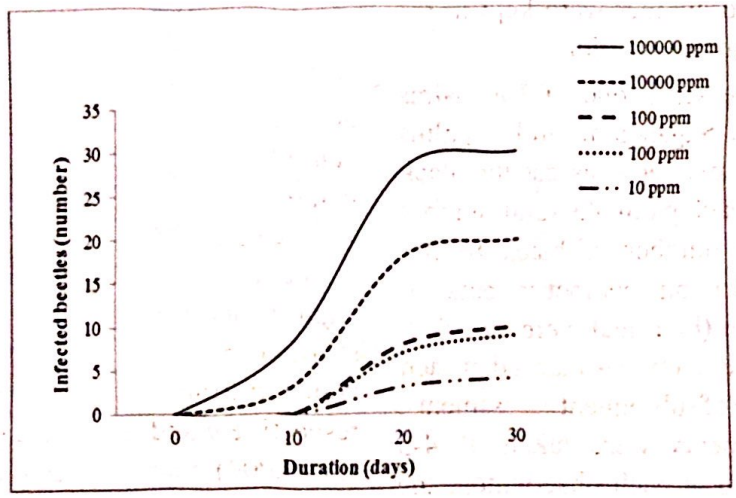


Figure 2 Number of dead beetles over time at different *Or*V concentrations

**The effect of releasing *Or*V infected beetles on damage levels**

Over 3-fold increase in the incidence of *Or*V infection in the beetles trapped in the pheromone-baited traps was observed at the end the experimental period in both sites (Figure 3). Also, over 90% of the infected beetles, kept at the laboratory were dead due to infection indicating that the field-released beetles too were successfully infected.

The mean percentage damage on the spear leaf and the first youngest leaf at Sagaragama and Niramesiya are given in Figures. 4 (A & B) and 5(A & B) respectively. In both sites, prior to release of the *Or*V-infected beetles, nearly 80% of the palms showed leaf damage, except the spear leaf at Niramesiya. After the release, percentage palms with damaged fronds decreased sharply up to the second year, and thereafter no further reduction in damage was observed. The damage levels reached more or less an equilibrium level around 40% level in both sites. A statistically significant negative relationship was observed in Sagaragama and Niramesiya between damage level and time. At Sagaragama, the quadratic regression for the spear leaf and the first leaf (spear leaf=78.33-2.406time+0.0471 time$^{**2}$, $R^2$=88.8,P=0.002; first frond=79.55-2.175time+0.0471 time$^{**}$2 $R^2$=80.5,P=0.008 respectively). The same negative trend was observed in Niramesiya (spear leaf=81.76-2.717time+0.05207 time $^{**2}$, $R^2$=79.3,P=0.002; first frond, Y=69.65-2.007time+0.03490 time$^{**2}$ $R^2$=87.9,P=0.009 respectively).

The results of this study confirmed that the dose of $10^5$ ppm of the local strain of *Or*V is capable of causing 100% mortality in black beetle adults. Further, release of laboratory-infected beetles increases the natural incidence of *Or*V infection in the field population considerably. Thus, it was revealed that the damage due to black beetle could be reduced by releasing infected beetles with a significant negative relationship between damage level and time.

The findings open up a new avenue of managing black beetle and add another component to the integrated management of the pest. This is the first study in Sri Lanka that showed release of *Or*V infected beetles could significantly reduce the damage to coconut palms. Studies elsewhere showed that the percentage reduction in damage due to release of *Or*V vary from location to location. In

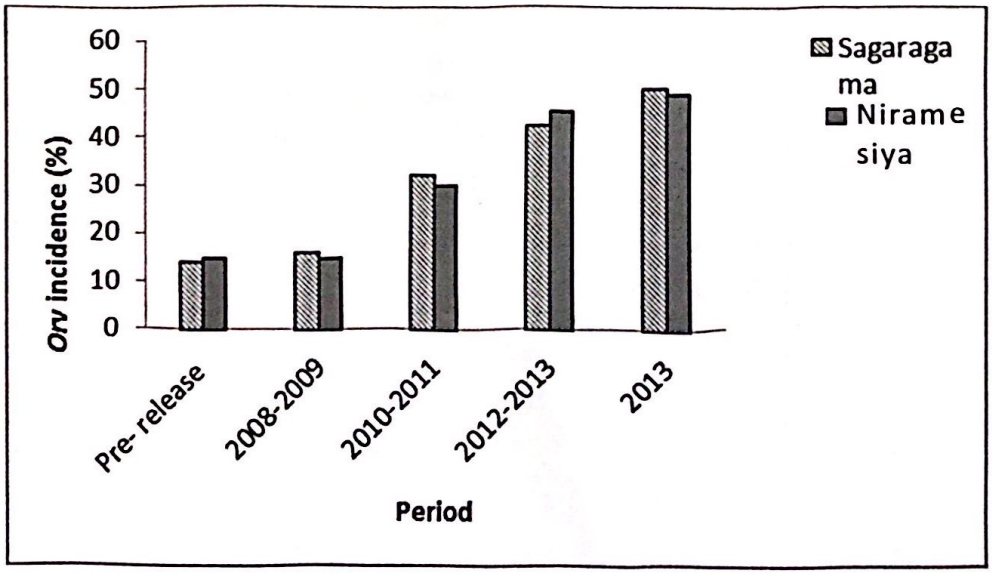


Figure 3 *Or*V incidence in adult *O. rhinoceros* population before and after release of infected beetles at the two sites

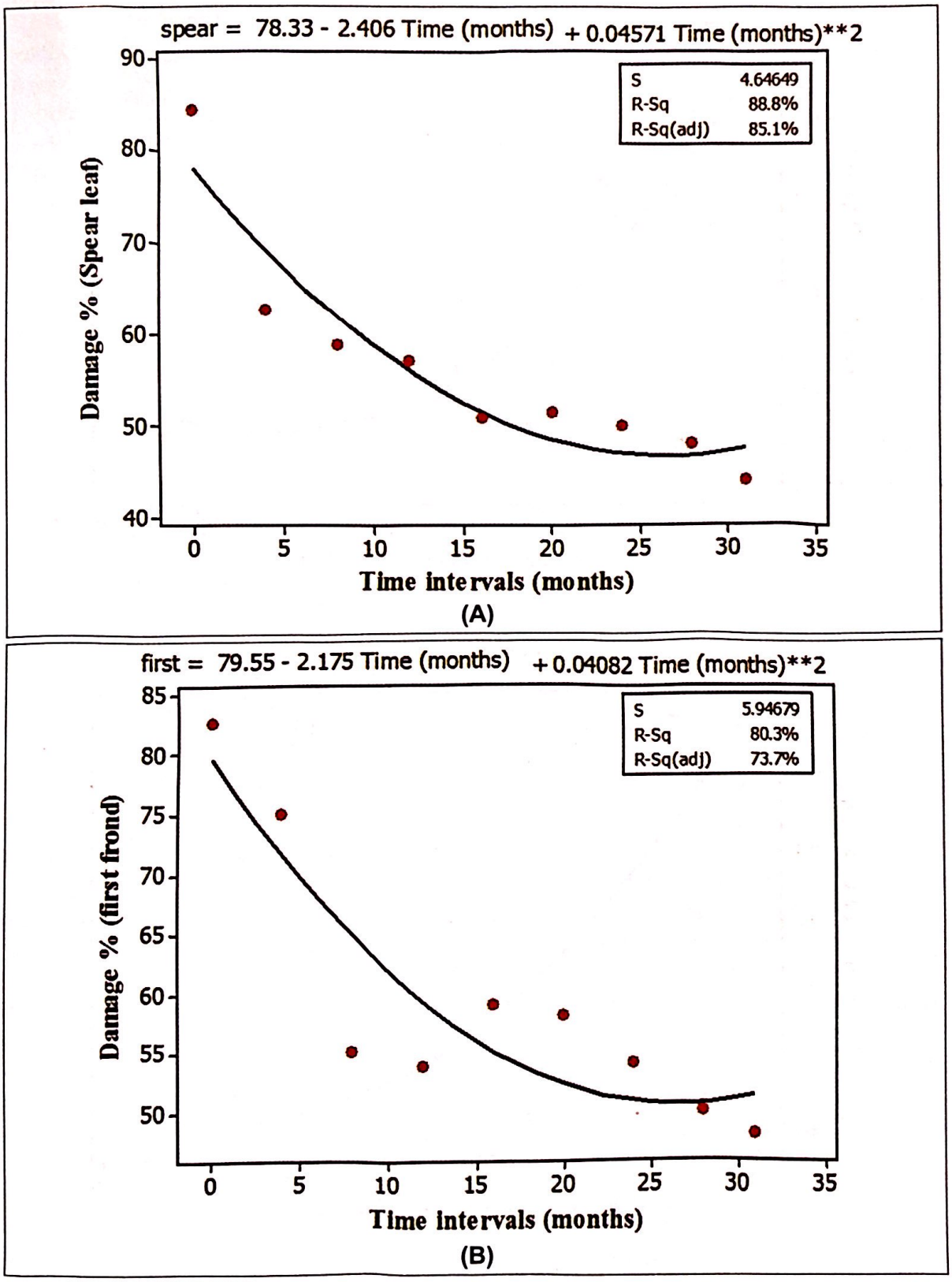


Figure 4 The fitted line plot for the percentage of palms with spear leaf damage (A) and first frond damage (B) at Sagaragama over time

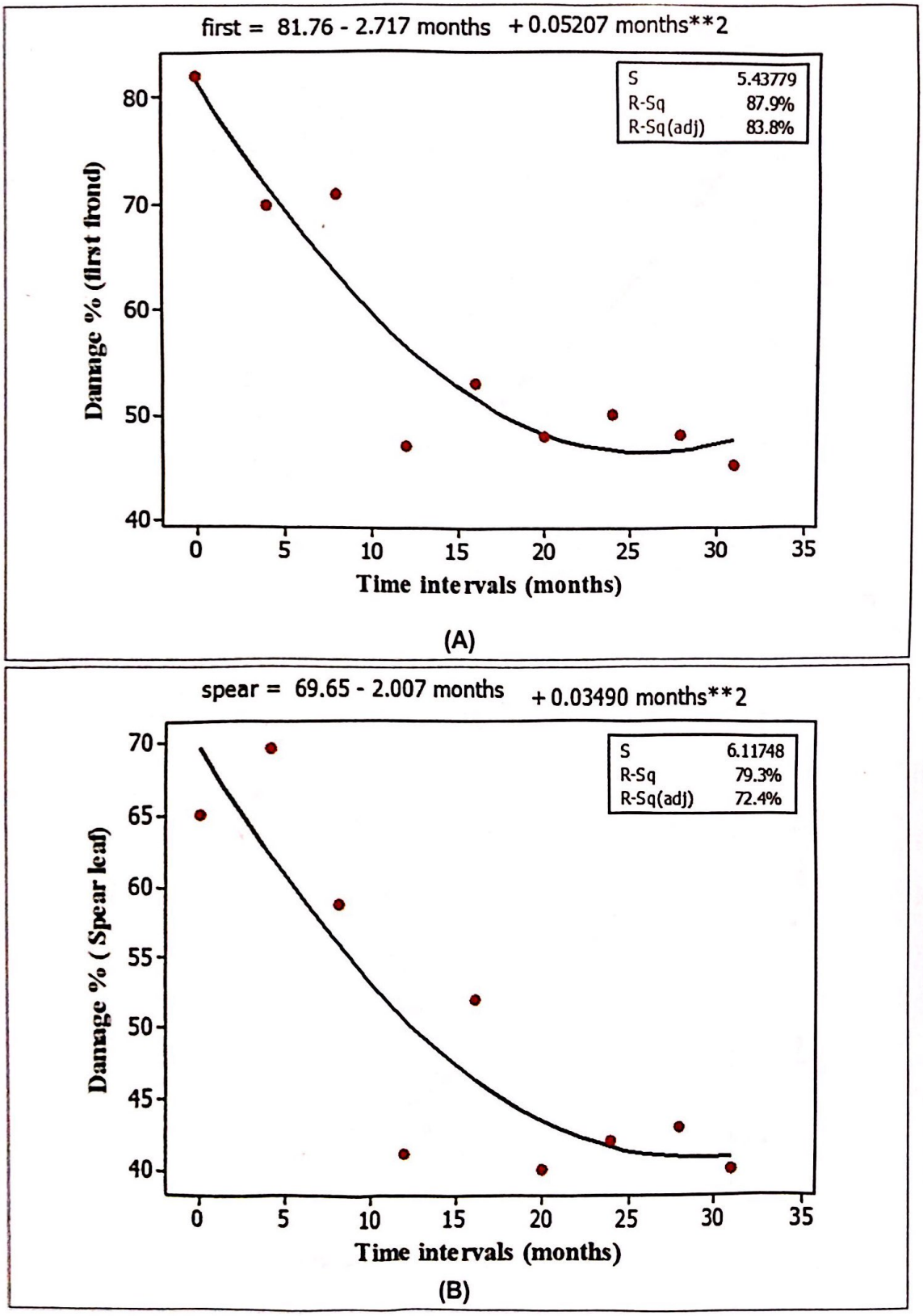


Figure 5 The fitted line plot for the percentage of palms with spear leaf damage (A) and first frond damage (B) at Niramisiya over time

Minicony Island, India, the leaf damage was reduced from 55.83% to 12.89% after three years of *Or*V introduction (Mohan *et al.*, 1989). The conspicuous reduction in palm damage up to 95% was reported in South Pacific islands; Fiji, Tonga, Wallis Islands, Tokelau Island, Palau Island and American Samoa (Bedford, 1980).

In view of augmenting *Or*V into the wild population of *O. rhinoceros* and thereby reducing damage to palms, the *Or*V fed beetles should act as flying virus reservoirs that effectively disseminate the disease among wild population. Thus, they have to be inoculated with an effective dose of the virus solution in the laboratory. In this study, *Or*V doses of $10^1$, $10^2$ and $10^3$ ppm did not even kill 50% of the inoculated beetles during the 38 days. Further, $10^4$ ppm dose too killed only 67% of the beetles. It was very unlikely that these doses would reach a death rate more than that even if the experimental period was extended beyond 38 days. In all these doses, the death rate reached a plateau around 30 days after inoculation. Considering the effectiveness of $10^5$ ppm dose in causing death in 100% of the beetles, that dose was chosen for inoculating beetles for field release. Further, the fact that this dose kills 100% of the beetles, it is an advantage in augmentation as the beetles will not survive to attack the palms for a longer period. In this study, all beetles got killed in 21 days after inoculation, but during that time, they were capable of disseminating the virus to the breeding sites they visited as well as the beetles fed on the same feeding hole together.

Different levels of natural incidence of *Or*V in *O. rhinoceros* population have been reported. In India, natural incidence ranged from 42.9% to 75% (Mohan *et al.*,1983). The natural incidence of *Or*V in Philippines, Borneo and central Sumatra was 29% (Zelazny, 1977). Compared to the above incidences, the natural level of the Sri Lankan local isolate is low (app. 15%). Low virulence of the local isolate may be one of the reasons for low natural incidence, but it was established in this study that augmentation of the *Or*V significantly enhances the incidence in the field and reduction in leaf damage levels over time. However, it may be necessary to repeat releases at 6-month intervals for at least 2 years to reduce damage levels by half. Since the damage levels remained the same thereafter despite further releases, it will be necessary to find out the period required to re-start the releases in future studies. It was not possible to maintain a control plot without releasing inoculated beetles similar to that of the released block because the variability in damage levels as well as black beetle populations even in close-by sites vary widely, hence comparison would not be reliable.

Young and Longworth (1981) reported 43% reduction in larval population occurred soon after the first release in Tonga and 94% in Minicony Island (Mohan *et al.*, 1989). The availability of breeding grounds is a crucial factor influencing the spread the virus disease. However, suitability and number of breeding grounds change over time to time due human interventions. Also, the rate of immigration and emigration of beetles affect both the level of the disease and the damage incidence. The dose and releasing frequency of the *Or*V could be another attributing factor. Therefore, the level of reduction in damage in any area could be mostly site and time specific. Releasing *Or*V could be ideal for the locations where plenty of breeding grounds are available, especially the ones that are not accessible to cause epizootics and brought down the pest population.

It could not be expected that the release of *Or*V alone is sufficient to manage the black beetle. It may be a component of an IPM programme and it is recommended to practise other measures such as eliminating breeding grounds, application of non-toxic repellent substances, extraction of beetles and monitoring and reducing pest population using pheromone-baited traps.

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