HOST - SPECIFICITY, PATHO - PHYSIOLOGY AND TRANSMISSION OF THE BACULOVIRUS (KERALA ISOLATE) INFECTING *Oryctes rhinoceros* Linn. (SCARABAEIDAE : COLEOPTERA)

By

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THESIS

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DECLARATION

I hereby declare that the thesis entitled **HOST-SPECIFICITY, PATHC-PHYSIOLOGY AND TRANSMISSION OF THE BACULOVIRUS (KERALA ISOLATE) INFECTING** *Oryctes rhinoceros* **Linn. (SCARABAEIDAE: COLEOPTERA)** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

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CERTIFICATE

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CERTIFICATE

We, the undersigned members of the Advisory Committee of Smt. SUMA PAULOSE a candidate for the degree of Doctor of Philosophy in Agriculture with major in Agricultural Entomology, agree that this thesis entitled **HOST-SPECIFICITY, PATHO-PHYSIOLOGY AND TRANSMISSION OF THE BACULOVIRUS (KERALA ISOLATE) INFECTING** Oryctes rhinoceros **Linn. (SCARABAEIDAE: COLEOPTERA)** may be submitted by Smt. SUMA PAULOSE in partial fulfilment of the requirement for the degree.

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Suma Paulose

SUMA PAULOSE

Dedicated to My Husband and Mol

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Introduction

INTRODUCTION

The Rhinoceros beetle, *Oryctes rhinoceros* Linn. which is widely distributed all over the coconut growing areas in the country is one of the most serious pests of the coconut palm. The adult beetles bore into the central spindle of leaves and unopened spathes, causing substantial damage, debilitation and yield reduction. The recommended control measures include leaf axil filling with a mixture of insecticide and sand thrice a year and treatment of the breeding media such as compost and farm yard manure with certain toxicants. These methods which are very cumbersome and labour intensive often lead to unwarranted repercussions such as environmental pollution and annihilation of the beneficial soil organisms. In view of several deleterious side effects arising from the use of chemical insecticides, it has become necessary to develop alternative strategies which are more ecofriendly and self sustaining.

In integrated pest management strategies, biocontrol involving the use of natural enemies and microbial pathogens deserve greater attention in view of the safety to non-target organisms, safety to the environment, economy, compatibility with other methods of control and selfsustainability which can bring about progressive reduction in pest population . In view of the above considerations, the use of insect pathogens has gained importance as a component of ecologically sound crop pest management programmes (Falcon, 1973). Among the microbial pathogens, viral insecticides which are strongly target specific in action, are considered to be ideal components in integrated pest management programmes against the Rhinoceros beetle.

Among many microbial pathogens tested against the Rhinoceros beetle, the *Baculovirus oryctes* discovered by Huger (1966 a and b) in Malaysia was later found to be a valuable agent for the biocontrol of the pest in several release sites . *B. oryctes* is reported to be one of the few successful microbial

control agents ever used for the suppression of this beetle pest. Introduction of this pathogenic virus from Malaysia to South Pacific Islands in the late sixtees caused a remarkable reduction in the population of the Rhinoceros beetle below the economic threshold.

Experimental release of the virus in Kerala and the Minicoy and other islands of the Union Territory of Lakshadweep gave excellent results against *O. rhinoceros* (Mohan *et al.*, 1983, 1989). Incidence of the baculovirus disease infesting adults and grubs of *O. rhinoceros* has been reported from several parts of Kerala to the extent of 40 to 75 per cent.

The basic information available on the Kerala isolate of the *B. oryctes* is very scanty. Such information is very valuable to exploit the potential of this pathogen for pest control. In this context, the present studies were undertaken to generate basic information on *B. oryctes* (KI) on several aspects such as morphology, patho-physiology, histopathology, gross pathology, viability, modes of transmission, cross-

infectivity to other pests of coconut, effect of certain physical factors on the virulence of the virus and safety to nontarget organisms.

Review of Literature

REVIEW OF LITERATURE

The literature available on insect viruses with special reference to *Baculovirus oryctes* is briefly reviewed in this chapter.

2.1 Role of baculovirus in the control of *Oryctes rhinoceros*

The *Baculovirus oryctes* infecting the Rhinoceros beetle is documented to be one of the most successful microbial control agents employed against an insect pest (Caltagirone, 1981). The baculovirus was originally recorded on *O. rhinoceros* grubs from Malaysia by Huger (1966 a and b) and was subsequently introduced into several of the South Pacific Islands to control the pest populations (Marschall, 1970; Hammes, 1971; Zelazny, 1973 a; 1977 b; Young, 1974; Bedford, 1976; 1977; 1986; Gorick, 1980; Uili, 1980; Gutierrez, 1981; Stechmann and Semisi, 1984; FAO, 1986). The virus had rapidly spread and established in all the introduced areas thereby effectively containing the

population of *O. rhinoceros* below the economic injury level (Hammes and Monsarrat, 1974; Zelazny, 1977 b; Bedford, 1980). Purrini (1989) introduced *B. oryctes* into the *Oryctes monoceros* 01. population in Tanzania. Villacarlos and Betonio (1990) reported the occurrence of baculovirus in rhinoceros population in Marcos Baybay and Leyle Islands of Philippines. The introduction of the disease into Maldives led to the suppression of the population in that area (Zelazny *et al.*, 1990, 1992).

The disease was reported to occur in the natural populations of the grubs and adults of *O. rhinoceros* in Kerala, India (Mohan *et al.*, 1983). The Indian isolate has been successfully used to control *O. rhinoceros* in the Minicoy and other Islands of the Union Territory of Lakshadweep. Later, it was also introduced to Nicobar Island (Mohan *et al.*, 1989). Rajamanickam *et al.* (1989) reported the natural incidence of *B. oryctes* in Tamil Nadu.

2.2 Morphology of the virus

Huger (1966 b) described the *B. oryctes* as a small particle of about 200 nm long and 70 nm wide. The virus particle consists of nucleocapsid surrounded by an envelope (235 X 110 nm). The genome is a double-stranded supercoiled DNA molecule with a molecular weight of 60 x 10^6 to 92 x 10^6 daltons (Monsarrat *et al.*, 1973 a and b; Revet and Monsarrat, 1974; Payne, 1974; Payne *et al.*, 1977). These characteristics strengthened the view that the virus should be included in the family Baculoviridae. The use of the original nomenclature *Rhabdionvirus oryctes* (Huger, 1966 b) has since been discontinued (David, 1975). Payne *et al.* (1977) reported that some of the virus particles had a small finger like protrusion at one end while the other had a hollow tail which could be seen on disruption of the envelope.

The virus is normally non-occluded; however, inclusion bodies containing virions were seen in the cytoplasm of cells of fat body, ovarian sheaths, inner spermathecal walls and midgut epithelium (Monsarrat *et*

al., 1973 a). A detailed characterisation of the *B. oryctes* KI genome was described and compared with that of the type isolate PV 505 by DNA reassociation kinetics and by the use of restriction endonucleases by Mohan and Gopinathan (1992). On the basis of differences in restriction enzyme profiles between the two genomes and previously reported differences in protein profiles and antigenic make up, a new taxonomic status of a variant of *B. oryctes* was proposed for the Indian isolate.

All baculoviruses have virions of the same basic structure-an enveloped rod shaped nucleocapsid in which an amorphous but definite layer exist between the nucleocapsid and the envelope (Tanada and Kaya, 1993).

2.3 Diagnosis of infection

B. oryctes infection in *O. rhinoceros* could be detected by electron microscopic observation of virions in infected faeces (Monsarrat and Veyrunes, 1976), histological examination (Huger, 1973); Monty, 1974), immunological testing (Croizier and Monsarrat, 1974; Longworth and Carey, 1980), bioassay method (Zelazny,

1972), adult midgut content smear and macroscopic examination of the adult midgut (FAO, 1978). Mohan *et al.* (1983), and Mohan and Pillai (1983) diagnosed the disease in the adult beetles by visual examination of the midgut and its contents, Giemsa stained smear of midgut contents, immuno-osmophoresis (IOP), bioassay test and electron microscopy. The smear test and immuno-osmophoresis were found to be ideal diagnostic methods for routine screening.

2.3.1 Symptomatology

Consequent on infection of grubs of *O. rhinoceros* by the Malaysian isolate of *B. oryctes*, it was found that the grub refused to take food. The abdomen gradually assumed a turbid glassy and sometimes pearly appearance. Diarrhoea was a concomitant symptom. Successive disintegration of the fat body and considerable increase in the amount of haemolymph brought about a sort of dropsied condition. In this stage, the specimens were very translucent when held near and viewed against light. As the disease approached the terminal phase, most grubs appeared

shiny, beige and waxy, especially in the abdominal region, which more often was broadened. Frequently, the turgor was considerably increased to such an extent that the hindgut was completely extroverted (Huger, 1966 b). Symptoms described by Mohan *et al.* (1985) with the Indian isolate of *B. oryctes* were similar to that of the Malaysian isolate. Most of the virus multiplication occurred in the midgut epithelium (Payne, 1974).

The diseased adult beetles generally showed no external symptom (Bedford, 1981). Zelazny (1977 a) reported that adults stopped feeding and flew less frequently. Males mated less often after they became infected. Monty (1974) reported malformed elytra, wings or abdominal walls in adults.

2.3.2 Gross pathology

The lethal infection time for *Oryctes* grubs that have consumed *B. oryctes* contaminated breeding medium depends on the larval instar: first instar grub die after nine days, second instar after thirteen days and

third instar after eighteen days and the older third instar after twenty five days (Zelazny, 1972). Mohan *et al.* (1985) reported that the second instar was the least susceptible stage to viral infection. The virus proved highly infectious to both grubs and adults (Gorick, 1980). Infected adults die sooner than healthy - 25 days as compared to 70 days of healthy (Zelazny, 1973 a). The relative virulence of the 22 isolates were tested by determining the LD_{50} for third instar larvae and adults. LD_{50} value for larvae and adult were 4.83 to 14.77 pico litres of haemolymph. The dosage-mortality responses did not reveal any significant differences among the different *B. oryctes* isolates, with the exception of one isolate which showed a different biological activity level against third instar larvae. All the isolates of the virus were sufficiently virulent and showed considerable potential as a microbial control agent against *O. rhinoceros* (Alfiler, 1989).

Studies on susceptibility of different larval instars of *Heliothis armigera* (Hb.) to nuclear polyhedrosis virus infection revealed that the earlier

instars were highly susceptible (Narayanan, 1979). The LT_{50} was shorter in young larvae than the older ones. Mathew (1980) observed a prolongation of incubation period of nuclear polyhedrosis virus in the larvae of *Anadevidia peponis* Fab. from 2.15 days in the first instar to 4.40 days in the sixth instar larvae. The viral infection caused 100 per cent mortality of the first, second, third and fourth instar larvae. The fifth and sixth instar larvae showed less susceptibility, the mortality being 90 and 70 per cent respectively.

Larvae of *Pandemis heparane* Denis and Schiff infected with nuclear polyhedrosis virus soon after hatching die in a few days, while death in older larvae is delayed for 30 days (Amargier *et al.*, 1981). According to Bergerjon *et al.* (1981) the level of resistance of *Lymantria dispar* Linn. to nuclear polyhedrosis virus infection increased as the age advanced. Chan *et al.* (1981) found that the infection of the first and second instar larvae of *L. dispar* was dose-dependant and a concentration of 1.9×10^4 to 10^6

polyhedra caused 58 to 92 per cent mortality of the larvae within eight days of treatment. In some nuclear polyhedrosis viral infections, the larval period is prolonged even beyond the period of normal larval stage (O'Reilly and Miller, 1989). The nuclear polyhedrosis virus of *Spodoptera exigua* (Hb.) was more virulent against eggs than larvae. The third instar larvae were more sensitive than first or fifth instar larvae (Im *et al.*, 1991). Cytoplasmic polyhedrosis virus was infective to all larval instars of *Heliothis virescens* (F.) except the prepupal. Three, four, five and six days old larvae were more susceptible than the older larvae (Sikorowski and Lawrence, 1994).

2.3.3 Patho-physiology

The *B. oryctes* infected adults of *O. rhinoceros* had a shorter life-span and laid fewer eggs than the uninfected ones (Zelazny, 1972; 1973 a). The effect of nuclear polyhedrosis virus on consumption, digestion and utilisation of food by the tobacco caterpillar *Spodoptera litura* (Fab.) was reported by Ramakrishnan and Chaudhari (1976). There was almost continuous

weight gain by both untreated and virus fed larvae during the first three days. The food intake, faecal production and weight gain of treated larvae were significantly lower than those of untreated larvae on the fourth and fifth days. Digestibility was not apparently affected by infection.

In India, Philip and Jacob (1980) carried out studies to assess the effect of granulosis virus on food consumption, growth rate and utilisation of food by the larvae of *Pericallia ricini* F. The higher consumption index observed in diseased larvae was due to short feeding period and low fresh weight of food consumed. The growth rate in diseased larvae was considerably lower than in healthy ones. Philip and Jacob (1981) determined the effect of the virus on larval weight and length. Diseased larvae had a mean weight of 0.101 ± 0.005 g while healthy larvae had a higher mean weight of 1.516 ± 0.051 g. Diseased larvae had a mean length of 2.50 ± 0.023 cm while healthy one had a mean length of 5.38 ± 0.037 cm.

Eid *et al.* (1985) studied the effect of oral administration of nuclear polyhedrosis virus to third instar larvae of *Spodoptera littoralis* (Boisd.), a major pest of cotton. There was a general reduction in food intake and weight gain. This decrease was dose-dependant and most pronounced among larvae that died before pupation. Larvae of *Pieris rapae* Linn. when fed the granulosis virus of *Pieris brassicae* at the beginning of the first, second, third or fourth instar, their food consumption was reduced significantly, but when they were fed with virus at the end of the fourth or beginning of the fifth instar, food consumption increased by 36.3 and 87.2 per cent respectively (Wan and Hd, 1986).

Narayanan and Gopalakrishnan (1988) reported the patho-physiological changes in *H. armigera* infected by the nuclear polyhedrosis virus. Diseased larvae in general consumed lesser amount of food material than healthy ones. The rate of feeding was reduced from the first day onwards and ceased completely on the sixth day. The growth parameters such as food consumption index, growth rate, approximate

digestibility, gross efficiency and net efficiency of ingested and digested food to the body substance were markedly less in diseased larvae than in the healthy ones.

Patil *et al.* (1989) studied the effect of nuclear polyhedrosis virus on growth, development and reproduction of *Mythimna separata* Wlk. Weight of larval, pupal and adult stages were decreased. Growth index, fecundity and egg hatchability declined considerably. The effect of *L. dispar* nuclear polyhedrosis virus infection on larval development was investigated by Burand and Park (1992). Virus infected neonate larvae were significantly smaller than uninfected larvae. In later instars, slow weight gain, delay in the onset of apolysis and moulting inhibition were observed.

2.3.4 Histopathology

Detailed histopathological investigations have been made in many baculovirus, but very few such attempts have been made in the case of *B. oryctes*. *B.*

oryctes was first observed in the nuclei of midgut epithelium of larvae and adults (Huger, 1973; Payne, 1974) and also in the walls of ovarian sheath and in the inner wall of spermatheca of *O. rhinoceros* (Monsarrat *et al.*, 1973 a). The virus multiplies in the nuclei of midgut epithelial cells. New cells which are produced in regenerative crypts, also become infected and their nuclei hypertrophy and the gut lumen eventually gets filled up with disintegrating cells and virus particles (Huger, 1973; Monty, 1974).

Pawar and Ramakrishnan (1977) made histopathological observations on the larvae and pupae of *S. litura* which were infected with nuclear polyhedrosis virus. Fat body,trachea and hypodermis were the main target tissues and in addition, the gonads, brain and ganglia of the larvae showed moderate infection. Foregut, hindgut, malpighian tubules and silk glands were not attacked. Smirnoff and Ackermann (1977) described histopathological changes in nuclear polyhedrosis virus-infected European skipper *Thymelicus lineola* (Ochs.). All tissues except the nerve cells and silk glands were infected and all nuclei of

infected cells were filled with polyhedra. Velichkova and Yanchev (1981) found out the nuclear polyhedrosis virus infection in fat body, hypodermal, tracheal and muscle tissues as well as the midgut epithelium and malpighian tubules of the larvae of *Mamestra brassicae* (L.).

Shreesam *et al.* (1983) reported that mainly the nuclei of the fat body, hypodermis, hypodermal glands and tracheal matrix were the target tissues for replication of the polyhedrosis virus of *M. separata*. Later the wing buds, imaginal discs, ovary, testis, silk glands, malpighian tubules and sarcolemma showed heavy infection. Finally, polyhedral inclusion bodies were also observed in nuclei of neurilemma, frontal ganglion, thoracic ganglion and cerebral complex. Histopathological studies conducted on the nuclear polyhedrosis virus infected *Opisina arenosella* Wlk. by Philip (1985), revealed that the hypodermis, fat body and trachea were the principal sites of virus multiplication. In addition to the above tissues,

muscles, malpighian tubules, connective tissues surrounding the gut, the gut epithelium, nerve ganglia and epithelial sheath of the gonads were also infected.

The nuclear polyhedrosis virus of *Orgyia pseudotsugata* Mc Dunn. infected fat body, epidermis and tracheal matrix (Su, 1986). The virus was replicated in the nucleus and in the cytoplasm. Nuclear contents in the fat body cells broke and got condensed into irregular mass one day after inoculation. Pyknotic nuclei with condensed chromatin occurred after two days and infected cells increased in size three days after inoculation. Disintegration of the nuclear membrane occurred four to seven days after inoculation. Similar changes took place in the cells of the epidermis and tracheal matrix, but the progress of infection tended to be more uniform than in the fat body cells. Beach *et al.* (1987) examined the histopathological changes in the nuclear polyhedrosis virus infected *Pseudoplusia includens* Wlk. Fat body, tracheal matrix and hypodermis showed various stages of infection four

days after inoculation. Occluding and mature polyhedra were observed in all the three tissues. Infection was not observed in muscle and nerve tissues. Hess and Falcon (1987) reported that granulosis virus of *Cydia pomonella* (L.) was reproduced in a moderately wide range of tissues: fat body, trachea, tracheoles, epidermis, malpighian tubules and even muscles.

The histopathological changes in *Heliothis zea* Boddie, *Spodoptera frugiperda* and *Trichoplusia ni* (Hb.). infected with ascovirus were studied by Federici and Govindarajan (1990). In all three isolates, infected cells and virion containing vesicles in the haemolymph were observed three days after inoculation. The isolate from *H. zea* and *T. ni* exhibited relatively broad tissuetropism infecting the tracheal matrix, epidermis, connective tissue and fat body. *H. zea* replicated more extensively in the epidermis. The isolate from *T. ni* replicated in the fat body during the early stages of disease. The isolate from *S. frugiperda* replicated only in the fat body and completely destroyed the tissues by 12 days

after inoculation. Federici and Stern (1990) reported the replication and occlusion of granulosis virus of Western grape leaf skeletoniser *Harrisina brillians* B. The principal site of infection was the midgut epithelium. Infection resulted in almost complete destruction of the midgut epithelium. Lobinger (1991) reported that cytoplasmic polyhedrosis virus of *Dasychira pudibunda* L. infected mainly the midgut epithelium. Miranpuri *et al.* (1992) reported the changes in haemolymph in response to infection with *Melanoplus sanguinipes* (F.) entomopox virus (MSEPV). Total haemolymph counts decreased in infected grasshopper after ten to fifteen days. Morphological changes in haemocytes were observed late in infection including a small percentage of enlarged haemocytes and cell with extensive pseudopodia.

Ding and MA (1993) reported the effect of sublethal dose of nuclear polyhedrosis virus on larvae of gypsy moth. Electron micrograph of ultrasection showed that the fat body, trachea, ganglia, brain, ovary and prothoracic glands of the adult females were infected.

2.4 Transmission of virus

2.4.1 Transovum and transovarial transmission

Viruses are transmitted through generations in two possible ways. Transmission of virus through the contaminated egg surface is called transovum whereas transmission of the virus internally through the egg itself is designated as transovarial (Steinhaus, 1954).

Simmons and Sikorowski (1973) reported that the larvae of *H. virescens* reared from eggs contaminated with cytoplasmic polyhedrosis virus were found to weigh 50 per cent less than those reared from virus free eggs. The larval stage in a virus infected group was four days longer than from virus free eggs. Diseased pupae weighed on an average of 60 mg less than the healthy ones. Adult emergence was reduced by ten per cent in virus infected pupae. Adults live nine days less than healthy one and egg production was reduced by 68 per cent in diseased females. The result of an investigation made by Larionov and Bakhvalov (1974) with the egg of *Lymantria monacha* Linn. by electronic

and luminiscent microscopy confirmed that the nuclear polyhedrosis virus of *L. monacha* can be transmitted through eggs. Hamm and Young (1974) observed that the nuclear polyhedrosis virus of *H. zea* was transmitted by surface contamination of eggs with inclusion bodies which had passed through the digestive tract of adult fed on polyhedra. Scanning electron micrographs showed polyhedra on the surface of eggs obtained from treated moths.

The treatment of eggs of *H. virescens* for eight minutes with 0.2 per cent sodium hypochlorite solution completely prevented the transmission of cytoplasmic polyhedrosis virus between generations. Thus the virus was transmitted on the surface rather than within the eggs (Mercy and Dulmage, 1975). Etzel and Falcon (1976) could not find transovum transmission in granulosis virus of *C. pomonella*. Nuclear polyhedrosis virus was transmitted by both transovum and transovarial routes in *M. separata* (Neelgund and Mathad, 1978). Tatchell (1981) studied the transmission of granulosis virus of *P. brassicae*. The

granulosis virus when applied as a paste to the tip of the abdomen of the female was readily transmitted to the progeny. Mortality of the progeny decreased with increasing time after treatment from 76.6 per cent immediately after contamination to two per cent on day sixteen.

Surface contamination of egg masses of the cotton pest *S. littoralis* in the laboratory with nuclear polyhedrosis virus resulted in transmission of the disease to larvae that hatched (El-Nagar *et al.*, 1985). Transmission of nuclear polyhedrosis virus of *Spodoptera mauritia* Boisd. from parent to progeny occurred mainly through surface of egg. Transovarial transmission was also observed (Nair and Jacob, 1985). Nuclear polyhedrosis virus of *H. armigera* when applied at different concentrations on the egg, the larval mortality was noticed from the fourth day onwards (Narayanan, 1985 b). The female moth of *O. arenosella* infected orally or contaminated externally served as effective carrier of the pathogen to the next generation mainly through surface contamination of eggs (Transovum) (Philip, 1985).

The transmission of a nuclear polyhedrosis virus from generation to generation was studied in *Spodoptera litura* (Fab.) by Santharam and Jayaraj (1989). Virus infected adults were produced by feeding fifth instar larvae with subnormal concentration of virus. Eggs and larval mortality was observed in the progeny from infected adult, the transmission being transovum and transovarial. The healthy adults were able to transmit the nuclear polyhedrosis virus to their progeny by egg surface coating when fed with nuclear polyhedrosis virus orally, more so when both sexes were fed. The larval mortality was more in the case of second and third day laid eggs.

Padmavathamma and Veeresh (1989) reported the effect of contamination of eggs of diamond backmoth *Plutella xylostella* (L.) with nuclear polyhedrosis virus. Larval mortality was directly proportional to the virus concentration applied and the larval susceptibility was influenced by the age of the eggs when they were contaminated with nuclear polyhedrosis virus. Im *et al.* (1991) reported that nuclear

polyhedrosis virus of *S. exigua* was more virulent against eggs than larvae. Aphid lethal paralysis virus (ALPV) and *Rhopalosiphum padi* (L.) virus (RHPV) were transmitted transovarially. ALPV infections significantly reduced the longevity and fecundity of the aphid (Laubscher *et al.*, 1992). The nuclear polyhedrosis virus of *Anticarsia gemmatalis* (Hub.) was not transmitted from adult to progeny (Fuxa and Richter, 1993).

2.4.2.Transmission through parasites and predators.

The effect of nuclear polyhedrosis virus on the relationship between *T. ni* and the parasite *Hyposoter exiguae* Vier. was investigated by Beegle and Oatman (1974; 1975). Polyhedra or free virions were never found within any parasite tissue. Parasitic larvae within the host exposed to virus before parasitisation perished when their host died of virus infection. Parasite larvae in host exposed to virus after parasitisation completed their development before their host died of virus infection.

Capinera and Barbosa (1975) reported that the transmission of nuclear polyhedrosis virus to the gypsy moth larvae by the predator *Calosoma sycophanta* (L.). The ability of the carabid *C. sycophanta* to transmit virus to larvae of *L. dispar* via ingestion of faecal deposits containing infective polyhedral bodies was investigated. Field collected adults of *C. sycophanta* that were fed infected larvae of gypsy moth *L. dispar* were found to excrete polyhedra in sufficient quantity to infect third instar larvae of *L. dispar*.

Adults of *Trichogramma cacoeciae* Marchal when exposed to a fresh dry deposit of an aqueous suspension of the nuclear polyhedrosis virus of *M. brassicae* at concentrations 1, 5 or 10 times the LC_{100} for *M. brassicae*, there was no reduction in their parasitising ability. The development of parasite in eggs of *Sitotroga cerealella* (Oliv.) was not affected when these eggs were dipped in a suspension of the virus at different times after they had been parasitised. There were no adverse effect on the larval feeding capacity, adult female fecundity or egg viability of *Chrysopa carnea* Steph. when the larvae

were sprayed directly with the highest tested concentration of the virus suspension or when exposed to the fresh dry deposit or provided with a contaminated diet (Hassan and Groner, 1977).

Raimo *et al.* (1977) exposed larvae of *L. dispar* reared on an artificial diet to parasitism by females of *Apanteles melanoscelus* (Ratz.) that had been contaminated with the nuclear polyhedrosis virus associated with the moth in the field. Three methods of contaminations (ovipositor, total body surface and exposure to infected host) at two exposure periods (2 and 24 hours) were tried. There was no significant difference between parasite emergence from larvae parasitised by contaminated and uncontaminated females.

Studies conducted to find out the interaction between the nuclear polyhedrosis virus infection and parasitism by *Eucelatoria* Sp. nr *armigera* Cog. and *H. armigera* have shown that the virus infection in *H. armigera* did not affect the development of the parasitoid. Maggot development, adult emergence, sex

ratio, longevity, larviposition and behaviour of adult flies emerging from diseased larvae were normal (Narayanan, 1980).

Cooper (1981) reported the role of predatory pentatomid bug *Oechalia schellembergii* (Guer.) in disseminating a nuclear polyhedrosis virus of *Heliothis punctigera* Wallgr. The predacious pentatomid *O. schellembergii* that had fed on the virus killed larvae of *H. punctigera* polyhedra were detected in the excreta three to six days after a meal. Nymphs retained virus for longer period as food residues were retained in the midgut until after the final moult. Bioassay indicated that virus in the excreta of the adult was highly infective from four days after meal.

In California, field and laboratory tests were conducted by Vail (1981) to determine the relationship between the parasite *Voria ruralis* (Fall.) its host *T. ni* and the nuclear polyhedrosis virus. By dissection it was observed that upto 100 per cent of the parasitism of field collected *T. ni* was in hosts infected with the virus. Laboratory tests confirmed

that the parasites can survive, develop in and emerge from diseased larvae. Although histological sections revelaed nuclear polyhedrosis virus in the gut lumen of puparia of *V. ruralis* that had fed on diseased host, the virus was voided from adults soon after emergence and before oviposition. Tests revealed that *V. ruralis* did not act as a biological vector and it acted as a mechanical vector only under very restricted conditions.

Campoletis chlorideae transmitted the nuclear polyhedrosis virus to *H. armigera* larvae directly by oviposition causing 100 per cent mortality (Odak *et al.*, 1982). Hamm *et al.* (1985) tested the compatibility of non-occluded virus of *S. frugiperda* with the parasite *Cotesia marginiventris*. Adults of *C. marginiventris* transmitted the virus from infected to uninfected *S. frugiperda* larvae. The progeny of the parasite failed to complete their development in host larvae inoculated with the virus before or during parasitisation. Fewer parasites completed development in host larvae that were inoculated four days after parasitisation than in larvae inoculated five days

after parasitisation and in parasitised controls. Fewer and smaller parasite larvae were found in virus infected host than in parasitised control when host larvae were dissected six days after parasitisation.

In Canada, Levin *et al* (1983) conducted studies which showed that 84 per cent of the parasite *Apanteles glomeratus* (L.) that completed their development in larvae of *P. rapae* infected with granulosis virus, transmitted the virus to the larvae of *P. rapae* in which they subsequently oviposited as adults. Adults of *A. glomeratus* did not show discrimination between healthy and diseased larvae, although healthy larvae were attacked first with a great frequency than the infected ones. Females of *A. glomeratus* distinguished between parasitised and unparasitised healthy but did not discriminate between parasitised and unparasitised infected ones. *A. glomeratus* play a significant role in the transmission of the granulosis virus in the field population of *P. rapae*.

Caballero *et al.* (1991) observed that the parasites *Apanteles telengi* Tobias, *Aleiodes gasteratus* (Jurine) and *Campoletis annulata* Tschek parasitised significantly more healthy than granulosis virus infected larvae of *Agrotis segetum* (Schiff). Females of the three parasitoid species that have oviposited in granulosis infected *A. segetum* larvae transmitted the virus to healthy ones in subsequent oviposition. Male parasites did not transmit the granulosis virus. White and Webb (1994) reported the survival of a dipteran parasitoid in a gypsy moth population subjected to virus infection. A population *L. dispar* in Quercus, Maryland was examined for parasitoid and infection by *L. dispar* nuclear polyhedrosis virus. Total parasitism was 19.5 and 22.8 per cent for two cohorts of *L. dispar* and 70 per cent of parasitised larvae were also infected with the virus.

2.5 Reproductive potential and transmission of virus during mating

Zelazny (1976) reported the transmission of *B. oryctes* during the process of mating. In the field,

mated females were more frequently infected than unmated females. Adults developing from larvae that had survived exposure to various dosages of the virus were not infected. Virus infection did not occur in larvae hatching from eggs that had been surface contaminated with the virus. Larvae hatching from the eggs laid by virus infected females were very rarely infected. In the *O. rhinoceros* population, the virus is transmitted most frequently during mating, possibly when the healthy partner contacts by mouth the virus material that had been excreted by the infected partner. The virus can be transmitted in a similar way when infected and healthy beetles feed together in palm trees. Virus infected beetle can pass the infection to healthy larvae when visiting a breeding site.

The *B. oryctes* infected adults produced 0.3 mg virus/day in the faeces (Monsarrat and Veyrunes, 1976). Mohan (1991) studied the persistence of *B. oryctes* in organic matter. The infective half life of *B. oryctes* in cattle dung was approximately five days and total inactivation of the virus occurred on the eighth day.

The virus isolated from the English grain aphid *Sitobion avenae* and the rose grain aphid *Metopolophium dirhodum* could be transmitted to healthy aphid by contact with honey dew secreted by virus infected individuals (Allen and Ball, 1990). Sikorowski and Lawrence (1994) reported that both cytoplasmic polyhedrosis virus infected males and females transmit virus to FI generation.

2.6 Cross-infectivity of the virus

The *B. oryctes* virus was infective to the larvae of European *Oryctes nasicornis* (Huger, 1966 b). In Ivory coast, larvae of *Oryctes boas* Fa. showed sensitivity to the virus which was close to that of *O. rhinoceros* larvae, but *O. monoceros* larvae were less susceptible (Julia and Mariau, 1976). However, in Seychelles the same species was susceptible to the *B. oryctes* (Windsor, 1975). The virus has a host range which includes members of Scarabaeidae such as *Scapanes australis grossepunctatus* (Boisd.) (Bedford, 1973), *Strategus aloeus* (L.) (Lomer, 1986), *Papuana uninodis*, *Papuana hubneri* (Zelazny *et al.*, 1988),

Heteronychus arator and *Costelytra zealandica* (Crawford *et al.*, 1985). The virus was not infective on the elephant beetle *Xylotrupes gideonulysses* L. (Bedford, 1973). The virus multiplied in widely divergent cell lines from the mosquito *Aedes albopinctus* Skuse (Singh, 1967) and the fall army worm *S. frugiperda* (Vaughn *et al.*, 1977), but Crawford (1981) found that the virus replicated in *H. arator* only. Rajapakse *et al.* (1982) reported that the cashew stem and root borer *Plocoederus ferrugineus* L. infection could be effectively controlled with *B. oryctes* in Sri. Lanka. But Bakthavatsalam and Sundararaju (1990) reported that *B. oryctes* KI and *B. oryctes* PV 505 were not infective to cashew stem and root borer *P. ferrugineus*.

The nuclear polyhedrosis virus isolated from the alfalfa looper *Autographa californica* Speyer was infective to *T. ni*, *P. xylostella*, *S. exigua*, *H. zea*, *Estigmene acrea* Drury and *Bucculatrix thurberiella* Linn. (Vail *et al.*, 1972). Hunter *et al.* (1973) reported that a polyhedrosis virus isolated from the

almond moth *Ephestia cautella* (Wlk.) was found to be cross infective to the Indian meal moth *Plodia interpunctella* (Hb.). Witt and Janus (1976) reported that the baculovirus from the alfalfa looper *A. californica* was successfully transmitted to the wax moth *Galleria mellonella* L.

H. zea, T. ni and the sphingid *Manduca sexta* Joh. were found highly susceptible to the nuclear polyhedrosis virus of *G. mellonella* (Fraser and Stairs, 1982). Dhana (1984) reported that the baculovirus of *Sceliodes cordalis* was unable to cross-infect the larvae of *Ctenopseustis obliquana, Planotortrix excessana, Epiphyas postvitlana* and *C. pomonella*. Cross-infectivity studies have shown that the larvae of *H. armigera* were highly susceptible to nuclear polyhedrosis virus isolated from *Adisura atkinsoni* Moore (Narayanan, 1985 a). The nuclear polyhedrosis virus of *O. arenosella* was found cross transmissible to *S. litura S. mauritia, A. peponis, P. ricini* and *Diacrisia obliqua* (Wlk.) (Philip, 1985). *S. litura* and *S. mauritia* were found to be suitable alternate hosts for large scale multiplication of the virus.

Sherlock (1985) reported that cytoplasmic polyhedrosis virus from *Noctua pronuba* (L.) was infective per os to *Phlogophora meticulosa* but not to *A. segetum*. Kellen and Hoffmann (1987) conducted studies to evaluate comparative response of two alternative hosts, *Ephestia figulilella* and *E. elutella* to a suspension of two non-occluded viruses of *Amyelois transitella*. All tests indicated that alternative hosts were highly resistant to the virus. Guzo *et al.* (1991) reported that the cell lines of *Diabrotica undecimpunctata*, *Manduca sexta L.*, *L. dispar*, *H. virescens*, *S. frugiperda* and *Trichogramma confusum* were susceptible to *A. californica* nuclear polyhedrosis virus.

Im *et al.* (1991) reported that the third instar larvae of *S. exigua* were susceptible to the nuclear polyhedrosis virus from *A. californica*, *M. brassicae* and *T. ni*. The virus did not infect any of the other lepidoptera under experimentation. The nuclear polyhedrosis virus isolated from *Anagrapha falcifera* Skirby was tested against six species of

lepidopteran pests and four insect cell lines. The LC_{50} in PIBs/ mm^2 were 23.9, 42.2, 47.3 and 51.04 for *Cadra figulilella, Amyelois transitella, C. pomonella* and *P. interpunctella* respectively. *Cadra cautella* W. and *Ephestia elutella* were most susceptible (Vail *et al.*, 1993).

The baculovirus isolated from *Ocnogyna baetica* (Rambur) was not infectious to *A. segetum*, *Mythimna loreyi* (Dupon.), *S. littoralis* and *S. exigua* (Vargas - Osuna *et al.*, 1994).

2.7 Thermal inactivation of virus

Continuous exposure to high temperature was found to adversely affect the viral stability and viral multiplication (Bird, 1955; Thompson, 1959; Ignoffo, 1966).

Lathika and Jacob (1974) reported that suspension of nuclear polyhedrosis virus of *S. mauritia* which had been exposed for ten minutes at 70°C and smeared on young leaves of the grass *Ischaemum aristatum* remained infective to second instar larvae of *S. mauritia*. The

nuclear polyhedrosis virus of *P. ricini* was highly infective even after 96 hours of exposure to a constant temperature of 35°C (Nair and Jacob, 1976).

In U.S.A, Lewis and Rollinson (1978) conducted studies on the effect of temperature on the virulence of nuclear polyhedrosis virus of *L. dispar*. The virus was stored as powder and as water suspension under different conditions for different lengths of time. Suspension retained their potency for five years under refrigeration and for two years at room temperature. Air dried powder stored at 4°C retained its potency for one year and thereafter, the potency diminished gradually. Air dried powder and suspension held at 38°C for six months also lost their potency. Pawar and Ramakrishnan (1979) observed a gradual decline in the infectivity of *S. litura* nuclear polyhedrosis virus with prolonged exposure to 40 and 50°C.

The nuclear polyhedrosis virus of the velvet bean caterpillar *A. gemmatilis* replicated effectively at 26.7°C and caused 30 to 50 per cent mortality than at

low temperature (Boucias *et al.*, 1980). Stairs and Milligan (1980) reported that 50 per cent of inactivation of nuclear polyhedrosis virus of *G. mellonella* was obtained with exposure to 42°C for 329 minutes. The granulosis virus of cabbage moth could tolerate exposure to 60°C for one hour (Liang, 1983).

The *B. oryctes* could be stored in deep freezers at -20°C (Bedford, 1976; Young, 1974). Crawford and Sheehan (1984) reported negligible reduction in infectivity of virus stored in sterile tissue culture fluid at 4°C for one year. Infectivity of virus in tissue culture fluid at 28°C was found to be significant for field use in the tropics upto three months. The storage of *B. oryctes* as macerated cadavers mixed in saw dust at 26°C reduced viral activity to 0.091 per cent of its initial level in one week and to 0.027 per cent in two weeks. No activity was detectable after one month. Drying and warming increased the rate of inactivation (Zelazny, 1974). The thermal inactivation point of the virus is 54°C (Mohan *et al.*, 1985).

The nuclear polyhedrosis virus of *O. arenosella* when exposed to a constant temperature of 35°C, the infectivity declined rapidly beyond 84 hours and complete inactivation occurred at 156 hours of exposure (Philip, 1985). Knittel and Fairbrother (1987) reported the effect of temperature on the survival of nuclear polyhedrosis virus of *A. californica*. No virus replication or formation of polynuclear inclusion bodies occurred at 37°C. Geissler *et al.* (1991) observed that at room temperature, nuclear polyhedrosis virus of *M. brassicae* and granulosis virus of *A. segetum* could be stored for a short time only. Intermediate storage for about 50 days, however, was possible in the refrigerator (6°C) without any major decline in efficacy. Freezers (-18°C) are recommended for long term storage.

The temperature of exposure to 10, 22, 35 or 50°C had no effect on the sensitivity of the virus of *Heliothis, Baculovirus heliothis*. Normally encountered field temperatures should not adversely affect the stability of the virus (Ignoffo and Garcia, 1992).

2.8 Safety of the virus

The safety of baculovirus to man, domestic animals, beneficial insects, parasites and predators has been established in several studies. After reviewing several reports, Ignoffo (1973) concluded that most of the commercial microbial insecticides so far tested were virtually harmless to vertebrates and forms of life other than target pest, but safety cannot be guaranteed in all living systems for all times.

2.8.1 Safety to productive insects

Aratake and Kayamura (1974) reported that cytoplasmic polyhedrosis virus of *Lymantria fumida* Btler. was moderately infective to larvae of *Bombyx mori* (L.). The nuclear polyhedrosis virus of the armyworm *M. separata* did not cause any harmful effect to silkworm *B. mori* through topical, intra-haemocoelic or oral administration at a concentration of 10×10^5 to 10×10^8 PIBs/larvae (Dhaduti and Mathad, 1979). The virus was also found to be safe to colonies of *Apis cerana indica* F. (Dhaduti and Mathad, 1980).

Narayanan (1980) reported that the nuclear polyhedrosis virus of *H. armigera* was safe to the silkworm *B. mori*. The virus was also found safe to the eri silkworm *Philosamia ricini* B. by topical, intra-haemocoelic and oral administration at a concentration of 10×10^8 PIBs/larvae (Dhaduti and Mathad, 1981).

Laboratory studies were conducted to determine the susceptibility of the silkworm to two commercial preparations of the virus (Elcar and Virion/H). Both had no harmful effect on second or third instar larvae of *B. mori* (Padhi and Maramorosch, 1983).

Allen and Ball (1990) reported that the insect viruses isolated from English grain aphid *Sitobion (Macrosiphum) avenae* and the rose grain aphid *Metopolophium dirhodum* has not multiplied in crop plants on which both *S. avenae* and *M. dirhodum* fed or in pupae of the honeybee *Apis mellifera*.

Bijjur *et al.* (1991) administered nuclear polyhedrosis virus of *H. armigera* to silkworms *B. mori*

and Samia cynthia ricini B., and the Indian honey bee *Apis cerana indica* F. and in all the tests, the virus was found to be safe.

Protein synthesis was investigated in pupae of *B. mori* infected with polyhedrosis virus by Milkhailov and Zemskov (1992). Susceptibility of infection decreased markedly during the pupal period. Following injection of the virus, all young pupae acquired polyhedrosis and finally died, whereas most of the older pupae did not exhibit disease and completed metamorphosis normally. Later in the pupal period, when the bombycids were infected the production of polyhedrosis in diseased pupae was lower. The rate of development of infection in *B. mori* is directly dependant on the environment under which the insect is reared. Silkworms reared at high temperature survive the infection by the flacherie virus, densonucleosis virus, nuclear polyhedrosis virus and cytoplasmic polyhedrosis virus (Kobayshi and Chaeychomsri, 1993).

2.8.2 Safety to parasites and predators

The nuclear polyhedrosis virus of *H. armigera* was tested against twelve species of parasitoids, four species of predatory insects and one species of predatory mite and it was found to be safe to all of them (Narayanan, 1980). Hotchkin and Kaya (1983) reported that the development of the embryonic and larval stages of the internal gregarious parasite *Apanteles militaris* (Walsh.) were adversely affected by the presence of nuclear polyhedrosis virus in larvae of *Mythimna unipuncta* (Haw.). The initial effects were the cessation of parasite growth and general tissues disruption, followed by melanisation of the parasitic tissue. Srinivasan (1985) found out that the nuclear polyhedrosis virus of *Heliothis* was safe to the associated parasitoids *Apanteles* Sp.,*Bracon greeni*, *Bracon. hebetor* Say., *Chelonus blackburni* (Cameron), *Eriborus Sp.*, *Campoletis chlorideae* and *Eucelatoria bryani* Sabrosky.

Consumption of nuclear polyhedrosis virus infected prey (larvae of *P. includens*) by the predator *Nabis*

roseipennis variously affected development, survival and reproduction of the predator (Ruberson *et al.*, 1991). Nymphs that fed infected larvae developed significantly faster at 25°C than nymphs fed uninfected prey. Nymphal survival was not affected by prey infection. Pre-oviposition periods were significantly longer for predator females maintained on infected prey through out their lives than for those receiving uninfected prey during pre-imaginal or adult (or both) stages.

2.8.3 Safety to birds, fishes and other mammals

Narayanan *et al.* (1977 b) observed that the nuclear polyhedrosis virus of *Amsacta albistriga* (Wlk.) was harmless to the common carp, *Cyprinus carpio* L. When the same virus was exposed to white mice and poultry birds, the animals were found healthy and there was no change in their bodily appearance, behaviour and feeding activity. Histopathological examinations showed no evidence of tissue damage and they were similar to the corresponding tissues of the healthy animals (Narayanan *et al.*, 1977 a; 1978). The

baculovirus of the European skipper *T. lineola* had no effect on mammals, fishes and beneficial insects (Smirnoff and Ackermann, 1977).

In France, Food and Agricultural Organisation (1978) conducted safety test of *B. oryctes*. The virus was safe to human cells, pig cells, mouse, hamster, fish and calf. No pathogenicity was observed in living inoculated mice or in brain, liver, spleen, lung, heart, stomach, bladder, intestine, muscle, kidney, gonads or blood of mice up to 60 days after inoculation. Gourreau *et al.* (1979) also reported that the virus was safe to white mice. Further pathogenicity studies on adult pigs confirmed the safety of the virus towards vertebrates (Gourreau *et al.*,1982). Regupathy et al. (1978) reported that the nuclear polyhedrosis virus of *S. litura* had no harmful effects on poultry birds, when they were fed the virus at a dose of 15.14×10^9 PIBs/bird. Preliminary studies have shown that nuclear polyhedrosis virus of *H. armigera* did not produce any deleterious effect on albino rats, white leghorn broiler chicks and two species of fishes common carp

Cyprinus carpio L. and tilapia *Tilapia mozambica P.* by oral adminstration. There was no significant difference between the virus treated and control organism in weight gain, food consumption, weight of different organs and blood or urine chemistry (Narayanan, 1979).

Lautenschlager *et al.* (1980) observed the natural occurrence of the nuclear polyhedrosis virus of the gypsy moth *L. dispar* in wild birds and mammals. Infectious polyhedra were found in the gut content of birds, blue jays (*Cyanocitta cristata* Lin.), towhees (*Pipilo erythrophthalmus* Jerd.), red backed voles (*Clethrionomys gapperi* Lin.) and mammals like white footed mice (*Peromyscus leucopus* Lin.) reccon (*Procyon lotor* Lin.), and chip munta (*Tamias striatus* Hume.). These animals served as carriers of the virus. In the Federal Republic, Doller and Groner (1981) conducted safety test of nuclear polyhedrosis virus of *M. brassicae*. The virus did not replicate in vertebrates and there was no risk to vertebrates in the use of the virus for the biological control.

In China, under the aegis of the Insect Virus Laboratory, a survey was conducted in 1981 to assess the safety of the granulosis virus of the cabbage butterfly *P. rapae*. The virus was administered at 50 mg/kg body weight as a single oral dose to pigs, cows, lambs, chicken, rabbits, mice, birds, frogs, fishes, shrimp and silkworms. None showed ill effects and growth was normal during two to three months following the administration. The virus was inoculated into human lung cells, rabbit kidney cells and chicken embryonic cells in tissue cultures and neither cytopathogenic changes nor virus replications were observed under electron microscope. The baculovirus isolated from *A. californica* was safe to the non-target arthropod shrimp *Paaemoneles vulgaris* (Says.) by direct exposure (Couch *et al.*, 1984).

Groner and Doller (1984)observed that the polyhedra of *M. brassicae* (L.) was not infective to mice and chicken. The nuclear polyhedrosis virus of *O. arenosella* was found safe to white mice, white rat and embryonated chick eggs (Philip, 1985)

Easwaramoorthy and Jayaraj (1990) reported that male and female albino rat fed with two granulosis virus, one infecting *Chilo infuscatellus* (Snell.) and the other infecting *Chilo sacchariphagus indicus* at a dose equal to 100 x 2.5 $\times 10^{14}$ inclusion bodies /75 kg man did not show any mortality, abnormality in general behaviour, food consumption, body weight gain, gross neuropsies, clinical chemistry and haematological examination. Also when the rats were injected with the virus intra-peritoneally at 1.1 X 10^{12} inclusion bodies/rat, no harmful effects could be detected.

Philip and Nair (1990) found out that the white mice and embryonated chick eggs were safe to nuclear polyhedrosis virus of *S. mauritia*.

Materials and Methods

MATERIALS AND METHODS

The study was conducted at the College of Horticulture, Vellanikkara and College of Veterinary and Animal Sciences, Mannuthy during the period 1992 to 1995.

3.1 Mass rearing of *Oryctes rhinoceros* Linn.

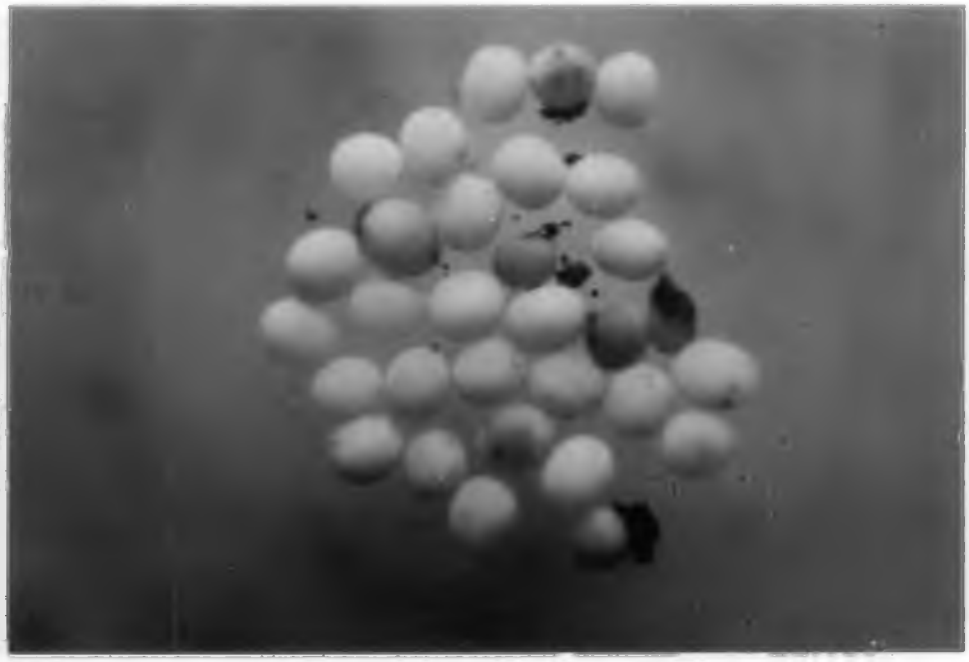
Field collected adults of the *O. rhinoceros* beetles could not be used directly for egg laying because of the likelihood of viral and fungal infections. Therefore, field collected grubs were reared through pupation to obtain disease free adults. These were then taken to the insectary to establish the stock culture and for mass rearing under laboratory conditions. The temperature in the insectary ranged from 24 to 30°C.

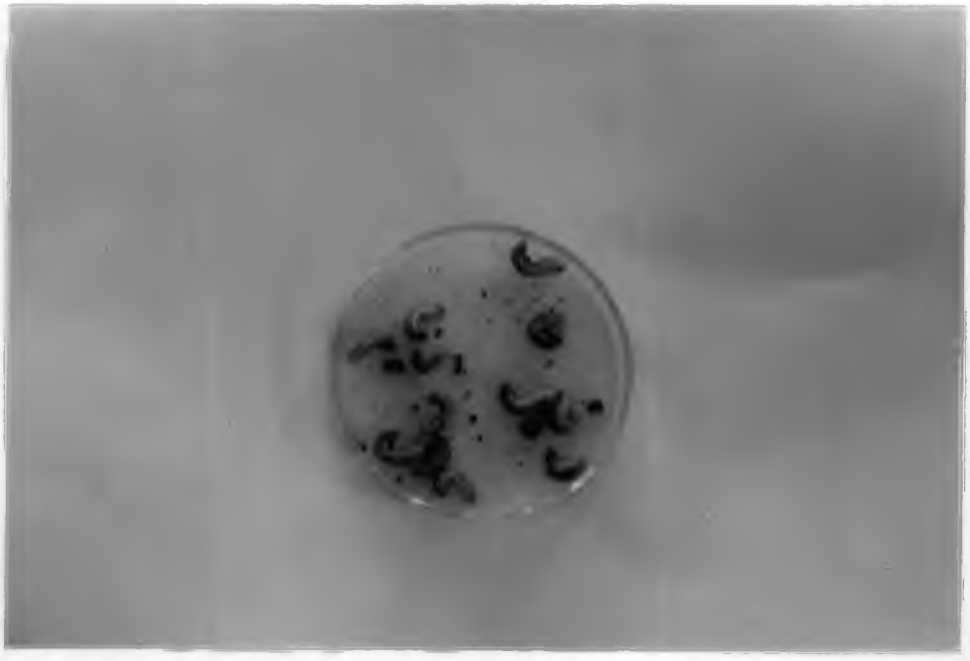
Flower pots each of 24 cm diameter were half filled with steam sterilised saw dust treated with captan (1 gm/2.5 litres) to prevent *Metarrhizium anisopliae* infections (Muller-Kogler, 1971). Six

female and three male beetles were released into each pot. Beetles were fed on ripe banana splits placed on top of the saw dust layer, at the rate of three to four pieces per pot. The pots were covered with masonite tops held in position with metal clips. The contents of each pot was removed every week and the medium examined carefully for locating and collecting eggs (Plate 1). Any container with diseased adults were discarded. The eggs thus collected from the oviposition chambers (pots) were placed in groups - 10 eggs/litre of saw dust - cowdung mixture in 1:1 proportion (v/v) (Alfiler and Zelazny, 1985). The young larvae on hatching (Plate 2) burrowed down into the food medium where they were left undisturbed for two months for further development through the first and most of the second instar. The grubs were thereafter transferred to metallic tins containing the same breeding medium and covered over with muslin cloth. The insects were left in these containers for the rest of their larval development. The containers were examined every day and after removal of faecal pellets fresh medium was added. The grubs showing

Plate 1. Eggs of *O. rhinoceros*

Plate 2. Just emerged first instar larvae of *O. rhinoceros*





symptoms of any disease were immediately removed from the medium and burnt. The grubs render the whole medium compact during the cocoon formation stage. Within the cocoon, the insect completed the prepupal and pupal stages and also the early adult stage during which the exoskeleton hardens. During this stage, the container was left undisturbed for a period of about five weeks. Thereafter, the containers were examined every day. The breaking up of the cocoon into loose material signalled adult emergence.

All precautions were taken for hygienic breeding and prevention of contamination by any pathogens. The rearing containers were disinfected using five per cent sodium hypochlorite solution.

3.2 Mass rearing of *Baculovirus oryctes*

The initial baculovirus culture required for the experiment was isolated from virus infected *Oryctes* grubs and adults collected from around the CPCRI Regional Centre, Krishnapuram, Kayamkulam. The infection was passed into first instar grubs by oral inoculation technique. Once the grub became

translucent on infection, they were collected and stored as such at - 20°C in deep freezers (Bedford, 1976).

3.2.1 Preparation of virus suspension

When the virus inoculum was required for experimental purposes, the frozen grubs were thawed quickly by putting into warm water (40°C). The gut was removed by dissection, weighed and triturated in chilled phosphate buffer (0.05M, pH 8.0 with 0.5M EDTA) containing the antibiotics-streptomycin 250 mg/litre and penicillin 200 mg/litre. The concentration of the inoculum was expressed as gut equivalent.

3.3 Inoculation techniques

This study was conducted in adults of the Rhinoceros beetle by using the following two methods.

3.3.1 Swim technique

In this method, adults were infected by allowing them to wade through virus suspension containing two per cent sucrose for 30 minutes (Mohan *et al.*, 1986).

3.3.2 Oral inoculation technique

Beetles were gently anaesthetised with ether and 0.2 ml of the midgut inoculum containing four per cent sucrose was administered orally using a polythene cannula attached to a tuberculin syringe.

The mortality rates of the adult in the two methods of inoculation were recorded and compared. For this experiment, 30 adults were used, there being four replications for each technique. Oral inoculation technique was followed for treating the grubs and adults in the subsequent experiments of this study.

3.4 Storage of virus

The Greave's centrifugal method of freeze drying was adopted for preserving the virus (Swain, 1972). The cultures were dried in special ampoules of hard glass. The ampoules were plugged with cotton wool and sterilised in autoclave. The culture (2.5 ml) was transferred into each ampoule using sterile capillary pipette and a fresh loose plug of sterile cotton wool inserted.

The chamber for primary drying was prepared by charging the desiccant trays with phosphorus pentoxide (3 g per ml of water to be absorbed). The ampoules were placed in the vertical slots of the centrifuge. The centrifuge was covered with belljar and switched on followed by the starting of the rotary pump. A low pressure of 0.1 mm of Hg was obtained in 2-5 minutes by which time the material got frozen. The centrifuge was then switched off and the rotary pump allowed to run for eight hours to complete primary drying. The open end of the ampoules were drawn out to form a thin capillary neck near the open end by flaming. On cooling, the open end was applied to one of the rubber adapters in the manifold for secondary drying.

The desiccant was removed from trays and replaced with fresh phosphorus pentoxide. The bell jar was pressed into position. The air release valve was closed and the pump run for 6-18 hours to ensure complete drying. The ampoules were tested for vacuum. The ampoules were sealed, while attached to the manifold with the pump running. The capillary neck was

heated with a small gas flame till the glass melted and then pulled gently allowing the flame to severe the thin filament so formed, to seal the ampoules (Plate 3). The air release valve was then opened and pump switched off. The ampoules were retested for vacuum with high frequency tester. The dried material was preserved in refrigerator at 4-5°C. The freeze drying of the virus was carried out at the Institute of Animal Health and Veterinary Biology, Palode, Thiruvananthapuram.

3.4.1 Viability study of the freeze dried virus

The viability of the freeze dried virus was tested for sixteen months at two months interval. The freeze dried materials were dissolved in phosphate buffer and prepared a suspension of 10^{-1} dilution. The inoculum (0.2 ml) was given orally, using a polythene cannula attached to a tuberculin syringe. The inoculated grubs were transferred to separate containers. Disease free first instar grubs obtained from laboratory cultures were used for various experiments. Thirty grubs were fed on viral suspension replicated

Plate 3. Freeze dried virus in ampoules

four times. An equal number of the test insect inoculated with buffer alone served as control. The grubs were examined daily for typical symptoms. The standard procedure of probit analysis was followed in the present study.

3.5 Morphology of the virus

The morphology of the virus was studied under electron microscope using the midgut smear. This was done at the Tata Memorial Cancer Hospital, Bombay.

3.6 Symptomatology

First, healthy grubs and adults were inoculated with the virus. An equal number of grubs fed on buffer alone served as control. Five grubs each from the treated and control groups were picked up randomly at intervals of 48 hours and symptoms charted.

3.7 Gross pathology

Bioassay of the grubs was done as described by Zelazny (1972). Bioassay was performed with the crude virus preparation. The study was carried out in all

the three instars of *O. rhinoceros*. Gut from acutely diseased grubs were weighed out and triturated in chilled phosphate buffer. Log dilutions of the triturate in buffer (10^{-1} to 10^{-6}) were then prepared and 0.2 ml of each dilution was orally administered using a polythene cannula attached to a tuberculin syringe after slightly anaesthetising with ether. They were then introduced into individual plastic containers. All grubs used in this experiment were laboratory reared and disease free ones. Grubs of uniform age and size were used for each assay. A healthy grub of each instar was introduced into each box. Each instar was exposed to all dilutions and untreated ones served as control. There were four replications of thirty grubs each. The grubs were examined daily. Death of grubs with typical disease symptoms were recorded. In doubtful cases, mortality due to virus was confirmed by microscopic examination of smear from diseased grubs. The data was used for probit analysis and LC_{50} and LT_{50} were computed.

Bioassay of adults were also carried out following the same method.

3.8 Patho-physiology

3.8.1 Consumption Index

The mean temperature during the experimental period was 29 $\pm$ 3°C. Individual grubs were grown inside cylindrical plastic containers of 12 cm height and 9 cm diameter. Dark containers were used to provide a natural breeding environment. Windows of 4 cm square were cut on the cap and polythene netting pasted over to ensure free aeration.

The grubs were treated with 0.2 ml of midgut inoculum as described earlier. An equal number of grubs treated with buffer alone served as control. The weight of cowdung offered and the weight of grubs were recorded every 48 hours. The left over food, faeces and aliquot were quantitatively separated and weighed at intervals of 48 hours and fresh cowdung was provided into each container. The mean weight and length of grubs were determined at 48 hours interval. Forty grams of the cowdung was kept at a time in every container and the natural loss of weight of the cowdung was determined after 48 hours. The experiment was

conducted separately for all instars. There were ten grubs per treatment with three replications.

These observations were used in computing several quantitative nutritional parameters as discussed by Waldbauer (1968). The characters for which correlations were computed were weight of food consumed, weight of faeces, consumption index (CI), growth rate (GR), approximate digestibility (AD), efficiency of conversion of digested food (ECD) and efficiency of conversion of ingested food (ECI).

(i) Consumption index

$$C\ I = \frac{F}{TA}$$

(ii) Growth rate

$$G\ R = \frac{G}{TA}$$

(iii) Approximate digestibility

$$A\ D = \frac{\text{Weight of food ingested} - \text{weight of faeces}}{\text{Weight of food ingested}} \times 100$$

(iv) Efficiency of conversion of digested food to body substance

$$E\ C\ D = \frac{\text{Weight gained by the larva}}{\text{Weight of food ingested - weight of faeces}} \times 100$$

(v) Efficiency of conversion of ingested food to body substance

$$E\ C\ I = \frac{\text{Weight gained by the larva}}{\text{Weight of food ingested}} \times 100$$

Where

F - Fresh weight of food eaten

T - Duration of feeding period (days)

A - Mean fresh weight of the animal during feeding period

G - Fresh weight gain of animal during feeding period.

3.9 Cytopathology

Infected grubs were collected, washed in running tap water, anaesthetised with ether and dissected along the dorsal plane. Care was taken to avoid any mechanical damage to the swollen gut. The midgut was

slit dorsally and the midgut fluid, if present, was aspirated. The midgut epithelium was gently scraped with the edge of a microscopic slide and suspended in minimum volume of phosphate buffer. A smear of the tissue suspension was made, air dried, fixed in methanol for 3-4 minutes and stained with Giemsa stain for one hour (Lillie, 1965), washed in distilled water and examined under microscope.

3.10 Histopathology

The grubs of *Oryctes* were inoculated with 0.2 ml of midgut inoculum as described earlier. The insect tissue and cells were subjected to histopathological examination at two days interval in all instars to study the changes due to the infection. The foregut, midgut and hindgut and the fat bodies were examined in detail as major changes could be detected in these areas. The tissues were fixed in hot alcoholic Bouin's fluid. After ten minutes, the specimens were transferred to fresh fixative at room temperature and kept for 24 hours (Drake and Mc Ewen, 1959). Then the tissues were dehydrated in graded series of ethyl

alcohol - 50 per cent alcohol for 30 minutes, 70 per cent alcohol for 30 minutes, 95 per cent alcohol for one hour and absolute alcohol for one hour. The tissues were cleared in xylol and embedded in paraffin wax. The embedded specimens were cut at 5 µm. The cut sections were fixed on slide and stained with Haematoxylin and Eosin (Lee, 1968). The sections from the tissues of the control were similarly processed and stained. The stained specimens were examined under microscope with the low and high power and the changes were studied comparing with control.

3.11 Transmission of virus

3.11.1 Transovum transmission

The experiment was conducted using newly deposited eggs. One batch of the above eggs were collected from virus treated adults. They were surface sterilised by soaking in ten per cent formalin for one hour. Another batch of eggs laid by same group of adults were left unsterilised. Four replications each of 50 eggs obtained from the sterilised and

unsterilised lots were kept separately for hatching. Symptoms of infection in the emerging grubs were observed. Similar lots of eggs laid by adults obtained from healthy grubs kept without surface sterilisation served as control. The grubs were reared in laboratory on fresh food material and grub mortality, pupation and adult emergence were recorded.

3.11.2 Transovarial transmission

Eggs obtained from infected adults were divided into two batches. The first batch of egg was thoroughly homogenised in a mortar and pestle. The second batch of egg was surface sterilised by immersion in ten per cent formalin for one hour and a homogenate was prepared as described above. The viral activity of the above homogenate was tested against third instar grubs of *O. rhinoceros*. Another set of adults treated with buffer alone served as control. The grubs in each treatment were reared separately. There were four replications of 50 grubs each for every treatment. The grub mortality, pupation and adult emergence were recorded.

3.11.3 Transtadial transmission

Early stages and late stages of different instars were inoculated with the virus at a concentration of 10^{-3} gut equivalent. Grubs used in this experiment were laboratory reared and disease free. Grubs of uniform age and size were used. There were four replications each of 30 grubs. The grub mortality, pupal mortality and adult emergence were recorded.

3.11.4 Transmission through predator *Platymerus laevicollis*

Adults of *Oryctes* were inoculated with virus suspension as mentioned earlier. Each predator confined in glass containers was allowed to feed on adult at 0, 3 and 5 days after inoculation. The predators host preference towards the infected grubs was observed and the predator was examined for 15 days for any evidence of infection. Twentyfive grubs were treated with four replications. The predators which fed infected adults were ground and fed to healthy laboratory reared grubs of *Oryctes* to study the predator mediated transmission

of the virus. Another set of predator confined in glass containers was allowed to feed on uninfected beetle which served as control.

3.12 Reproductive potential and transmission of virus during mating

Normal and active adults obtained from healthy grubs were selected and pre-conditioned by starving them for 24 hours. The adult beetles of either sex were kept separately until they were treated with virus at a concentration of 10×10^{-3} gut equivalent mixed with four per cent sucrose solution. The experiment was conducted in the following parental combinations:

Healthy male	x	Diseased female
Healthy female	x	Diseased male
Healthy male	x	Healthy female
Diseased male	x	Diseased female

Five pairs in each of the above combinations were confined in a glass jar for mating and egg laying. The eggs laid by the contaminated adults were divided into two batches. One batch in each case was

surface sterilised in 10 per cent formalin for one hour. The other batch of eggs in each parental combinations was kept without surface sterilisation. An equal number of eggs treated with buffer alone served as control. Percentage of hatchability, grub and pupal mortality and adult emergence were recorded. There were three replications of fifty eggs each. Histopathological examination of the ovary and testis were conducted.

3.13 Cross-infectivity of the virus

Cross-infectivity of *B. oryctes* was carried out on red palm weevil *Rhyncophorus ferrugineus*. (Curculionidae), the coconut root grub *Leucopholis coneophora* (Melolothidae), the coconut caterpillar *Opisina arenosella* (Cryptophasidae) and the predator *P. laevicollis* (Reduviidae).

The test insects were inoculated with the virus suspension at a concentration of 10×10^{-3} gut equivalent. The treated larvae were then transferred to fresh uncontaminated food and reared until adult

emergence or death. There were twenty five insects in each treatment and three replications for each host. An equal number of the test insect inoculated with buffer alone served as control.

3.14 Thermal inactivation of the virus

Twenty ml of aliquots of 10 x 10^{-3} log dilution of triturated diseased tissue in buffer was held in thin walled glass tubes and subjected to a constant temperature of 37°C for 24 hours in a constant water bath with stirrer (Noordam, 1973). One ml of the heat-treated viral suspension was collected at four hours intervals upto 24 hours. The virus was cooled to room temperature and was orally fed to the grub at the rate of 0.2 ml as mentioned earlier. Healthy and middle aged third instar grubs were used for the bioassay. Twenty grubs were fed on the viral suspension at different intervals replicated twice. A negative control group with unheated viral suspension was also included.

Viral infection was not found after four hours of heating. Hence the virus was tested at shorter

intervals of 30 minutes. The data were subjected to statistical analysis and LT_{50} values computed.

3.15 Dosage requirement in adult equivalent per unit volume of the breeding media

Infected freshly emerged adult beetles were separately introduced into dealwood boxes (size 30 x 30 x 30 cm) at 2, 4, 6, 8 and 10 per container having sterile and well rotten cowdung and confined for two weeks. After this period, the adults were removed from the breeding medium. Ten healthy third instar grubs were then introduced into the food medium contaminated by the infected adults. The disease incidence in the grubs was monitored and recorded at intervals of two, three, four, five and six weeks of inoculation.

3.16 Safety of the virus

3.16.1 Safety to silkworm *Bombyx mori*

These studies were conducted on third instar larvae of the mulberry silkworm *B. mori*. Twenty

larvae were inoculated with the virus by spot feeding technique developed by Jacob (1972) (Plate 4). Middle aged mulberry leaves were fixed on a thick cardboard using clips with the laminar area exposed on the card surface. Pieces of paper gum tape, 2.5 cm square, with a circular hole of 6 mm diameter punched in the middle were pasted over the exposed surface of leaves. With a syringe, 0.05 ml of 10×10^{-3} virus suspension was placed on each of the circular exposed leaf disc and the suspension was allowed to dry up at room temperature. One larva each was confined to every inoculated spot by using an inverted penicillin vial. Larvae similarly fed on 0.05 ml buffer alone served as control. The larvae which had completely ingested the leaf disc were transferred to individual labelled glass containers. Fresh uncontaminated mulberry leaves were provided daily. There were four replications for each treatment. Larval mortality, pupation and adult emergence were recorded.

Plate 4. Spot feeding technique for inoculating *B. Oryctes* to *Bombyx mori* larvae

3.16.2 Safety to chick embryo

3.16.2.1 Selection of egg

In the study, seven to nine days old embryonated chick eggs were used. Only naturally very clean, medium sized, white shelled eggs with a fertility rate of above 95 per cent was selected for the experiment. This ensured embryos of normal dimension. The eggs were incubated in the vertical position with the broad end facing upward and during the entire pre-inoculation incubation period, they were turned twice daily through 180° in order to assist symmetrical embryonic development and to prevent adhesion of the embryonic membrane.

3.16.2.2 Candling

Candling consisted of viewing the eggs against a concentrated light source (100 watt lamp enclosed in a box with an oval opening), so that the shadow of the embryo and its associated structures were clearly visible.

3.16.2.3 Sterilisation

A swab lightly squeezed in tincture of iodine was used for sterilising the egg shell.

3.16.2.4 Inoculation

The virus was introduced into the chorio-allantoic membrane, allantoic cavity, amniotic cavity and egg yolk following the standard embryonated egg inoculation technique developed by Hoskins (1967) (Fig. 1). Each treatment was replicated five times with 20 eggs in each replication. Twenty eggs receiving 0.1 ml sterile normal saline served as control.

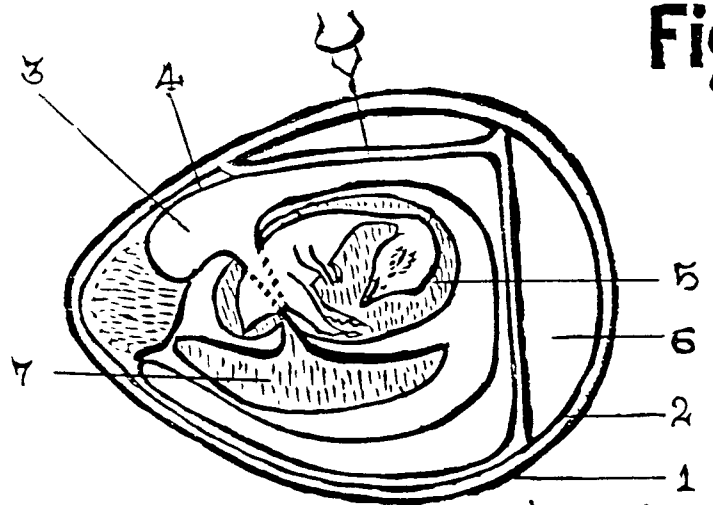
3.16.2.4.1 Chorio-allantoic membrane inoculation

The eggs are candled and marked the edge of air space and a point on the side of the egg which was not adjacent to major underlying blood vessels. The marked areas were sterilised and a simple hole was drilled in the shell overlying the centre of the air space puncturing the underlying shell membrane with a sterilised needle. Gentle suction was applied to the hole overlying the air space using a rubber teat.

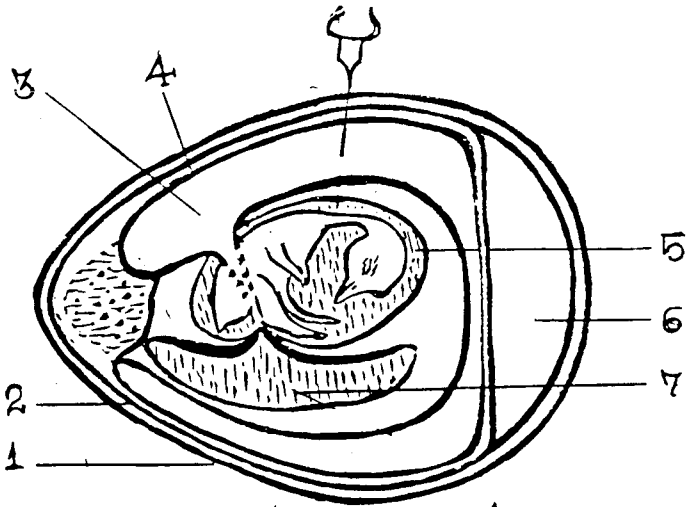
Routes of inoculation of *B. oryctes* to embryonated chick egg.

1. Shell
2. Shell membrane
3. Allantoic cavity
4. Chorio-allantoic membrane
5. Amniotic Cavity
6. Air space
7. Yolk sac.

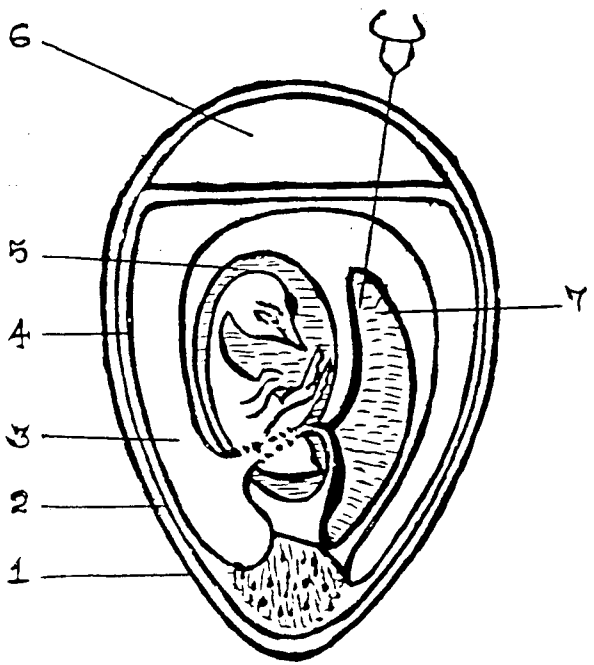
Fig. 1



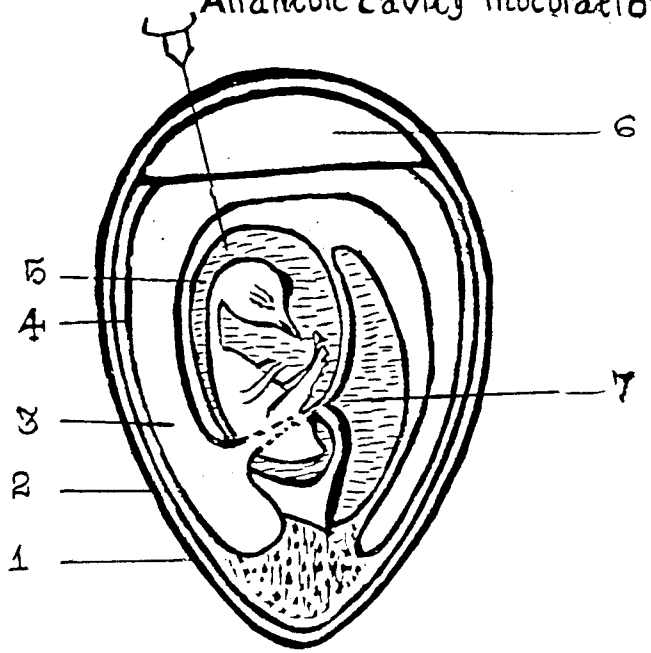
Chorio-allantoic membrane inoculation



Allantoic cavity inoculation



Yolk sac inoculation



Amniotic cavity inoculation

The virus suspension at a concentration of 10×10^{-3} gut equivalent containing antibiotic was inoculated directly on to the dropped chorio-allantoic membrane with a hypodermic syringe. Then the egg was gently rotated to distribute the inoculum evenly. All the holes were sealed and incubated.

3.16.2.4.2 Allantoic cavity inoculation

Candling, sterilisation and drilling were same as chorio-allantoic membrane inoculation. The viral suspension (0.1 ml) was inoculated directly into the allantoic cavity using 1.25 cm long 26 guage needle by inserting only one cm of the needle. This was done by holding the syringe at right angles to the long axis of the egg and entered via the lateral hole.

3.16.2.4.3 Amniotic cavity inoculation

Candling and sterilisation were the same as chorio-allantoic membrane inoculation. The shell round the entire air space periphery was cut keeping 1-2 mm above the edge of the latter, but at the same time not damaging the underlying shell membrane. The shell cap

was carefully detached and discarded. The entire shell membrane exposed at the base of the air space was painted with a cotton swab soaked in sterile liquid paraffin. This made the membrane transparent and enabled the embryo and its associated structures situated underneath very clearly visible. The shell membrane and the adjacent chorio-allantoic membrane were carefully teared with spatulate forceps and the amniotic sac enveloping the embryo was grasped with extreme care because of its fragility. The virus suspension was directly inoculated into the amniotic cavity using tuberculin syringe at the rate of 0.1 ml. Proper entry into the amniotic sac could be ascertained by observing the movements of the latter as the needle is moved to and fro. The egg was sealed with a coverslip and waxed as described above.

3.16.2.4.4 Yolk sac inoculation

Candling and marking the edge of the air space done as described earlier. The shell in the air space region was sterilised. A simple hole was drilled with a sterile needle into the shell overlying the centre of

the air space and punctured the underlying shell membrane. The viral suspension (0.1 ml) was directly inoculated into the yolk sac by using 3.5 cm long 26 guage needle and the hole was then sealed and inoculated.

3.16.2.5 Harvesting the material

The general condition of the embryo (dead/alive) was observed daily till the end of the test period.

3.16.2.5.1 Allantoic fluid

Eggs were chilled at +4°C for few hours. The shell at the air sac was broken with forceps and removed. The allantoic fluid was collected using a Pasteur pipette and examined for any change in treated and control.

3.16.2.5.2 Amniotic fluid

The shell was broken away over the air sac down to the level of the chorio-allantois. The chorio-allantois was stripped off and the allantoic fluid was spilled out in a petridish. The amnion was grasped

with forceps and the amniotic fluid was aspirated with a Pasteur pipette and examined.

3.16.2.5.3 Chorio-allantoic fluid

Diluted tincture iodine was applied to the shell over the artificial air space. The shell was removed and the shell membrane was stripped off with a pair of fine scissors. The chorio-allantoic membrane was cut and collected ensuring that the whole of the inoculated area was removed.

3.16.2.5.4. Yolk sac

The shell over the air space was broken and the shell membrane and the chorio-allantois removed. The yolk sac was picked up with forceps after dissecting out the embryo. It was then washed in sterile saline (0.85 per cent NaCl) and examined.

3.16.3.1 Safety to white mice

Four to six weeks old randomly mated non-inbred lines of white mice were used in the study. A virus suspension of 10×10^{-3} gut equivalent was orally

administered at the rate of 0.2 ml/animal. Ten animals receiving 0.2 ml of sterile distilled water alone served as control. The animals were maintained in separate cages in groups of five each. Each treatment and control were replicated five times. Standard food and adequate quantity of water were supplied *ad libitum*. The general appearance and behaviour of the white mice were recorded daily and the body weight was recorded at weekly intervals for 21 days.

At the end of the test period of 21 days, the test animals were sacrificed and detailed postmortem examination was conducted to see any lesion. Wet weight of the important organs namely liver, spleen, kidney, testis and ovary were recorded. Gross pathology and histopathological observations on the above organs were also made.

3.16.3.2 Safety to white rat

Twentyfive numbers of four to seven week old randomly mated non-inbred lines of white rats were chosen. A virus suspension containing 10×10^{-3} gut

equivalent was orally administered at the rate of 0.4 ml per animal. Ten white rats receiving 0.4 ml of sterile buffer served as control.

The animals were maintained in separate cages in groups of five each and each treatment was replicated five times. Standard food and water were supplied *ad libitum*. The animals were examined daily for general appearance and behaviour for 21 days. Body weight was recorded at weekly intervals. At the end of the test period of 21 days, the animals were sacrificed and detailed postmortem examination was conducted. Wet weight of the important organs were recorded. Gross pathology and histopathological observation on the above organs were also made.

Results

RESULTS

4.1 Techniques of inoculation of *Oryctes rhinoceros*

The results of the experiment are given in Table 1 and Fig.2. It was observed that mortality due to infection varied depending on the method of inoculation. In the oral inoculation technique, the cumulative mortality was 98.66 per cent and in the swim inoculation technique, the corresponding figure was 74.66 per cent. Mortality was noted from 20th day onwards and completed on 28th day in oral inoculation technique while in the swim technique mortality started on the 23rd day and was completed on 30th day. In the oral inoculation technique, 98.66 per cent of adults died before 28 days, while in the swim technique, the death rate was only 68.66 per cent. In control group, two per cent mortality was observed and this was not due to viral infection.

4.2.Storage of virus

Freeze drying method was used for storing *Baculovirus oryctes*.

Table 1. Relative efficacy of two methods of inoculating adults with *B. oryctes*

Methods of inoculation	Percentage of mortality	Day on which mortality started	Day on which mortality completed	Time taken for complete mortality (days)
Oral	98.66	20	28	9
Swim	74.66	23	30	8
Untreated control	*2.00			

* not due to virus infection

Effect of two methods of inoculating adults of *O. rhinoceros* with *B. oryctes*

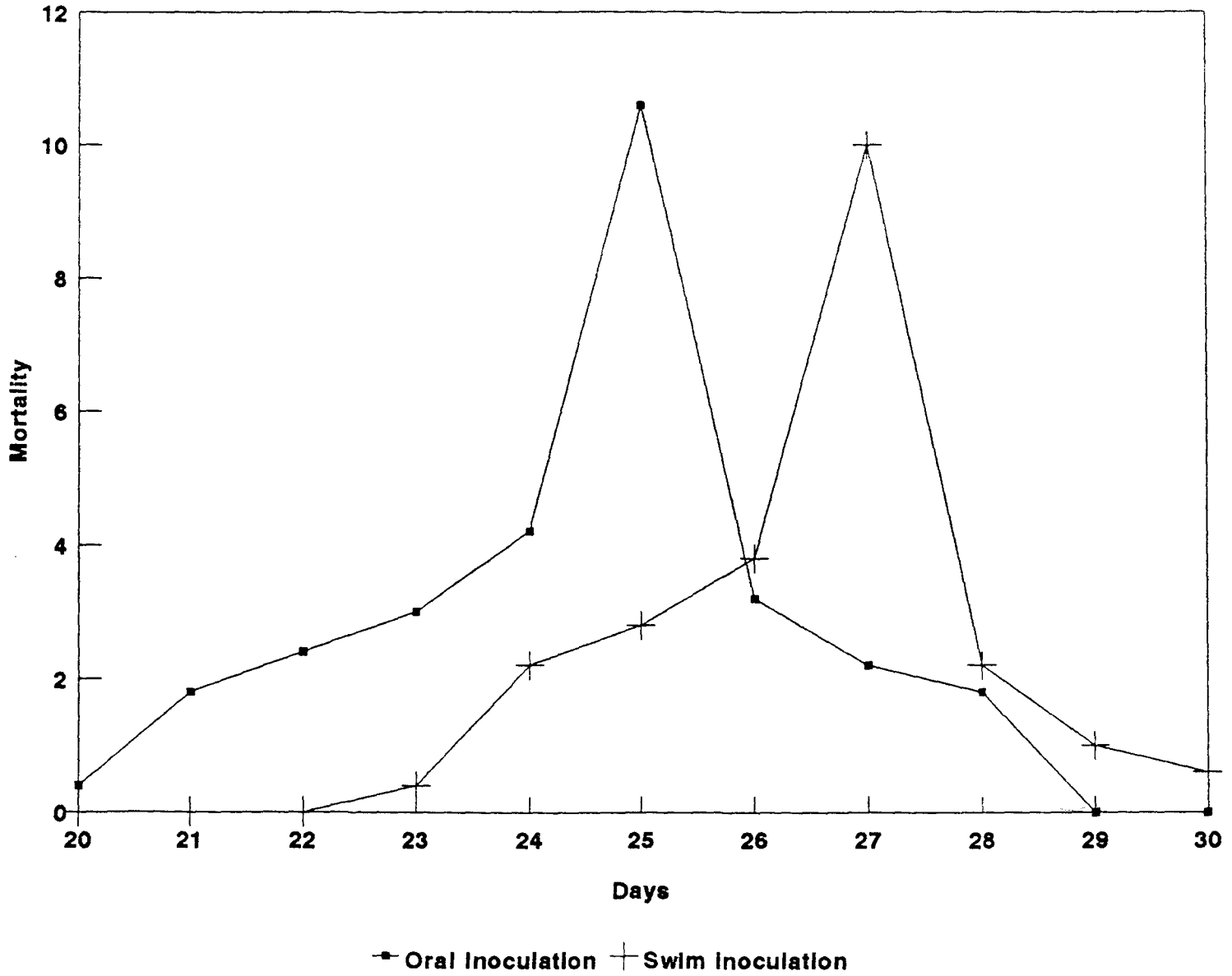

12
10
8
6
4
2
0
Mortality
20
21
22
23
24
25
26
27
28
29
30
Days
Oral Inoculation
Swim Inoculation

Fig.2

4.2.1 Viability study of the freeze dried virus

The observations from the experiment and the results of its statistical analysis are given in Table 2. It was observed that the grub mortality in first instar grub ranged from 100 to 34.16 per cent over a storage period of sixteen months. At the second and fourth months, complete mortality was obtained. The time taken for cent per cent mortality was six days in both the cases. The fiducial limits were not high. The LT_{50} values were 8.652 and 8.795 months. On storing for 6 and 8 months, it was observed that the virus did not loose much of its viability, the mortality rate being 85 and 72.5 per cent respectively. The LT_{50} values were 8.940 and 9.315 months. The mortalities recorded at the 10th and 12th months were 61.66 and 52.50 per cent respectively, the LT_{50} values being 11.700 and 12.723 months. At the 14th and 16th months, the mortalities obtained were only 42.50 and 34.16 per cent. LT_{50} value in these cases could not be computed since the mortality for the concentration was less than 50 per cent.

Table 2. Viability of freeze dried *B. oryctes* under prolonged storage for upto 16 months

	Period of storage of the virus (months)								
	2	4	6	8	10	12	14	16	Control
Percentage of grub mortality	100	100	85	72.5	61.66	52.50	42.5	34.16	
Time taken for death	7-12	7-12	7-12	7-12	7-13	7-14	8-14	8-16	
LT_{50} (months)	8.652	8.795	8.940	9.315	11.700	12.723	--	--	
Fiducial limits	8.651	8.512	8.845	8.892	11.330	12.272	--	--	No mortality
	8.930	8.802	9.107	9.800	12.084	13.203	--	--	
Heterogeneity	16.334	8.390	3.080	1.336	0.265	16.552	--	--	
Regression equation	(a)	(b)	(c)	(d)	(e)	(f)			

a - y=2.262 X + 4.141

b - y=2.364 X + 4.999

c - y=2.617X + 4.088

d - y=3.468 X + 2.944

e - y=3.354 X + 2.178

f - y=1.849 X + 3.807

4.3 Morphology of the Virus

The electron micrography of the midgut epithelial cells showed the viral particle to be bacilliform in shape consisting of an inner rod measuring 210 to 250 nm long and 75 to 105 nm broad surrounded by a membranous envelope (Plate 5 and 6).

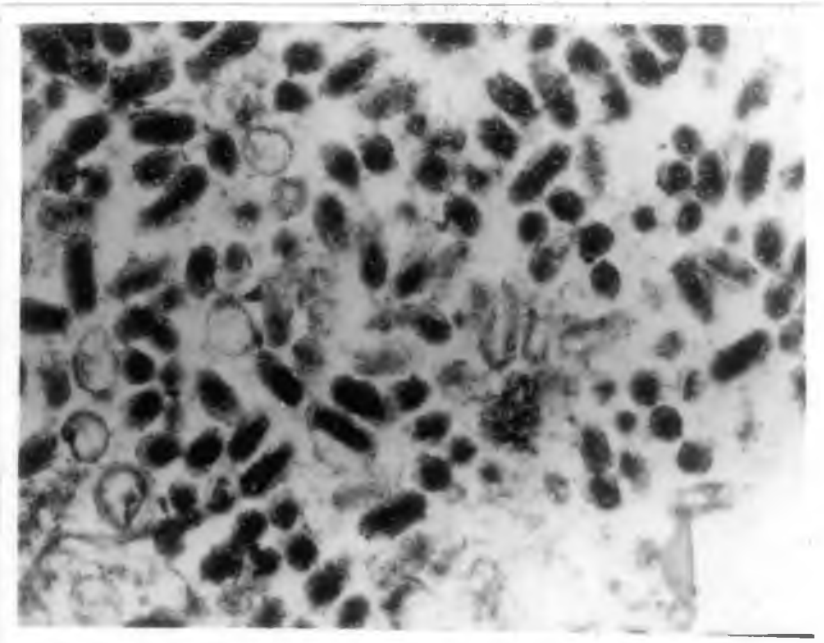
4.4 Symptomatology

4.4.1 Grubs

The early symptoms of viral infection in the grubs of *Oryctes* were manifested from the third to fourth day. The infected grubs initially refused to take food and they became paler than the healthy ones. Soon, the loss of appetite increased and they became lethargic. As the infection progressed, the larvae migrated to the top of the cowdung medium and gradually ceased to feed. The fat bodies disintegrated and the grubs appeared translucent especially in the abdominal region and became soft and supple. The infected grubs developed diarrhoea and as a consequence, extroversion of the rectum took place (plate 7). In the acute stage of

Plate 5. Electron micrograph of *B. oryctes* isolated from *O. rhinoceros* x 15,000

Plate 6. Electron micrograph of *B. oryctes* isolated from *O. rhinoceros* x 50,000



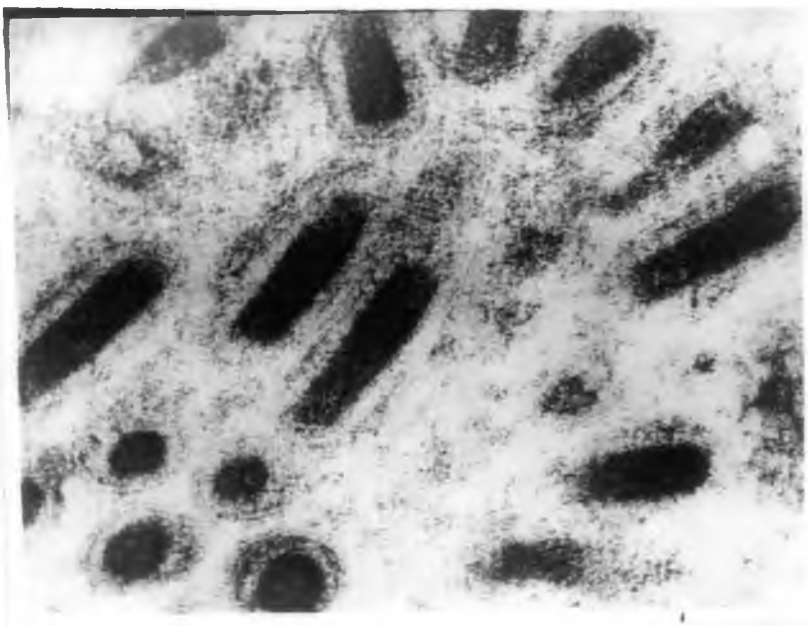
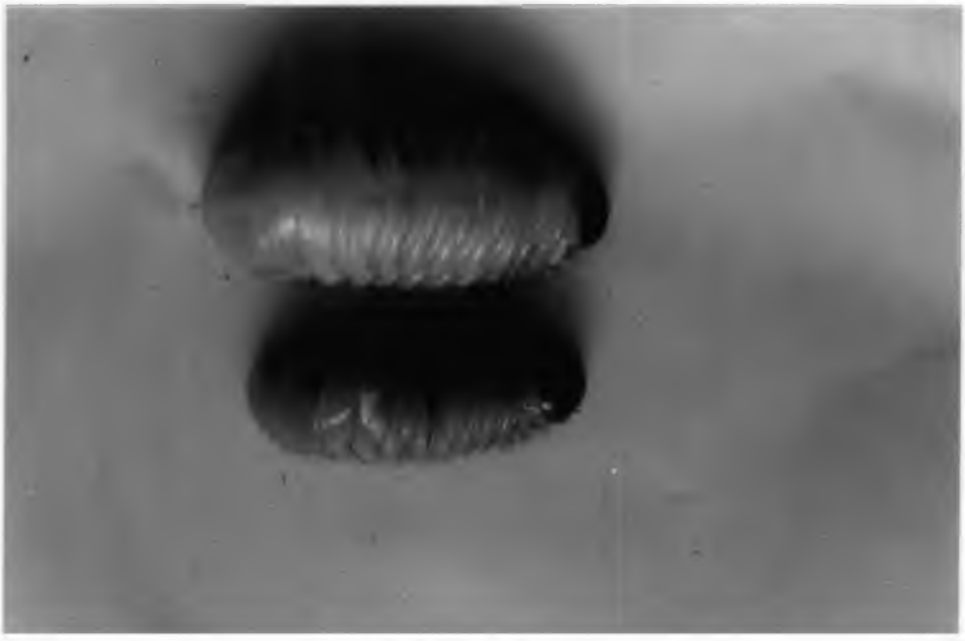


Plate 7. Infected grub of *O. rhinoceros* with extroverted rectum

Plate 8. Infected grubs of *O. rhinoceros* with fluid filled midgut



infection, the midgut became devoid of food and was filled with pale mucoid fluid (Plate 8). All the infected grubs moved over to the surface of the medium in the terminal stage of infection. Death started on seventh day in the first instar, twelth day in the second instar and ninth day in the third instar.

4.4.2 Adults

The infected adults did not show much external symptoms as in the case of grubs. The infected beetles refused to take food, developed diarrhoea and excreted mainly virus. The physical activity became much less, compared to control. The midgut of the beetle was white, swollen and filled with milky fluid. The infected females stopped egg laying. Malformed elytra and wings (Plate 9) were noted in two of the infected beetles. Death occurred in twenty to thirty days.

4.5 Gross pathology

The results of the experiment are furnished in Table 3 to 6 and illustrated in Fig. 3 to 8. The mortality due to viral infection in the first instar grub ranged from 39.17 to 100 per cent for the dose

Plate 9. Adults of *O. rhinoceros* A: Infected one with malformed elytra and wings B: Healthy one.


A
B

Table 3. Dosage mortality relationship of *S. oryctes* infecting first instar grub of *O. rhinoceros*

Gut equivalent dilutions	Per cent mortality	Intervals (days)	Mean time taken for death (days)	LT_{50} (days)	Fiducial limits		Regression equation	Hetero-geneity
10^{-1}	100	7-11	8.54	7.862	7.751	7.983	y=3.413 x + 5.875	10.36
10^{-2}	100	7-12	9.43	8.687	8.551	8.830	y=2.364 x + 6.141	83.34
10^{-3}	99.17	7-12	9.71	8.940	8.845	9.107	y=2.617 x + 5.088	38.06
10^{-4}	68.13	8-13	10.03	10.631	10.379	10.898	y=2.223 x + 4.171	18.96
10^{-5}	52.50	9-14	11.02	12.723	12.272	13.207	y=1.849 x + 3.807	16.552
10^{-6}	39.17	9-15	11.33	--	--	--	--	--

Effect of *B. oryctes* at 10^{-1} gut equivalent dilution of different larval instars and adults of *O.rhinoceros*

Effect of *B. oryctes* at 10^{-2} gut equivalent dilution of different larval instars and adults of *O.rhinoceros*

Table 4. Dosage mortality relationship of *B. oryctes* infecting second instar grub of *O. rhinoceros*

Gut equivalent dilutions	Per cent mortality	Intervals (days)	Mean time taken for death (days)	LT_{50} (days)	Fiducial limits		Regression equation	Hetero-geneity
10^{-1}	80.83	12-17	14.350	14.214	14.011	14.431	y=3.089 x + 3.770	12.71
10^{-2}	68.33	13-18	15.590	16.092	15.348	15.489	y=1.405 x + 5.085	14.06
10^{-3}	42.50	14-20	15.510	--	--	--	--	--
10^{-4}	35.00	14-20	16.670	--	--	--	--	--
10^{-5}	35.00	15-21	16.670	--	--	--	--	--
10^{-6}	29.16	15-23	17.657	--	--	--	--	--

Table 5. Dosage mortality relationship of *B. oryctes* infecting third instar grub of *O. rhinoceros*

Gut equivalent dilutions	Per cent mortality	Intervals (days)	Mean time taken for death (days)	LT_{50} (days)	Fiducial limits		Regression equation	Hetero geneit
10^{-1}	100	9-14	11.508	10.752	10.612	10.902	y=2.364 x + 5.994	83.902
10^{-2}	90.83	9-14	11.514	11.052	10.890	11.222	y=2.539 x + 5.079	39.579
10^{-3}	73.33	10-15	12.571	12.407	12.682	13.140	y=1.345 x + 5.291	10.626
10^{-4}	58.33	10-15	12.636	13.010	12.586	13.473	y=3.144 x + 2.652	12.877
10^{-5}	46.67	10-16	12.654	--	--	--	--	--
10^{-6}	30.83	10-17	13.405	--	--	--	--	--

Table 6. Dosage mortality relationship of *B. oryctes* infecting adult of *O. rhinoceros*

Gut equivalent dilutions	Per cent mortality	Intervals (days)	Mean time taken for death (days)	LT_{50} (days)	Fiducial limits		Regression equation	Hetero-geneity
10^{-1}	98.33	20-27	23.797	22.960	22.777	23.154	y=1.940 x + 5.122	10.49
10^{-2}	90.83	21-28	24.532	23.499	23.244	23.768	y=2.750 x + 3.445	15.77
10^{-3}	67.50	21-30	25.247	26.515	26.139	27.911	y=1.740 x + 3.722	15.97
10^{-4}	56.67	21-30	25.162	27.755	27.181	28.371	y=2.025 x + 3.157	10.34
10^{-5}	51.67	22-30	25.387	28.505	27.857	29.200	y=1.688 x + 3.338	12.39
10^{-6}	35.00	22-30	25.310	--	--	--	--	--

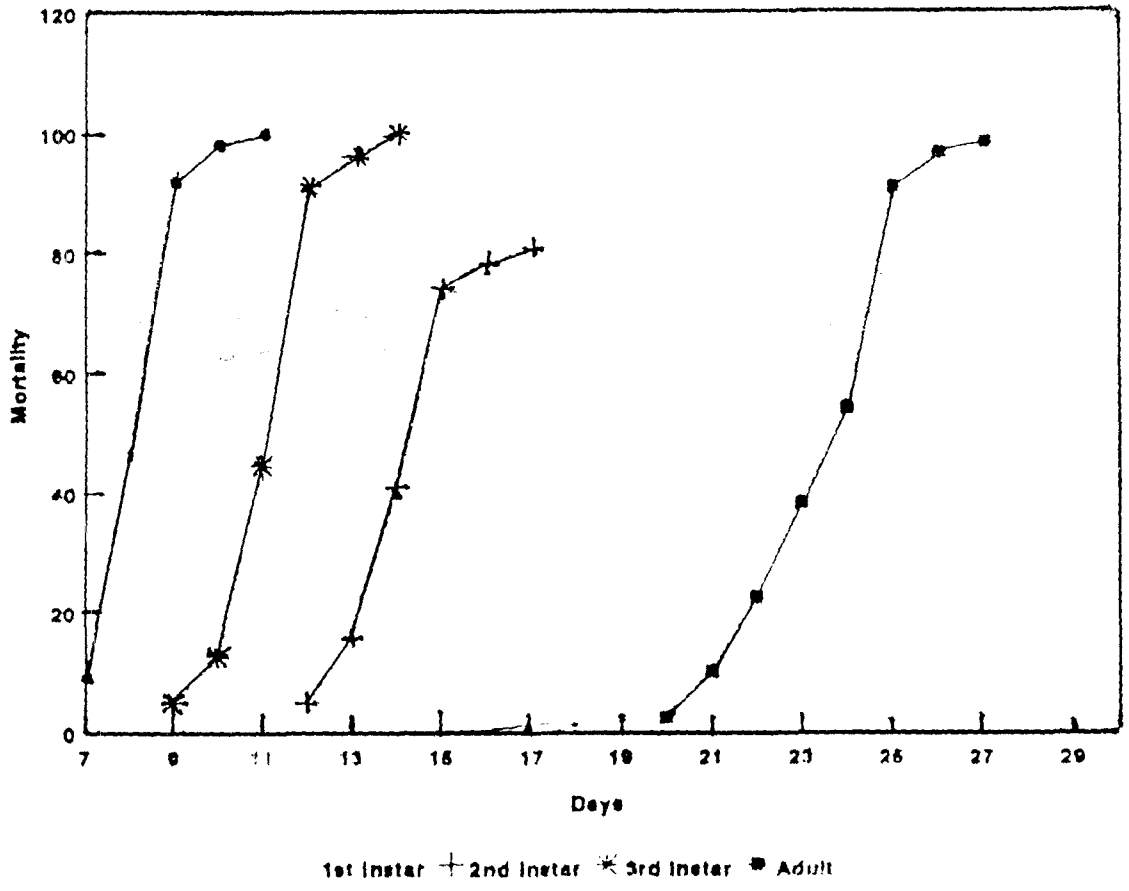

120
100
80
60
40
20
0
Mortality
7
9
11
13
15
17
19
21
23
25
27
29
Days
1st Instar
2nd Instar
3rd Instar
Adult

Fig-3

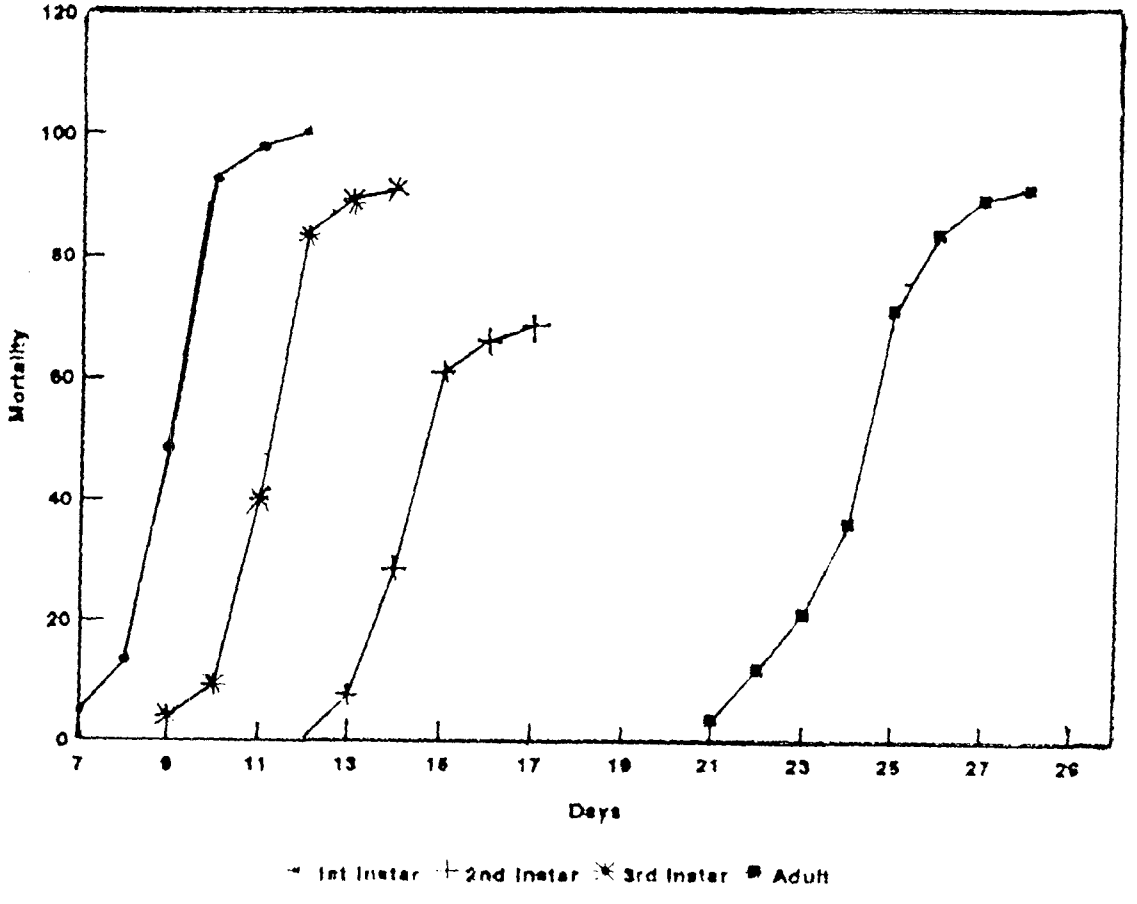

120
100
80
60
40
20
0
Mortality
7
9
11
13
15
17
19
21
23
25
27
29
Days
1st Instar
2nd Instar
3rd Instar
Adult

Fig.4

Effect of *B. oryctes* at 10^{-3} gut equivalent dilution of different larval instars and adults of *O.rhinoceros*

Effect of *B. oryctes* at 10^{-4} gut equivalent dilution of different larval instars and adults of *O.rhinoceros*

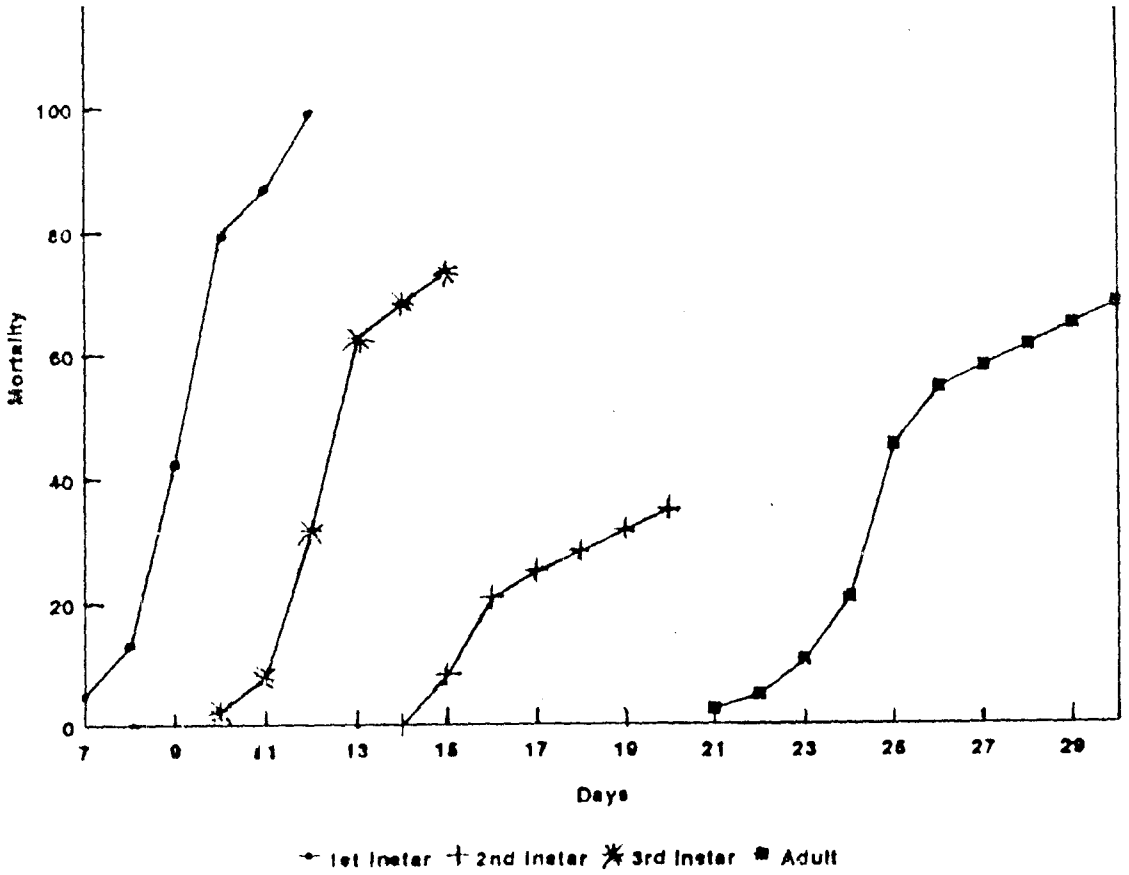

Mortality
100
80
60
40
20
0
7
9
11
13
15
17
19
21
23
25
27
29
Days
1st Instar
2nd Instar
3rd Instar
Adult

Fig.5

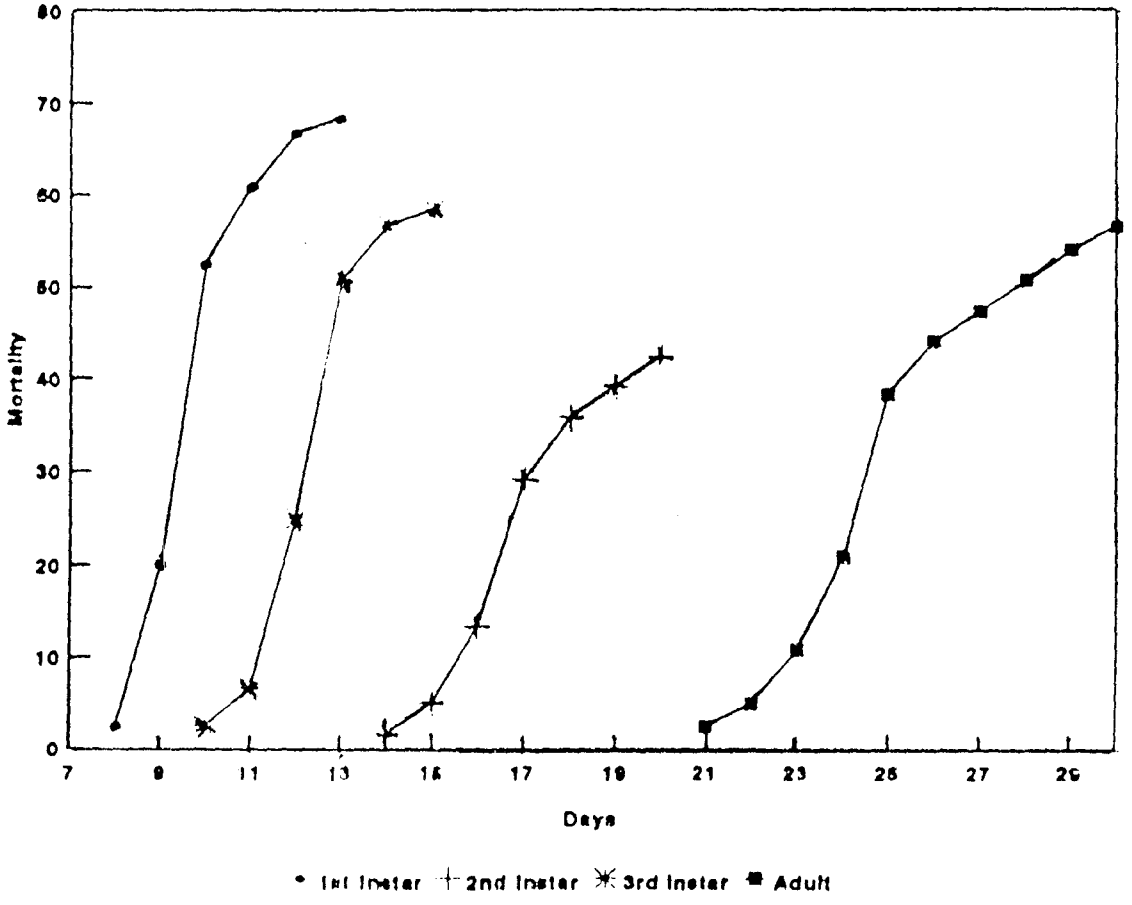

Mortality
80
70
60
50
40
30
20
10
0
7
9
11
13
15
17
19
21
23
25
27
29
Days
1st Instar
2nd Instar
3rd Instar
Adult

Fig.6

Effect of *B. oryctes* at 10^{-5} gut equivalent dilution of different larval instars and adults of *O.rhinoceros*

Effect of *B. oryctes* at 10^{-6} gut equivalent dilution of different larval instars and adults of *O.rhinoceros*

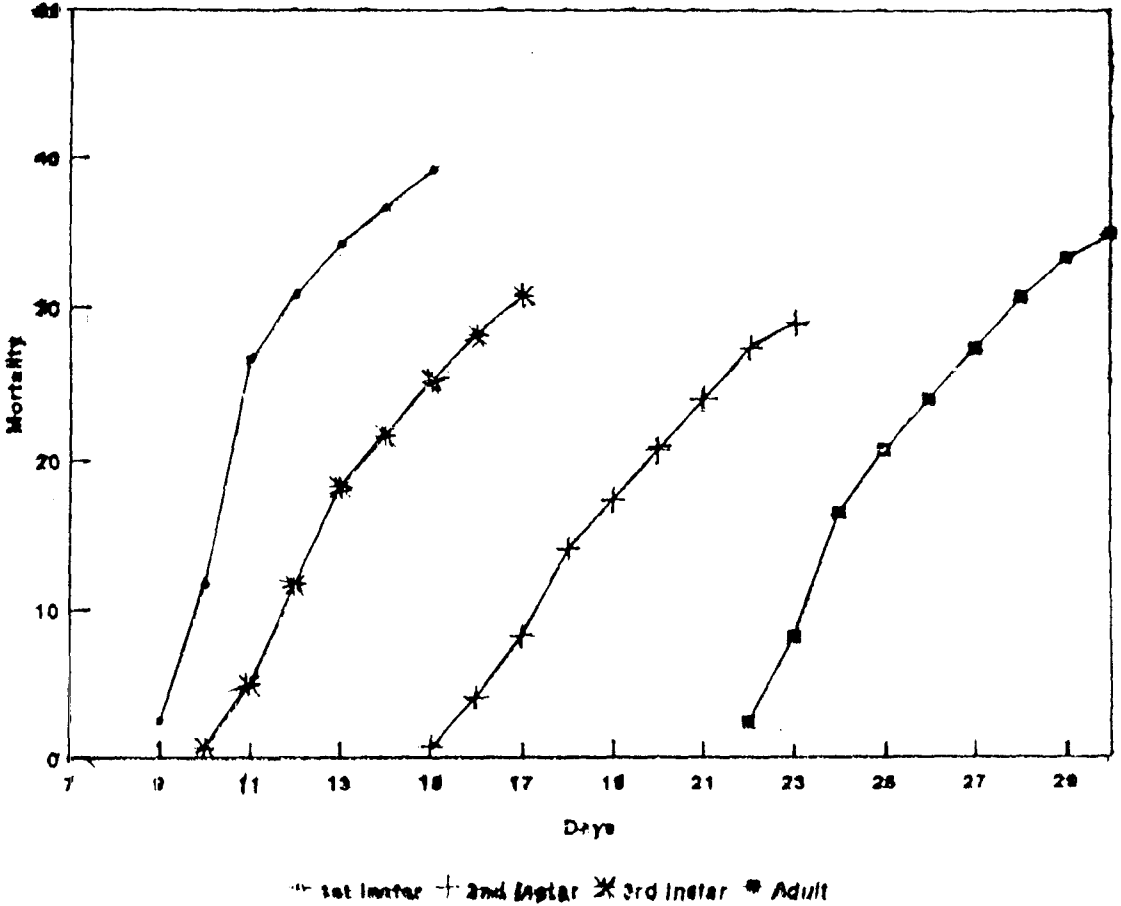

Mortality
0
10
20
40
7
9
11
13
15
17
19
21
23
25
27
29
Days
1st Instar
2nd Instar
3rd Instar
Adult

Fig.8

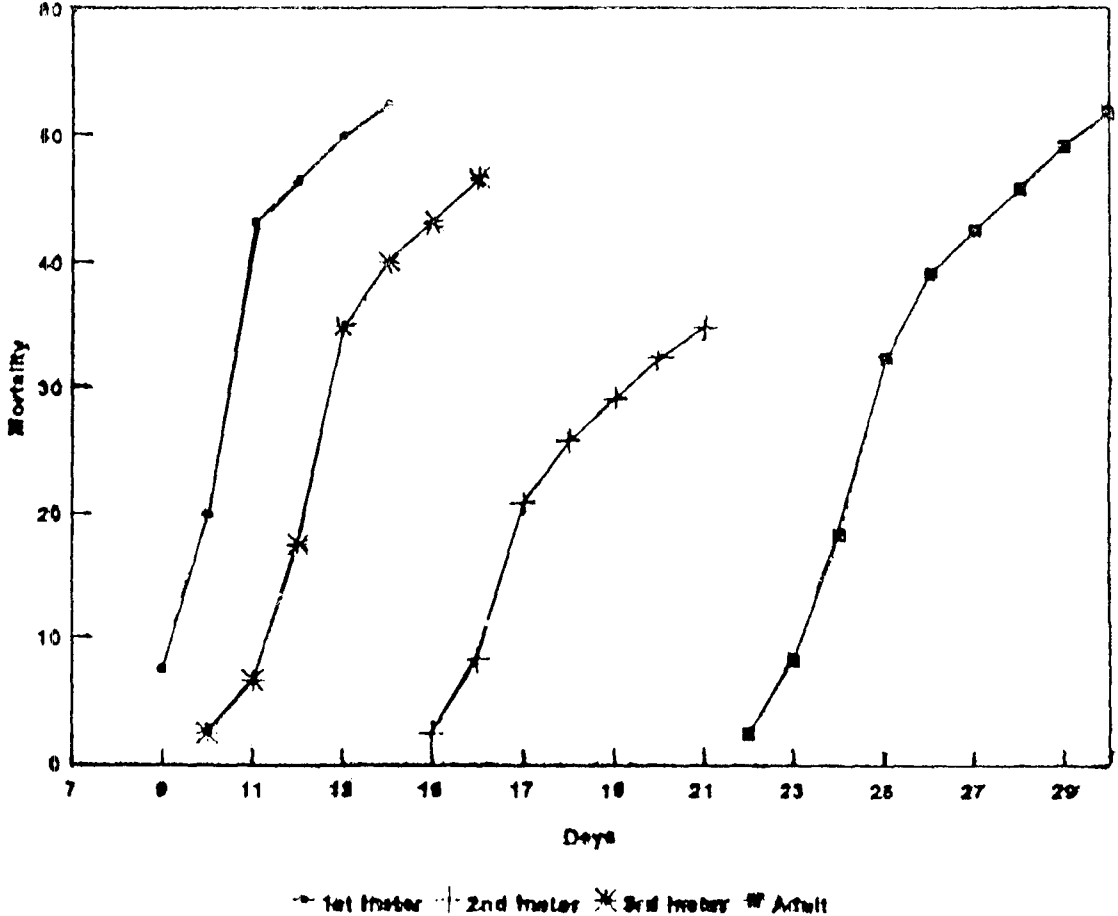

Mortality
0
10
20
30
40
50
60
7
9
11
13
15
17
19
21
23
25
27
29
Days
1st Instar
2nd Instar
3rd Instars
Adult

Fig.7

ranging from 10^{-6} to 10^{-1} viral dilutions of gut equivalent. The time taken for death ranged from 7 to 15 days. The mean time taken for death ranged from 8.54 to 11.33 days. For the different concentrations, the LT_{50} values ranged from 7.862 days to 12.723 days for the serial dilutions from 10^{-1} to 10^{-5}. The fiducial limits were also not seen to be high. In the first two higher doses of 10^{-1} and 10^{-2}, cent per cent mortality was obtained, but the number of days taken for 10^{-1} dilution was five and for 10^{-2} dilution, it was six days. The LT_{50} values for the two dilutions were 7.862 and 8.687 days respectively. In the 10^{-3} dilution also the extent of mortality was very high (99.17 per cent), with the LT_{50} value of 8.940 days. There was a drastic reduction in the mortality from 10^{-4} dilutions and down the scale. In 10^{-4} and 10^{-5}, the mortalities recorded were 68.13 and 52.50 per cent with the LT_{50} value of 10.631 and 12.723 days respectively. In the lowest dilutions, 39.17 per cent mortality was obtained. The LT_{50} of this dilution could not be calculated since the mortality recorded for the concentration was less than 50 per cent.

The mortality of second instar grub ranged from 29.16 to 80.83 per cent for the doses ranging from 10^{-6} to 10^{-1}. The time taken for death ranged from 12 to 23 days, with the mean ranging from 14.350 to 17.657 days. Complete mortality was not obtained in any of the dose. For the highest two doses, the percentage of mortality was above fifty per cent with the LT_{50} values of 14.214 and 16.092 days. The LT_{50} values for the concentrations from 10^{-3} to 10^{-6} could not be calculated as the mortality recorded was less than fifty per cent.

In the third instar grub, 30.83 to cent per cent mortalities were observed for the doses ranging from 10^{-6} to 10^{-1} viral dilutions and the mean time varied from 11.508 to 13.405 days. The LT_{50} values varied from 10.752 to 13.010 days for the dilutions ranging from 10^{-1} to 10^{-4}. In the highest dose, complete mortality was obtained with the LT_{50} value of 10.752. The LT_{50} values for the dilutions of 10^{-5} and 10^{-6} could not be calculated as the mortality recorded at the doses were below fifty per cent.

Mortality ranging from 35 to 98.33 per cent were observed in adults for the doses ranging from 10^{-6} to 10^{-1} dilutions. The time taken for the death ranged from 20 to 30 days and the mean time ranged from 23.797 to 25.310 days. In the highest two concentrations 98.33 and 90.83 per cent mortalities were obtained, with LT_{50} values of 22.960 and 23.499 days respectively. The LT_{50} value for the lowest concentration of 10^{-6} dilution could not be calculated as the maximum cumulative mortality recorded was below fifty per cent.

The LC_{50} value of baculovirus to first, second, third instar grubs and adults were 0.377 $\times 10^{-4}$, 14.417 $\times 10^{-4}$, 0.297 $\times 10^{-4}$ and 1.137 $\times 10^{-4}$ respectively. Taking the LC_{50} value to the first instar as standard, the concentrations required to cause fifty per cent mortality of second and third instar grubs and adults were calculated to be 38.241, 0.788 and 3.016 times higher respectively (Table 7).

Table 7. Relative susceptibility of different larval instars and adults of *O. rhinoceros* to infection by *B. oryctes*

	Heterogeneity	Regression equation	LC_{50} (Gut equivalent)	Fiducial limits		Order of relative efficacy
First instar	34.495	y=3.269 x + 4.210	0.377×10^{-4}	$10^{-4.586}$	$10^{-4.249}$	--
Second instar	45.050	y=3.299 x + 2.760	14.417×10^{-4}	$10^{-3.195}$	$10^{-2.454}$	38.241
Third instar	28.946	y=3.808 x + 3.013	0.297×10^{-4}	$10^{-4.272}$	$10^{-4.724}$	0.788
Adult	40.042	y=3.390 x + 2.261	1.137×10^{-4}	$10^{-4.272}$	$10^{-3.625}$	3.016

4.6 Patho-physiology

4.6.1 Consumption index

The differential rates of length, weight, growth, intake of food, efficiency of conversion of ingested food, digestibility and efficiency of conversion of digested food on exposure to baculovirus on first, second and third instar grubs from second day till death are presented in Tables 8-10.

In the first instar grub, there was significant difference in linear growth increment between the control and the infected grub. The mean increase in length of the control was 1.265 cm, but in the case of treated grub, it was only 0.628 cm during the test period of eight days. The weight gained by the treated grub was significantly lower (0.400 $\pm$ 0.008 to 0.452 $\pm$ 0.008 gm) from second to the eighth day over control grub (0.440 $\pm$ 0.001 to 0.879 $\pm$ 0.002 gm) from second to eighth day.

The rate of consumption of food (CI) in the case of control grub was significantly higher than the

Table 8. Comparison of rate of intake, digestibility and efficiency of conversion of food to body substance by *O. rhinoceros* first instar grub infected with *B. oryctes*

	Weight(gm)	(Length (cm)	RGR	CI	GR	ECI	AD	ECD
Control								
Initial (2nd day)	0.440± 0.001	0.895± 0.002	0.159± 0.003	0.785± 0.002	0.070± 0.001	8.923± 0.004	39.902± 0.074	35.197± 0.127
Final (8th day)	0.879± 0.002	2.160± 0.025	0.084± 0.009	0.842± 0.005	0.074± 0.002	8.925± 0.024	38.133± 0.153	38.229± 0.113
Treated								
Initial (2nd day)	0.400± 0.008	0.872± 0.019	0.125± 0.0003	0.663± 0.007	0.050± 0.001	7.598± 0.067	30.006± 0.120	33.283± 0.255
Final (8th day)	0.452± 0.008	1.500± 0.032	0.002± 0.0003	0.025± 0.002	0.002± 0.001	0.547± 0.007	0.600± 0.087	0.600± 0.006

RGR - Relative growth rate
CI - Consumption index
GR - Growth rate
ECI - Efficiency of conversion of ingested food
AD - Approximate digestibility
ECD - Efficiency of conversion of digested food

Table 9. Comparison of rate of intake, digestibility and efficiency of conversion of food to body substance by *O. rhinoceros* second instar grub infected with *B. oryctes*

	Weight(gm)	(Length (cm)	RGR	CI	GR	ECI	AD	ECD
Control								
Initial (2nd day)	2.282± 0.004	2.330± 0.020	0.040± 0.001	0.815± 0.002	0.091± 0.002	11.160± 0.031	44.936± 0.025	40.366± 0.139
Final (8th day)	3.627± 0.006	6.260± 0.017	0.274± 0.001	0.951± 0.008	0.099± 0.003	10.430± 0.003	43.622± 0.155	45.499± 0.150
Treated								
Initial (2nd day)	2.221± 0.003	2.320± 0.020	0.027± 0.001	0.807± 0.001	0.060± 0.001	7.520± 0.020	34.724± 0.013	34.736± 0.039
Final (8th day)	2.557± 0.001	3.619± 0.020	0.002± 0.001	0.042± 0.002	0.005± 0.001	1.214± 0.024	3.210± 0.054	3.029± 0.259

RGR - Relative growth rate
CI - Consumption index
GR - Growth rate
ECI - Efficiency of conversion of ingested food
AD - Approximate digestibility
ECD - Efficiency of conversion of digested food

Table 10. Comparison of rate of intake, digestibility and efficiency of conversion of food to body substance by *O. rhinoceros* third instar grub infected with *B. oryctes*

		Weight(gm)	(Length (cm)	RGR	CI	GR	ECI	AD	ECD
Control									
	Initial (2nd day)	4.360± 0.004	5.840± 0.024	0.018± 0.001	0.803± 0.001	0.080± 0.001	9.972± 0.002	42.085± 0.014	38.031± 0.009
	Final (8th day)	5.213± 0.007	9.181± 0.009	0.017± 0.002	0.902± 0.001	0.070± 0.002	9.834± 0.028	42.011± 0.031	42.132± 0.113
Treated									
	Initial (2nd day)	4.312± 0.017	5.832± 0.019	0.016± 0.001	0.702± 0.001	0.089± 0.001	9.995± 0.002	40.035± 0.009	35.028± 0.009
	Final (8th day)	4.572± 0.017	6.754± 0.020	0.002± 0.0001	0.102± 0.001	0.009± 0.004	0.906± 0.002	8.069± 0.078	2.275± 0.020

RGR - Relative growth rate
CI - Consumption index
GR - Growth rate
ECI - Efficiency of conversion of ingested food
AD - Approximate digestibility
ECD - Efficiency of conversion of digested food

treated grub, the index being 0.785 ± 0.002 on the second day and 0.842 ± 0.005 on the eighth day and 0.663 ± 0.007 on the second day and 0.025 ± 0.002 on the eighth day in the control and treated respectively. The relative growth rate (RGR) was 0.084 ± 0.009 and 0.002 ± 0.0003 in healthy and diseased respectively on eighth day.

Significant difference was observed in the efficiency of conversion of ingested (ECI) and digested (ECD) food to body matter between treated and the control grub. On the eighth day, the ECI was 0.547 ± 0.007 per cent in diseased and 8.925 ± 0.024 per cent in healthy and the ECD was 0.600 ± 0.006 per cent in diseased and 38.229 ± 0.113 per cent in healthy.

The rate of approximate digestibility (AD) was significantly higher in control. In control, the approximate digestibility was 38.133 ± 0.153 per cent on eighth day and in treated, it was 0.600 ± 0.087 per cent. From table 8, it is evident that the faecal production was relatively higher in the infected grub as compared to the amount of food ingested.

In the second instar grub, there was significant difference between the control and treated in respect of length and weight gain. The mean increase in length of the control was 3.93 cm but in the case of treated, it was only 1.299 cm. The mean increase in weight of the control was 1.345 g, but in the case of treated, it was only 0.336 g.

The consumption index was lower in the virus treated larvae, 0.807 $\pm$ 0.001 on second day and 0.042 $\pm$ 0.002 on 16^{th} day and in healthy it was 0.815 $\pm$ 0.002 on second day and 0.951 $\pm$ 0.008 on sixteenth day. Significant difference was noted in the case of RGR. In the control, it was 0.040 $\pm$ 0.001 on second day and 0.274 $\pm$ 0.001 on sixteenth day. But in diseased, it was 0.027 $\pm$ 0.001 on second day and 0.002 $\pm$ 0.001 on sixteenth day.

The efficacy of conversion of ingested and digested food and approximate digestibility also showed significant difference between treated and control. The ECI was reduced in both treated and control but drastic difference was noted in the treated ones. Thus

in control, it was 11.160 ± 0.031 per cent on the second day and 10.430 ± 0.003 per cent on sixteenth day as compared to the treated ones in which it was 7.520 ± 0.020 per cent on second to 1.214 ± 0.024 per cent on sixteenth day. The approximate digestibility on second day in diseased and healthy were 44.936 ± 0.025 and 34.724 ± 0.013 per cent and 43.622 ± 0.155 and 3.210 ± 0.054 per cent on sixteenth day respectively. The ECD was increased from 40.366 ± 0.139 on second day to 45.499 ± 0.150 per cent on sixteenth day in the case of control and decreased from 34.736 ± 0.039 to 3. 029 ± 0.259 per cent in the case of treated grubs.

The growth rate, relative growth rate and weight of control in the third instar grub was significantly higher than the treated grub. The growth rate in the control and treated on the second day was 0.080 ± 0.001 and 0.089 ± 0.001 and 0.070 ± 0.002 and 0.009 ± 0.004 on twelth day. The relative growth rate was 0.017 ± 0.002 in control on twelth day and 0.002 ± 0.0001 in the treated. Weight gained by the treated grub was significantly lower (4.312 ± 0.017 to 4.572 ± 0.017)

from second day to twelth day over control group (4.360 ± 0.004 to 5.213 ± 0.007).

The consumption index was also higher in control grub than treated. In healthy, it was increased from 0.803 ± 0.001 on second day to 0.902 ± 0.001 on twelth day and in the treated lot, it decreased from 0.702 ± 0.001 on second day to 0.102 ± 0.001 on twelth day. Similar to consumption index, the approximate digestibility and efficiency of conversion of ingested and digested food was significantly higher in control. On second and twelth day, approximate digestibility of control was almost same (42.085 ± 0.014 and 42.011 ± 0.031 per cent). But in treated there was greater reduction in the digestibility (40.035 ± 0.009 to 8.069 ± 0.078 per cent from second to twelth day). The ECI was high in diseased as compared to control on second day but significant difference was noted on twelth day (9.834 ±0.028 in healthy and 0.906 ± 0.002 per cent in diseased). On twelth day, ECD was also significantly higher in control (42.132 ± 0.113 per cent) than in diseased (2.275±0.020 per cent).

4.7 Cyto-pathology

Giemsa stained smears of infected midgut contents showed clumps of cells with highly disintegrated cytoplasm and purple stained hypertrophied nuclei. The infected nuclei contained a deep pink circular band along the periphery of the nucleus-ring staged nucleus and a central core of granular net work (Plate 10 and 11). Smears from the gut in the terminal stage of infection showed masses of hypertrophied nuclei with very little cytoplasm. The healthy nucleus was considerably smaller in size and purple stained.

4.8. Histopathology

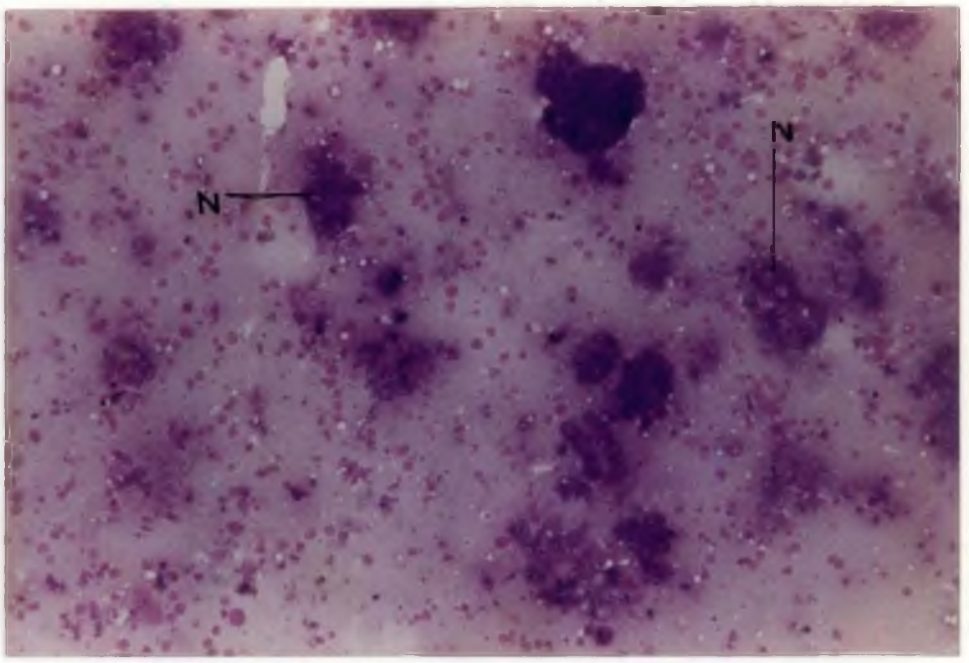
The histopathological studies were made on first instar grubs killed on the second, fourth, sixth and eighth days after inoculation with the virus. The foregut, midgut, hindgut and fat bodies were studied in detail as major changes could be noted in these tissues. The course of infection as studied in these tissues with the aid of light microscope is presented below.

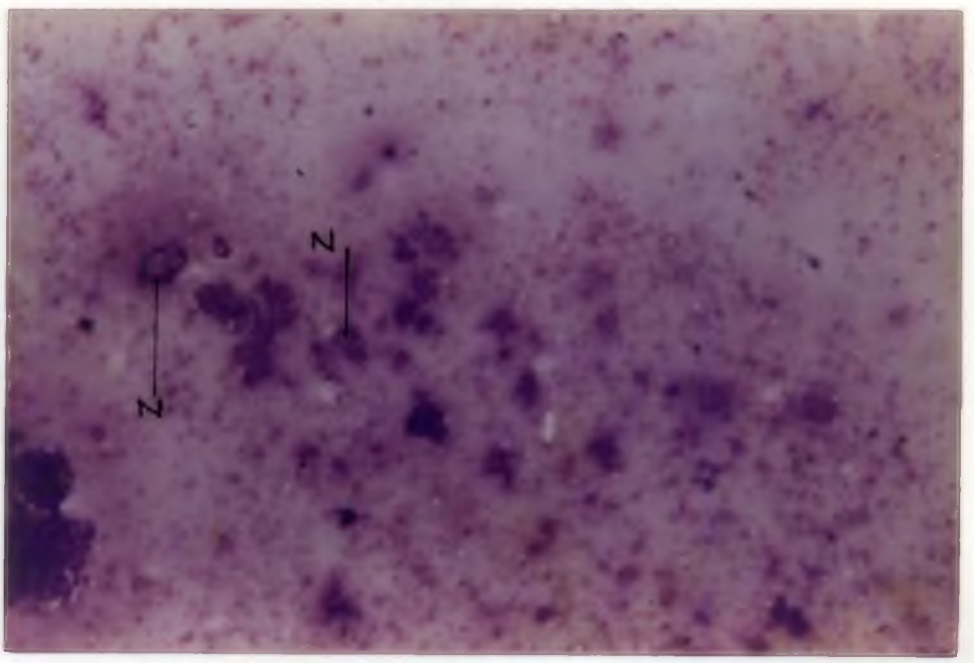
Plate 10. Infected midgut 10 x 16

N - Ring stage nucleus

Plate 11. Midgut smear 10 x 40

N - Ring stage nucleus


N
N


N
N

4.8.1 Changes observed in foregut

4.8.1.1 Control

The foregut showed normal epithelial lining of flattened cells. Secretory cells were normal. Inflammatory cells were not found. Muscle layer was thinner. The gut lumen contained food materials (Plate 12).

4.8.1.2 Second day of inoculation

The epithelial layer became irregular. A few inflammatory cells were seen in the stroma. Muscle layer was normal and gut lumen contained food materials (Plate 13).

4.8.1.3 Fourth day of inoculation

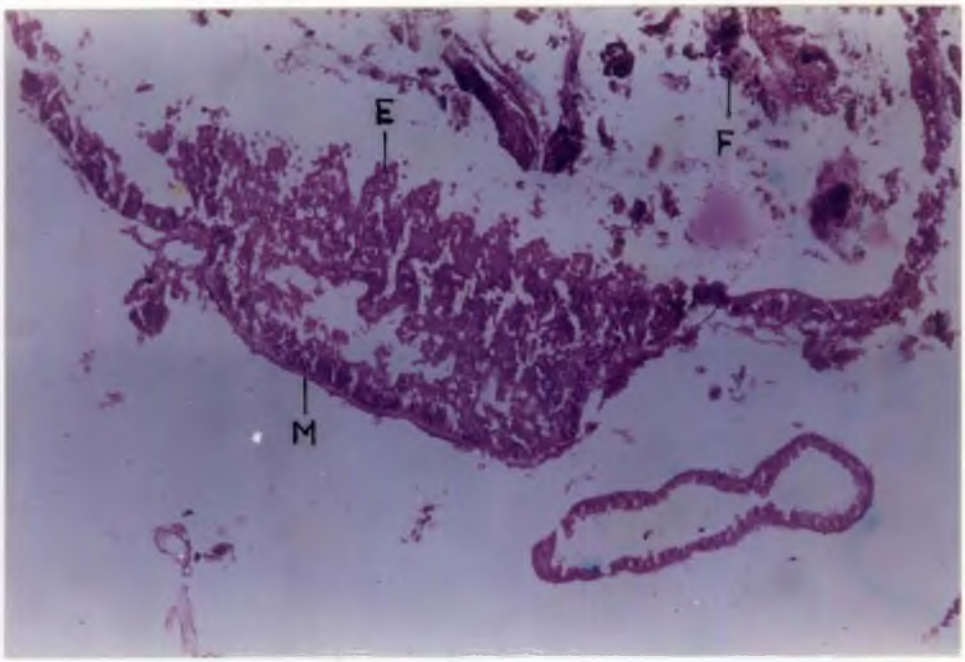
Unstained areas suggesting vacuole formation was found in the cytoplasm. Inflammatory cells were also seen. Gut lumen contained less amount of food materials (Plate 14).

Plate 12. Section of foregut of *O. rhinoceros* grub - control 10 x 16

M- Muscle
E- Epithelium
F - Food particles

Plate 13. Section of foregut of *O. rhinoceros* grub - second day of inoculation 10 x 16

M- Muscle
E- Epithelium
F - Food particles


E
F
M

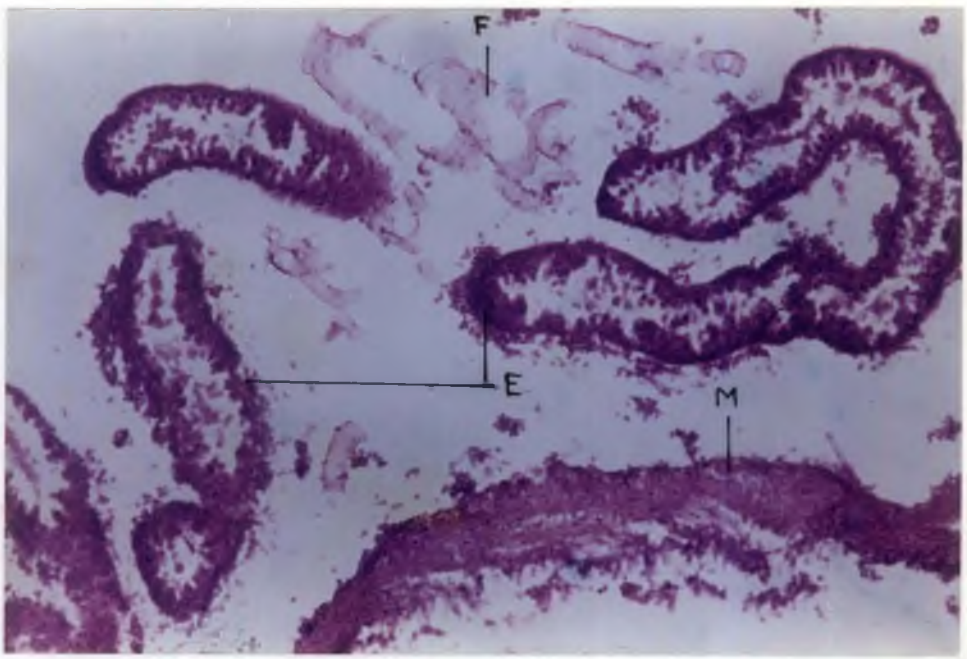
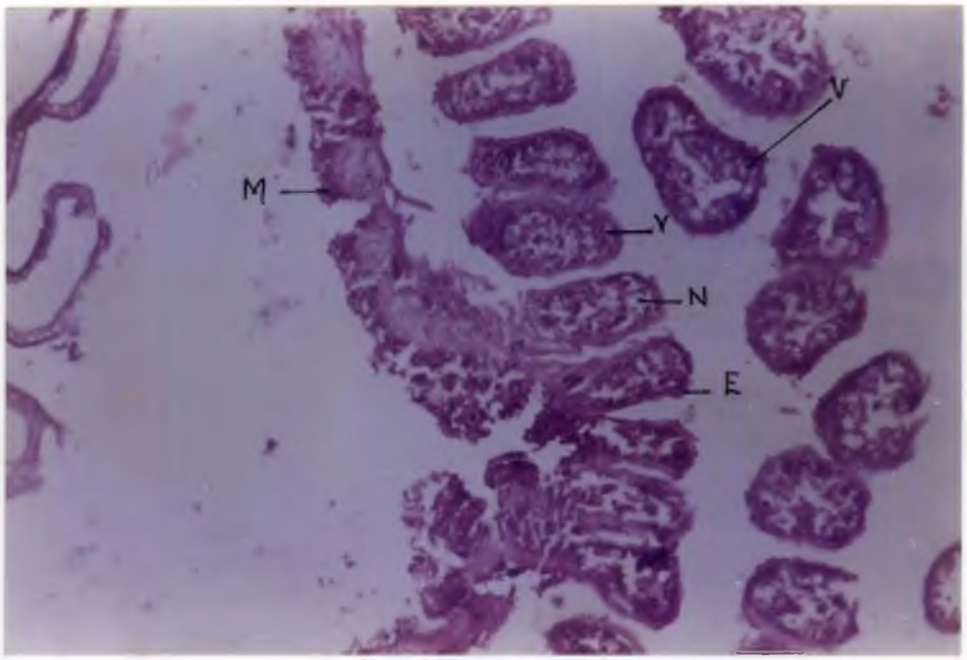

F
E
M

Plate 14. Section of foregut of *O. rhinoceros* grub - fourth day of inoculation 10 x 16

M - Muscle
E - Epithelium
V - Vacuole
N - Nucleus


V
M
Y
N
E

4.8.1.4 Sixth day of inoculation

Cytoplasmic vacuolation became more prominent. The cells became irregular and started desquamation. Nucleus was enlarged and deeply stained. The muscles remained unaffected. Food materials and desquamated cells were seen in the gut lumen (Plate 15).

4.8.1.5. Eighth day of inoculation

Cytoplasmic vacuolation was more prominent. Epithelial lining was disrupted and cells were desquamated. Nuclear vacuolation was also noted. More inflammatory cells were seen along with cells shed into the lumen. Food materials were not seen in the gut lumen (Plate 16).

4.8.2. Changes observed in midgut

4.8.2.1 Control

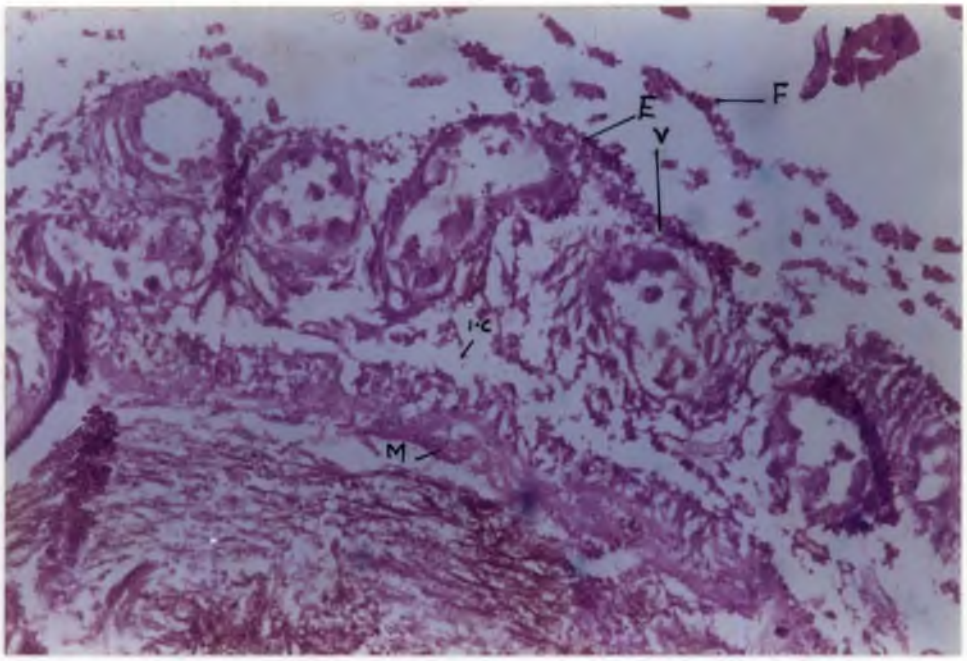
Sections of midgut of healthy larvae showed continuous epithelial lining of tall columnar cells. The gut lumen contained food materials. The secretory cells were filled with secretions. The nucleus was

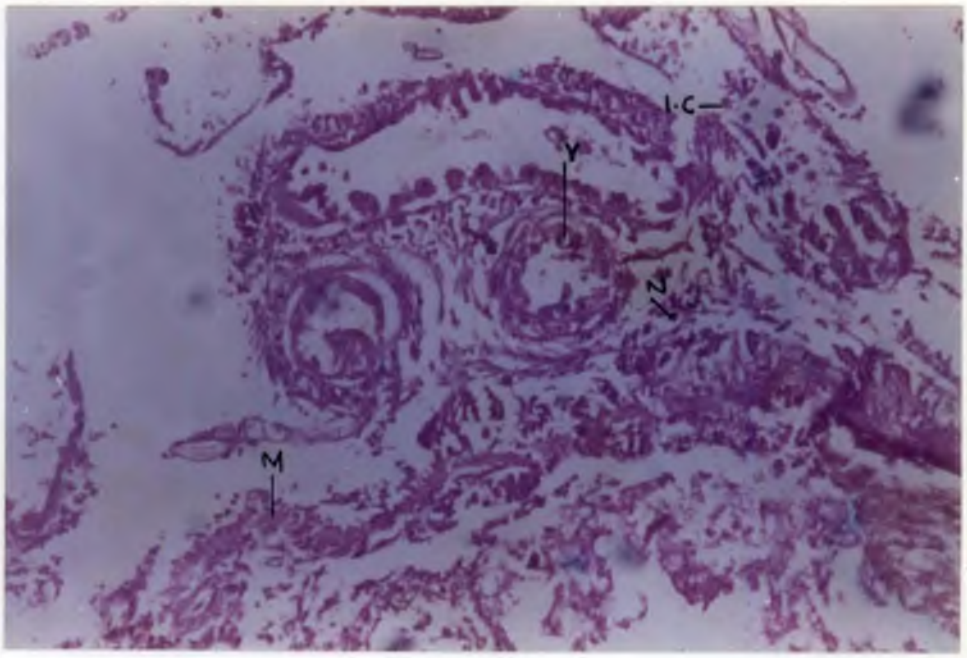
Plate 15. Section of foregut of *O. rhinoceros* grub - sixth day of inoculation 10 x 16

M- Muscle
V - vacuole
E- Epithelium
F - Food particles
IC - Inflammatory cells

Plate 16. Section of foregut of *O. rhinoceros* grub - eighth day of inoculation 10 x 16

M- Muscle
V - vacuole
F - Food particles
IC - Inflammatory cells


F
E
V
I·C
M


I·C
V
N
M

small and rounded in shape. Muscle layer was seen outside the epithelial layer (Plate 17).

4.8.2.2. Second day of inoculation

Changes were noted as early as the second day of inoculation. Vacuole like unstained areas were seen in the cytoplasm. The epithelial cells lost its normal contour. Nucleus became irregular and increased in size. The secretory cells were also enlarged in size. The gut lumen contained food materials (Plate 18).

4.8.2.3. Fourth day of inoculation

More and more cells showed cytoplasmic vacuolation. The epithelial cells became more irregular. The crypts showed varying depths. A few inflammatory cells were seen in the stroma. The nucleus got enlarged in size with eccentric unstained vacuoles. Muscle layers remained unchanged (Plate 19).

4.8.2.4. Sixth day of inoculation

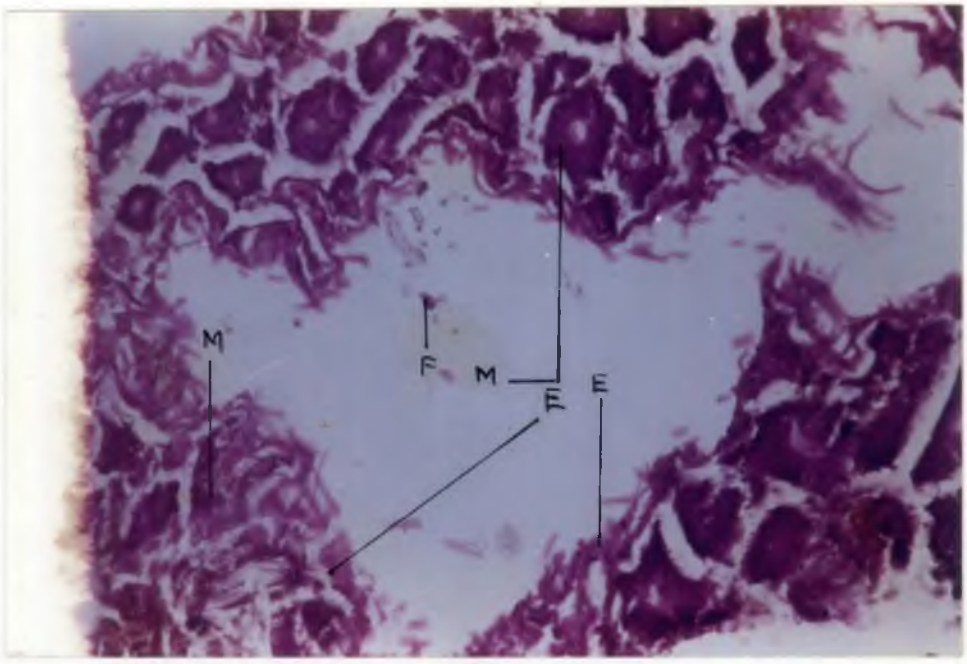
The epithelial lining was broken at many sites. Numerous inflammatory cells were seen in the stroma.

Plate 17. Section of midgut of *O. rhinoceros* grub - control 10 x 16

M - Muscle
E - Epithelium
F - Food particles

Plate 18. Section of midgut of *O. rhinoceros* grub - second day of inoculation 10 x 16

M - Muscle
V - Vacuole
F - Food particles
N - Nucleus


M
F
M
F
E

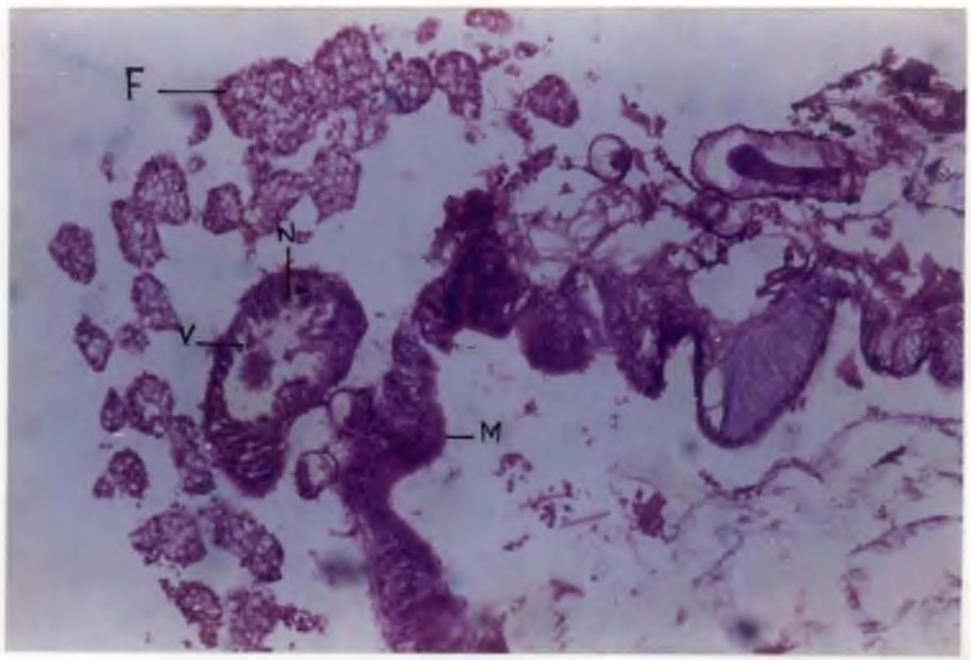
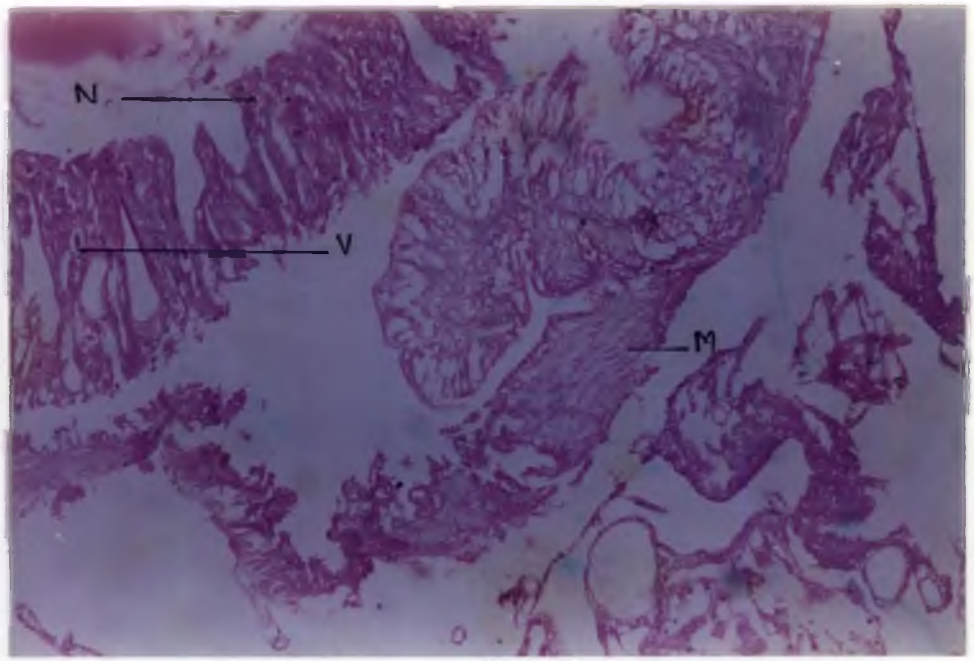

F
N
V
M

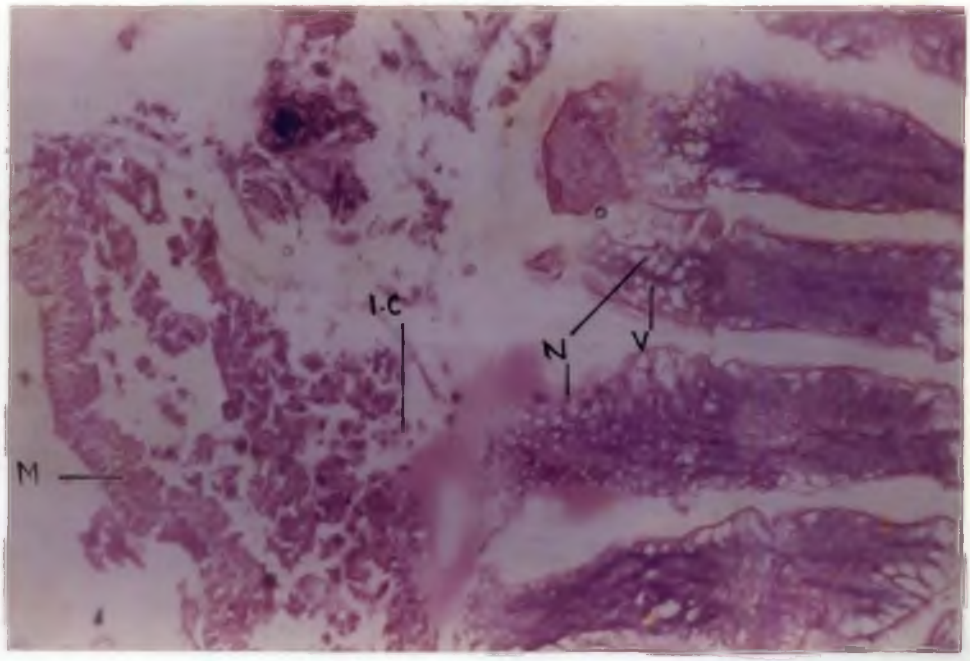
Plate 19. Section of midgut of *O. rhinoceros* grub - fourth day of inoculation 10 x 10

M- Muscle
V - vacuole
N - Nucleus

Plate 20. Section of midgut of *O. rhinoceros* grub - sixth day of inoculation 10 x 10

M- Muscle
V - vacuole
IC - Inflammatory cells
N - Nucleus


N
V
M


I.C
N
V
M

All the cells developed vacuolation. The epithelial lining started desquamation. The lumen contained clumps of infected cells but no food materials. The nucleus became irregular in shape and it stained deep pink. Some vacuoles were seen inside the nucleus of some cells (Plate 20).

4.8.2.5. Eighth day of inoculation

All the cells showed nuclear and cytoplasmic vacuolation. The epithelial lining was totally desquamated and there was complete loss of the normal architecture of the epithelial layer. Food materials were not seen in the gut lumen and instead was filled with infected cells which had deep stained large nucleus, scanty cytoplasm and vacuoles (Plate 21).

4.8.3 Changes observed in hindgut

4.8.3.1 Control

The section of the hindgut of healthy insects showed intact epithelial lining. They were more cuboid than the foregut. The lumen contained faecal matter. Inflammatory cells were not seen (Plate 22).

Plate 21. Section of midgut of *O. rhinoceros* grub - eighth day of inoculation 10 x 16

M- Muscle
V - vacuole
N - Nucleus

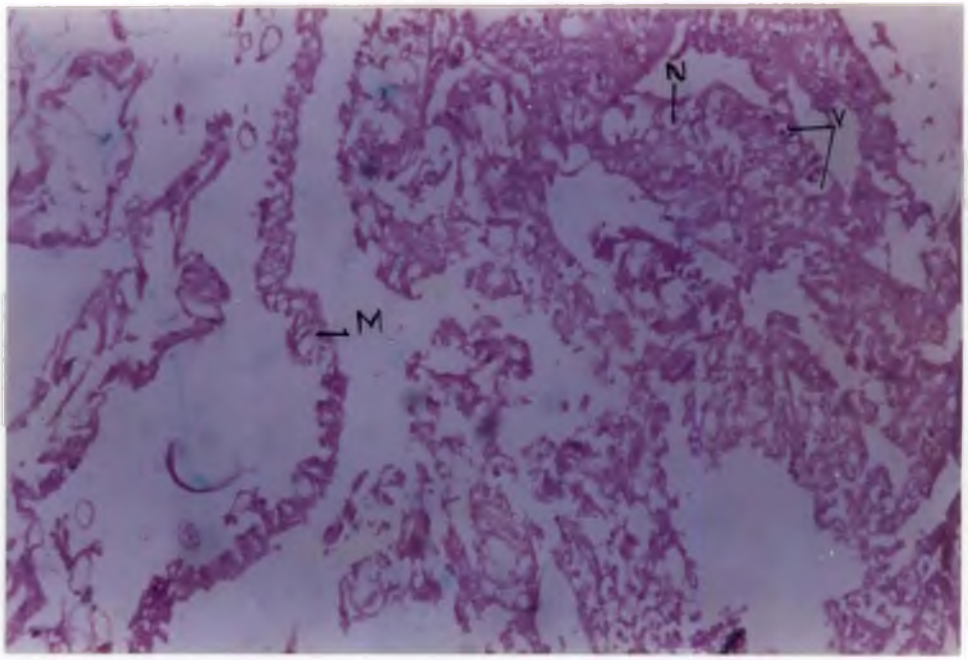
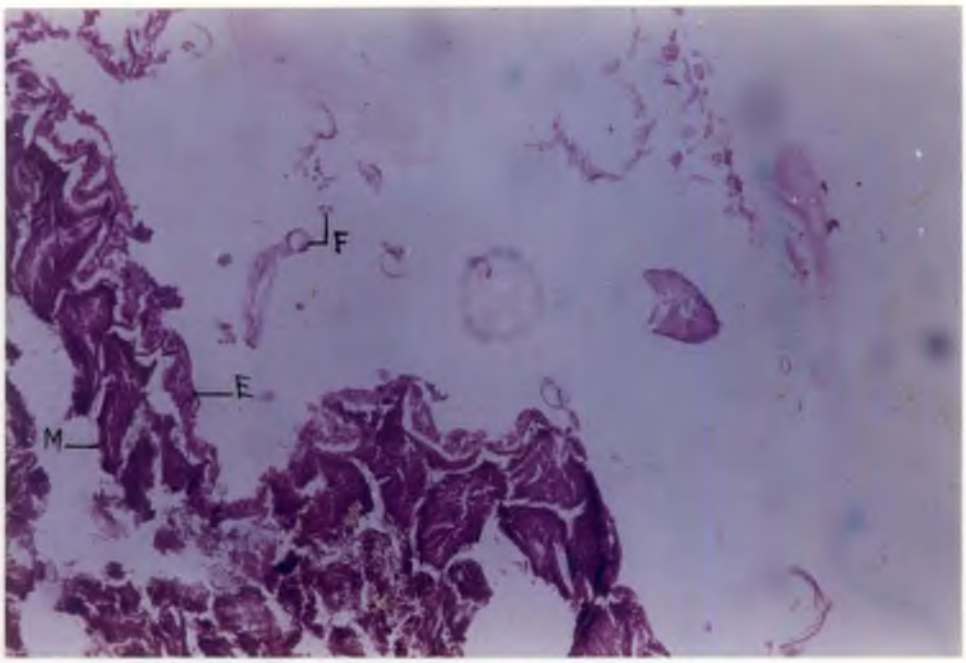

N
V
M

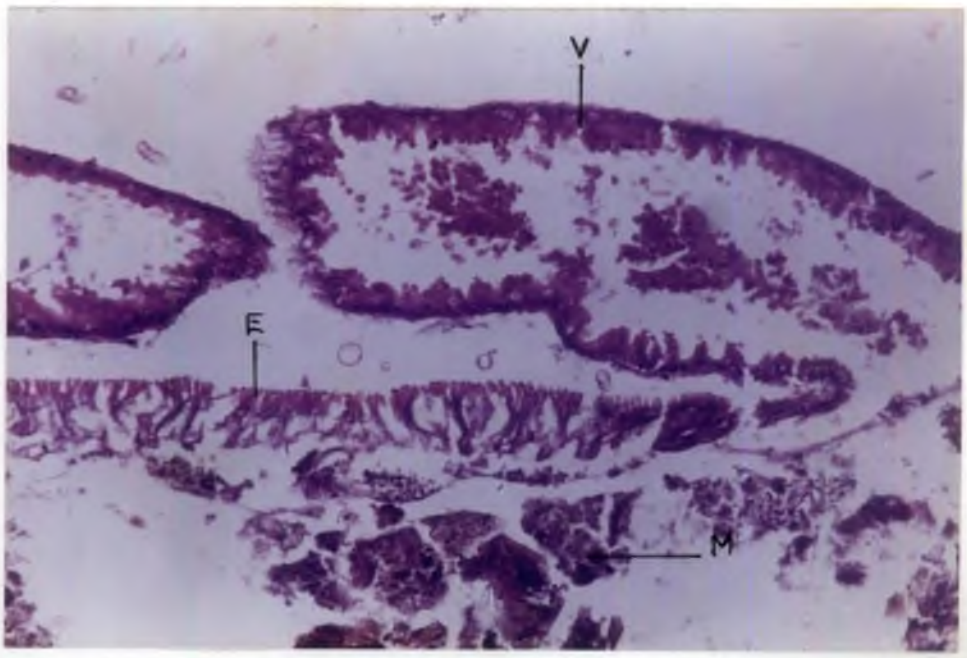
Plate 22. Section of hindgut of *O. rhinoceros* grub - control 10 x 10

M- Muscle
E- Epithelium
F - Faecal matter

Plate 23. Section of hindgut of *O. rhinoceros* grub - second day of infection 10 x 10

M- Muscle
V - Vacuole
E - Epithelium


F
E
M


V
E
M

4.8.3.2. Second day of inoculation

A few inflammatory cells were seen. Cytoplasmic vacuolation were seen in some cells. Nucleus started hypertrophying (Plate 23).

4.8.3.3 Fourth day of inoculation

More cells underwent vacuolation. Nucleus was enlarged in size and took deep stain. Inflammatory cells were seen in the stroma. The epithelial lining was irregular and disrupted at a few sites (Plate 24).

4.8.3.4 Sixth day of inoculation

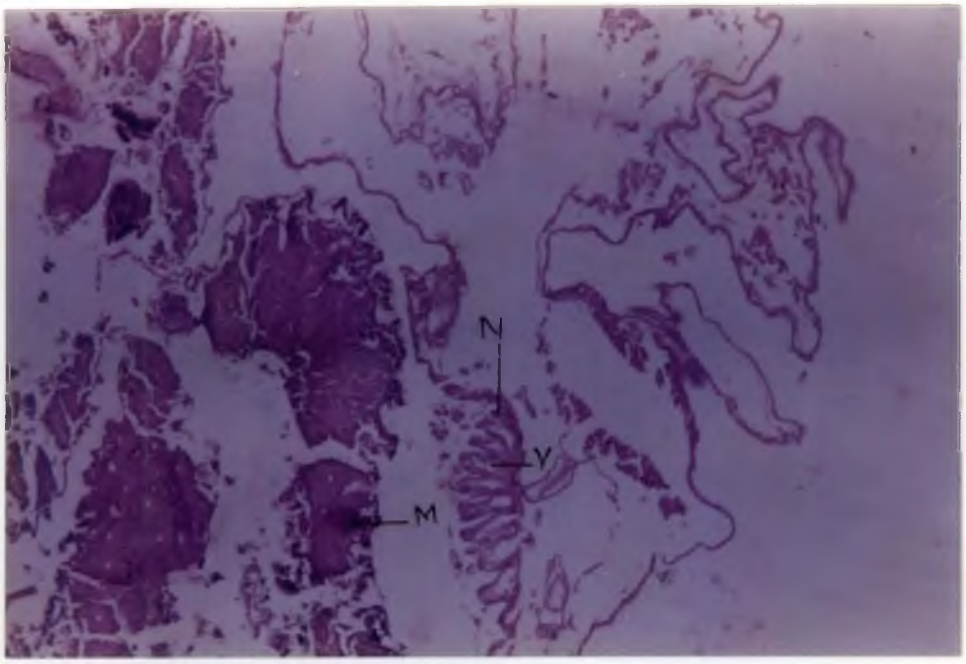
The epithelial lining was broken at many sites and almost all the cells showed cytoplasmic vacuolation. The gut lumen contained clumps of desquamated cells with large purple stained nucleus. Vacuolation was seen in some nuclei and the nuclei were placed eccentrically inside the cells. Muscle layer was oedematous (Plate 25).

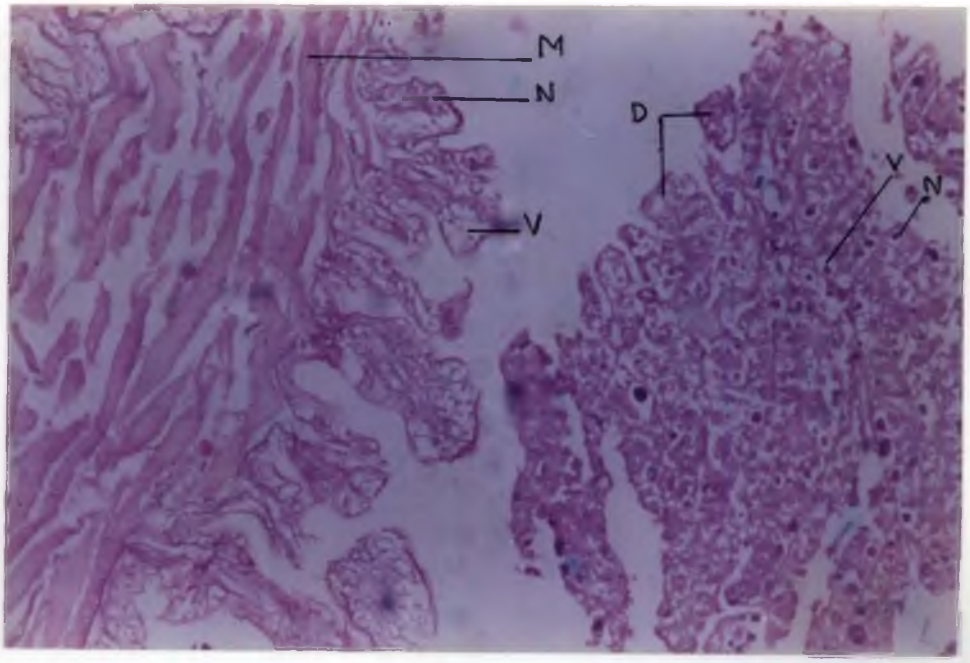
Plate 24. Section of midgut of *O. rhinoceros* grub - fourth day of incubation (10 x 10).

M- Muscle
V - vacuole
N - Nucleus

Plate 25. Section of hindgut of *O. rhinoceros* grub - sixth day of incubation (10 x 10).

M- Muscle
V - vacuole
N - Nucleus
D - Desquamated cells


N
V
M


M
N
D
V
V
N

4.8.3.5 Eighth day of inoculation

The gut lumen was filled with clumps of desquamated and disintegrated cells. Cytoplasmic and nuclear vacuolations were more prominent. There was complete loss of the normal architecture and it was unable to distinguish the various layers (Plate 26).

4.8.4 Changes observed in fat bodies

4.8.4.1 Control

The fat body was composed of masses of cells confined by delicate connective membrane (Plate 27).

4.8.4.2 Treated

Studies on the sections of fat body from the infected grubs showed marked histopathological changes. Cells of fat body showed necrosis with extensive vacuolation and disintegration. The nuclei in some cells has undergone pyknotic changes and in other cells the nucleus became hypertrophied. In the advanced stages of disease, the fat body was converted into a mass of necrosed tissue in the form of a loose net work consisting of loose connective tissues (Plate 28).

Plate 26. Section of hindgut of *O. rhinoceros* [illegible]
eighth day of inoculation 10 x [illegible].

M - Muscle
V - Vacuole
N - Nucleus

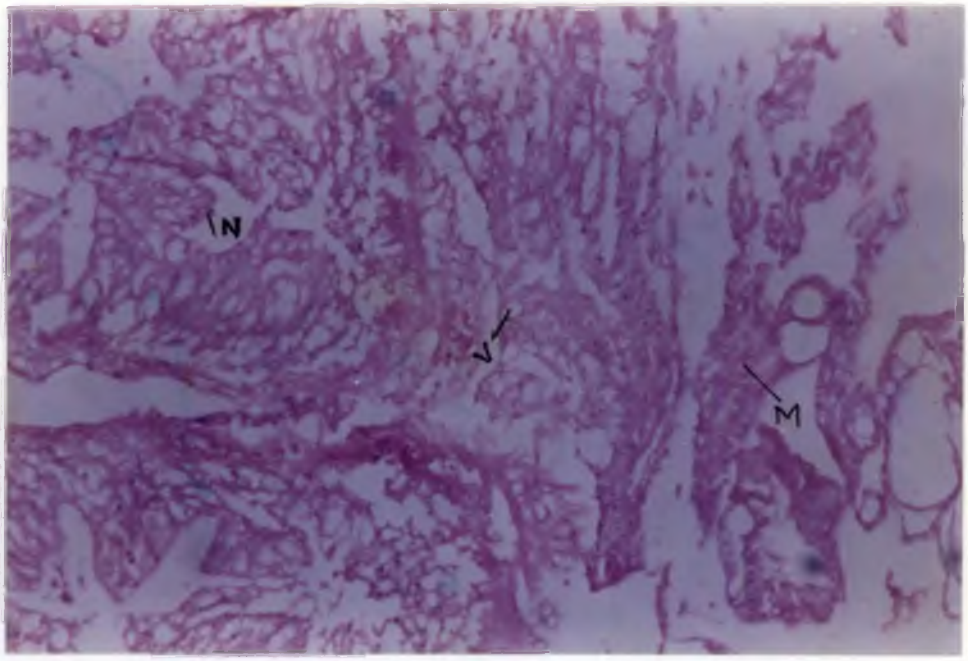
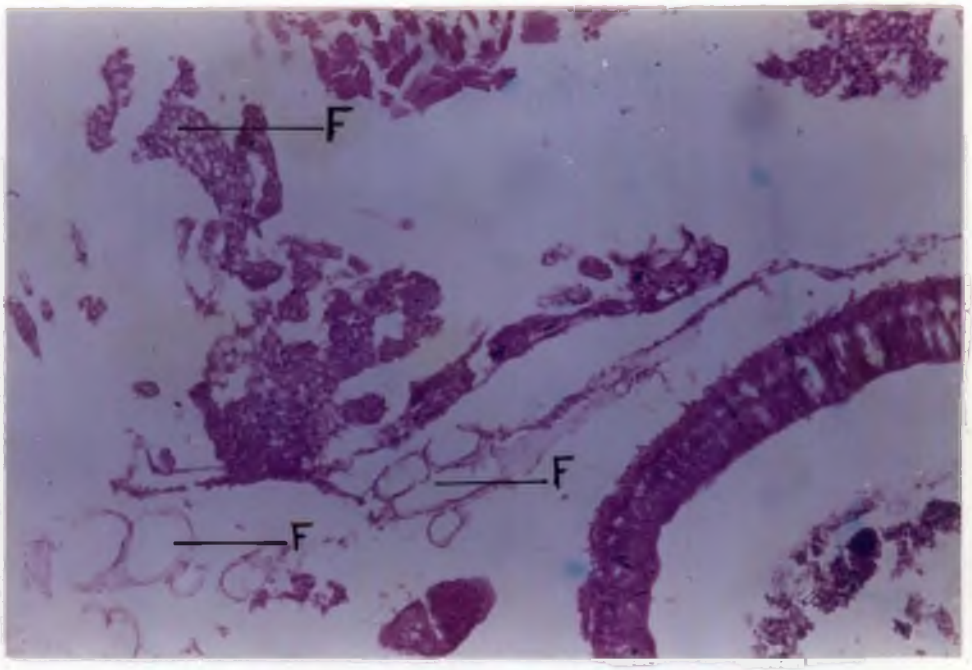

N
V
M

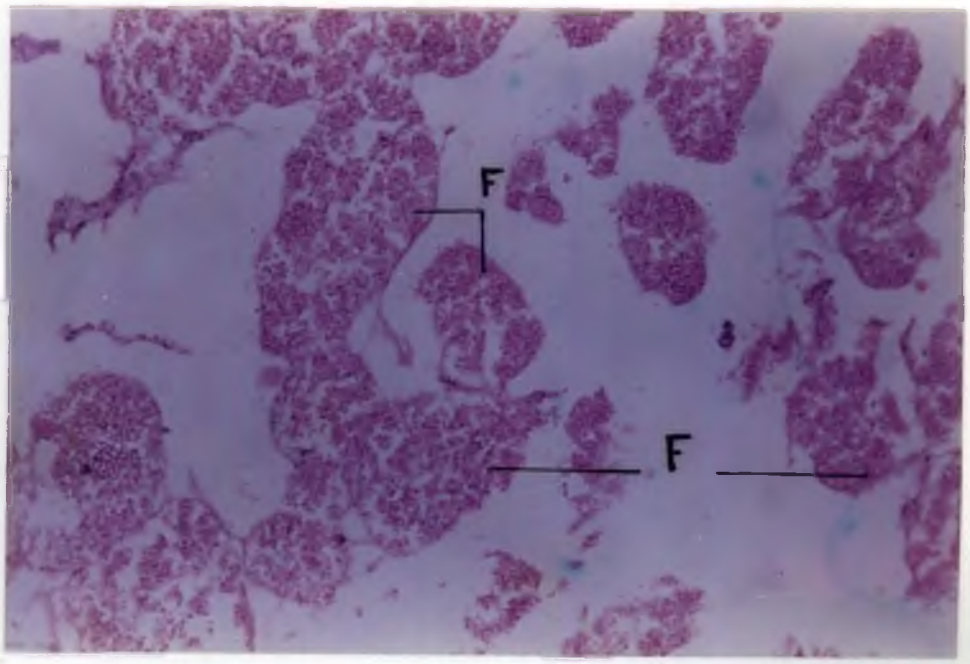
Plate 27. Section of normal fat body of *O. rhinoceros* (0 x 16.

F- Fat body

Plate 28. Section of infected fat body of *O. rhinoceros* (0 x 16.

F- Fat body


F
F
F


F
F

Similar changes were observed in the gut and fat bodies of second and third instar grubs and adults, but there was variation in the time taken for the development of the histopathological changes.

4.9. Transmission of virus

4.9.1 Transovum transmission

The results of the experiment are presented in Table 11. The number of eggs hatched out in the surface sterilised group was 171, in the unsterilised group it was 170 and 169 in the control. In neither group, there was evidence of viral infection in the grub or pupal stage. The percentage of adult emergence was 100, 98.89 and 97.78 in the lots of surface sterilised egg, unsterilised egg and in the control.

4.9.2 Transovarial transmission

The result of feeding third instar grub with homogenates of surface sterilised and unsterilised eggs obtained from virus infected adult beetles are presented in Table 12. Mortality due to viral infection

Table 11. Transovum transmission of *B. Oryctes*

Treatments	No.of eggs taken	No.of eggs hatched	Percentage of grub mortality		Percentage of pupal mortality	Percentage of adult emergence
			Virus	Others		
Eggs from treated adult (surface sterilised)	200	171	Nil	Nil	Nil	100
Eggs from treated adult (unsterilised)	200	170	Nil	1.11	Nil	98.89
Eggs from healthy adult (control)	200	169	Nil	2.22	Nil	97.78

Table 12. Transovarial transmission of *B. Oryctes*

Treatments	No. of grubs fed	Percentage larval mortality due to		Percentage pupal mortality due to		Percentage adult emergence
		Virus	Others	virus	others	
Homogenate of surface sterilised eggs of treated adult	200	Nil	Nil	Nil	Nil	100
Homogenate of unsterilised eggs of treated adults	200	Nil	3.0	Nil	Nil	97
Homogenate of eggs of healthy adult (control)	200	Nil	1.5	Nil	Nil	98.5

was not observed in the grub fed on homogenates of surface sterilised and unsterilised eggs. The percentage of adult emergence was 100, 97 and 98.5 in the group of grubs fed on homogenates of surface sterilised and unsterilised egg, and in the control respectively.

4.9.3 Transtadial transmission

The results of the study on transtadial transmission from first instar grub to second instar grub is presented in Table 13 and Fig 9.

When the early first instar grubs were treated with the virus it was observed that all grubs died within thirteen days, before initiation of moulting. The late first instar grubs on treatment with the virus showed a delay in the moulting and also the disease was transmitted to the second instar. In both the treated and control, moulting started from fifth day, the moulting being 15.83 and 45 per cent respectively. On the eighth day, all the control insects moulted normally whereas in the treated, it was only 30 per cent. Complete moulting occurred in the treated group

Table 13. Transtadial transmission of *G. Oryctes* from first instar grub to second instar grub

Days after treatment	Early instar (Cumulative percentage)				Late instar (Cumulative percentage)			
	Treated		Control		Treated		Control	
	Moulting	Mortality	Moulting	Mortality	Moulting	Mortality	Moulting	Mortality
5	Nil	Nil	Nil	Nil	15.83	Nil	45.00	Nil
6	Nil	Nil	Nil	Nil	25.00	Nil	85.00	Nil
7	Nil	5.83	Nil	Nil	27.50	Nil	95.00	Nil
8	Nil	15.00	Nil	Nil	30.00	0.83	100.00	Nil
9	Nil	27.00	Nil	Nil	34.17	3.33		Nil
10	Nil	88.33	Nil	Nil	42.50	6.67		Nil
11	Nil	94.17	Nil	Nil	56.67	10.83		Nil
12	Nil	98.33	Nil	Nil	81.67	24.17		Nil
13	Nil	100.00	Nil	Nil	92.50	51.67		Nil
14	Nil		Nil	Nil	100.00	62.50		Nil
15	Nil		Nil	Nil		70.00		Nil
16	Nil		Nil	Nil		73.33		Nil
17	Nil		Nil	Nil		75.00		Nil

Transtadial transmission of *B. oryctes* from first instar to second instar larvae of *O. rhinoceros* (Cumulative per cent)

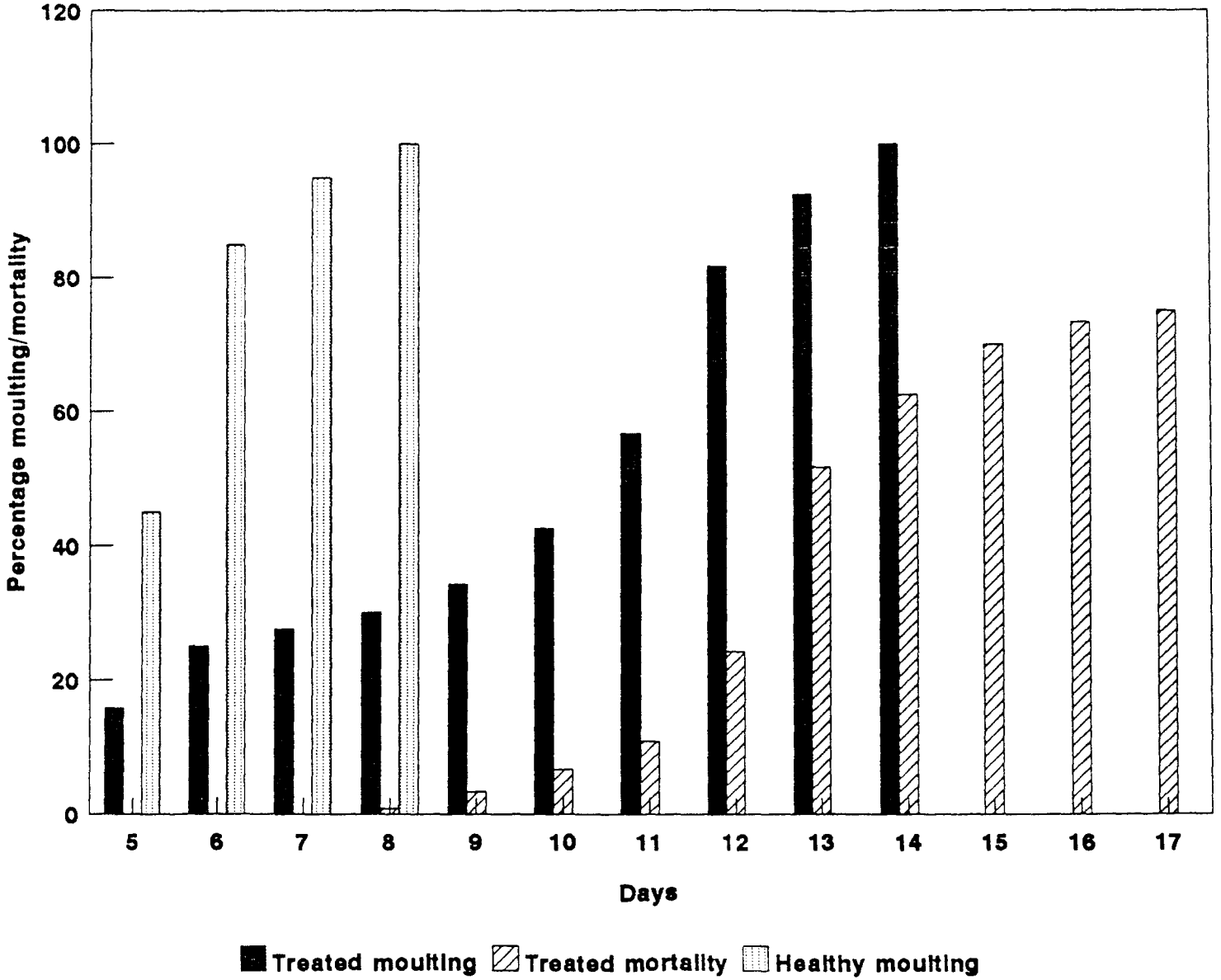

120
100
80
60
40
20
0
Percentage moulting/mortality
5
6
7
8
9
10
11
12
13
14
15
16
17
Days
Treated moulting
Treated mortality
Healthy moulting

Fig.9

by the fourteenth day. In treated grub death started from eighth day onwards, it being 0.83 per cent out of 30 per cent moulted. On the eleventh day, out of 56.67 per cent moulted only 10.83 per cent mortality was observed. On the fourteenth day, cent per cent moulting and 62.50 per cent death were observed. On seventeenth day 75 per cent mortality was recorded.

The result of the study on transtadial transmission from second to third instar grub is presented in Table 14 and Fig. 10. When the early second instar grubs were treated with the virus, it was observed that 77.50 per cent mortality occurred before moulting. When late second instar grubs were treated with the virus it was noted that there was delay in the moulting. In the treated ones moulting started from fifth day and was completed within 12 days. In control, moulting started from fifth day and it was completed on the ninth day. In diseased, on the fifth day only 19.17 per cent moulting was found but in control, it was 46.67 per cent. On ninth day, in control cent per cent moulting was recorded, but in treated it was

Table 14. Transtadial transmission of *O. Oryctes* from second instar grub to third instar grub

Days after treatment	Early instar (Cumulative percentage)				Late instar (Cumulative percentage)			
	Treatment		Control		Treatment		Control	
	Moulting	Mortality	Moulting	Mortality	Moulting	Mortality	Moulting	Mortality
5	Nil	Nil	Nil	Nil	19.17	Nil	46.67	Nil
6	Nil	Nil	Nil	Nil	29.17	Nil	86.67	Nil
7	Nil	Nil	Nil	Nil	37.50	Nil	96.67	Nil
8	Nil	Nil	Nil	Nil	65.83	Nil	99.17	Nil
9	Nil	Nil	Nil	Nil	80.83	Nil	100.00	Nil
10	Nil	Nil	Nil	Nil	92.50	Nil		Nil
11	Nil	Nil	Nil	Nil	96.67	Nil		Nil
12	Nil	3.3	Nil	Nil	100.00	0.83		Nil
13	Nil	10.00	Nil	Nil		3.33		Nil
14	Nil	33.33	Nil	Nil		6.67		Nil
15	Nil	63.33	Nil	Nil		10.83		Nil
16	Nil	70.00	Nil	Nil		20.83		Nil
17	Nil	75.00	Nil	Nil		45.83		Nil
18	Nil	77.50	Nil	Nil		55.00		Nil
19	Nil	Nil	Nil	Nil		61.67		Nil
20	Nil	Nil	Nil	Nil		65.83		NIl
21	Nil	Nil	Nil	Nil		69.17		Nil
22	Nil	Nil	Nil	Nil		70.83		Nil

Transtadial transmission of *B. oryctes* from second instar to third instar larvae of *O. rhinoceros* (Cumulative per cent)

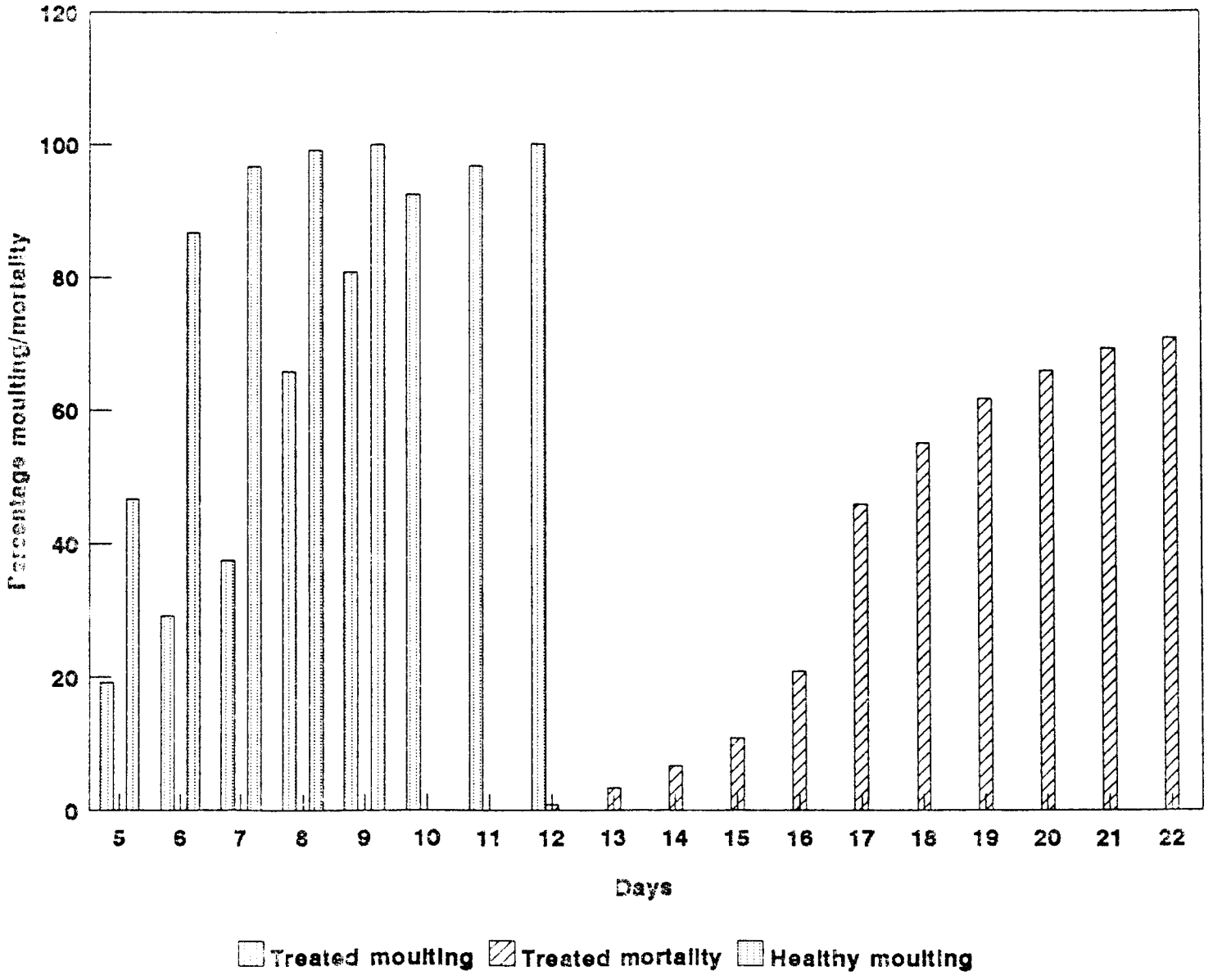

120
100
80
60
40
20
0
Percentage moulting/mortality
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
Days
Treated moulting
Treated mortality
Healthy moulting

Fig.10

only 80.83 per cent. In treated ones, mortality started from twelth day and it was only 0.83 per cent. Moulting recorded on eighteenth day was 55 per cent and has reached 70.83 per cent on 22nd day.

The result of the study on transtadial transmission from third instar grub to pupa is presented in Table 15 and Fig. 11. When the early third instar grubs were treated with the virus, it was observed that all grubs were dead within fourteen days before initiating moulting. When late third instar grubs were treated with the virus, it was noted that there was delay in the moulting and disease was not transmitted to the pupa. Moulting started from fifth day onwards in the case of both treated and control. In control, on the fifth day 48 per cent moulting was recorded but in diseased, it was only 16.67 per cent. On the sixth day, moulting recorded in both the control and the treated were 76.67 and 29.17 per cent respectively. On seventh, eighth and ninth day in treated grub moulting was not recorded but on the eleventh day, 36.67 per cent moulted and pupated normally. Mortality was not recorded in the pupal

Table 15. Transtadial transmission of *B. Oryctes* from third instar grub to pupa

Days after treatment	Early instar				Late instar (Cumulative percentage)			
	Treated		Control		Treated		Control	
	Moulting	Mortality	Moulting	Mortality	Moulting	Mortality	Moulting	Mortality
5	Nil	Nil	Nil	Nil	16.67	Nil	48.00	Nil
6	Nil	Nil	Nil	Nil	29.17	Nil	76.67	Nil
7	Nil	Nil	Nil	Nil	29.17	Nil	90.17	Nil
8	Nil	Nil	Nil	Nil	29.17	1.67	100.00	Nil
9	Nil	3.33	Nil	Nil	29.17	4.17		Nil
10	Nil	10.00	Nil	Nil	32.50	9.17		Nil
11	Nil	40.00	Nil	Nil	36.67	25.00		Nil
12	Nil	83.33	Nil	Nil		45.00		Nil
13	Nil	90.00	Nil	Nil		58.33		Nil
14	Nil	100.00	Nil	Nil		63.33		Nil

No pupal mortality

Transtadial transmission of *B. oryctes* from third instar larvae to pupae of *O. rhinoceros* (Cumulative per cent)

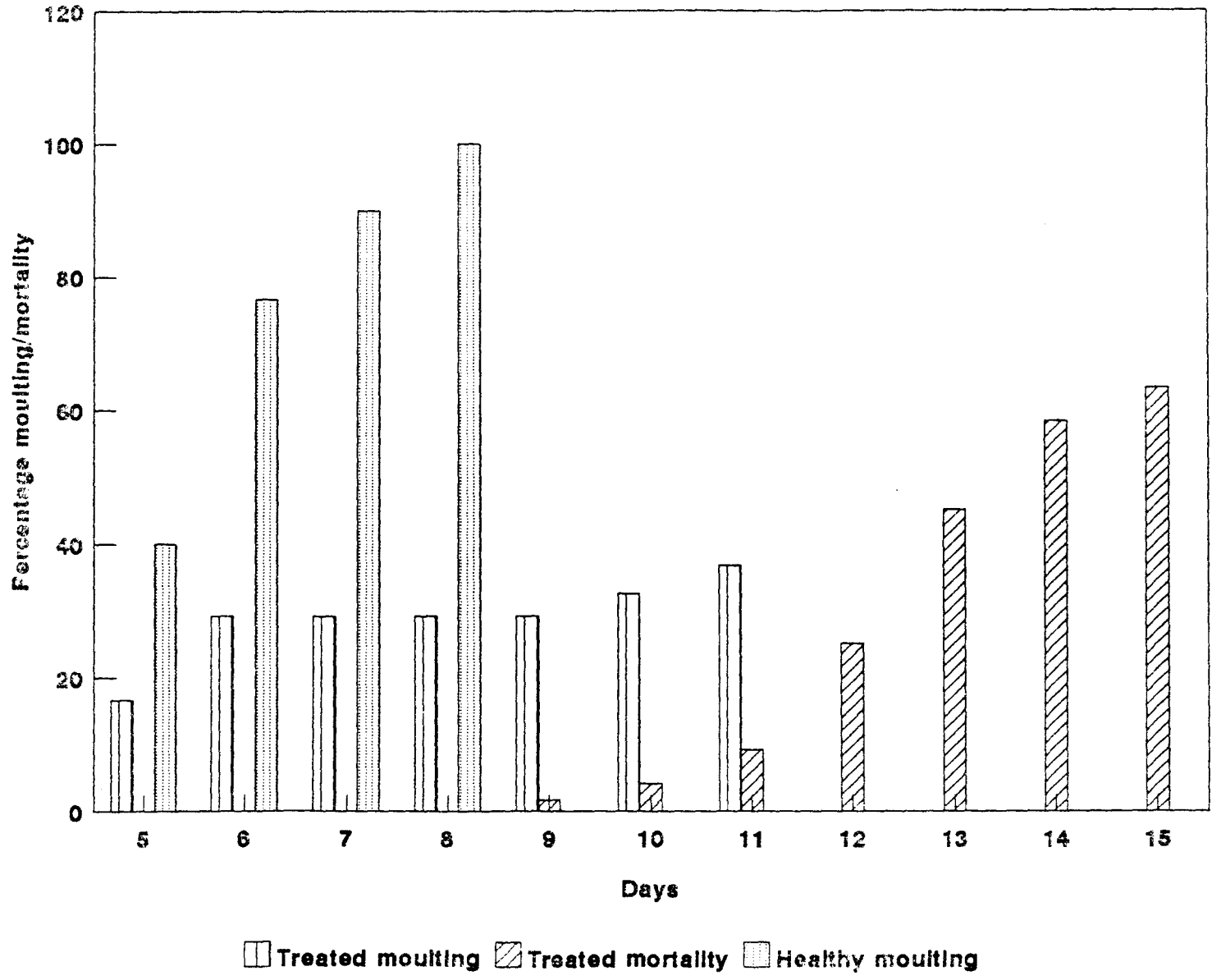

120
100
80
60
40
20
0
Percentage moulting/mortality
5
6
7
8
9
10
11
12
13
14
15
Days
Treated moulting
Treated mortality
Healthy moulting

Fig.11

stage. In control, all the grubs pupated within eight days. In treated, mortality was recorded from ninth day onwards and within 14 days 63.33 per cent died due to virus infection and 33.67 pupated normally.

4.9.4 Transmission through predator

The data on the transmission of the virus through predator *Platymerus laevicollis* is presented in Table 16 and Fig. 12. The insect showed varying rate of predation on the *Oryctes* beetle with different degrees of infection as 100, 87 and 63 per cent on 0, 3 and 5 days respectively after infection and cent per cent in control. The predator did not show any symptoms of infection. The grub fed on the homogenate of macerated predator did not develop viral infection. All pupated normally and emerged as adult.

4.10 Reproductive potential and transmission of virus during mating

Effect of viral infection on the reproductive potential is shown in Table 17. It was observed that when either or both the sexual partners were infected

Table 16. Transmission of *B. Oryctes* through predator *P. laevicollis*

Treatments	Predator exposed to virus infected beetles at different intervals (days)			
	0	3	5	Control
No. of beetles under test	100	100	100	100
No. of predators used	100	100	100	100
Percentage of adult fed by predator (preference)	100	87	63	100
Percentage mortality of predator due to virus infection	Nil	Nil	Nil	Nil
Percentage mortality of rhinoceros grub due to the feeding of homogenate of treated predator	Nil	Nil	Nil	Nil
Percentage of pupal mortality due to the feeding of homogenate of predator	Nil	Nil	Nil	Nil
Percentage of adult emergence	100	100	100	100

P. laevicollis having different intensity of *B. Oryctes* having diferent intensity of *B. oryctes* infection.

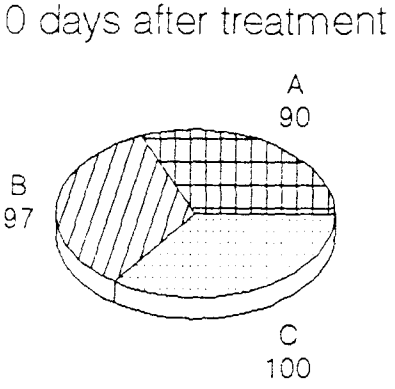
A - First day

B - Second day

C - Third day

D - Fourth day

E - Fifth day


0 days after treatment
A
90
B
97
C
100

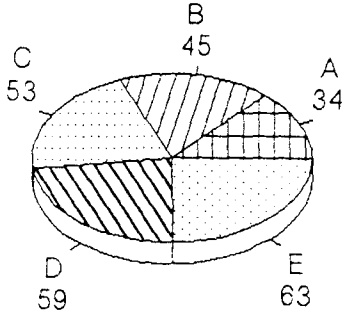
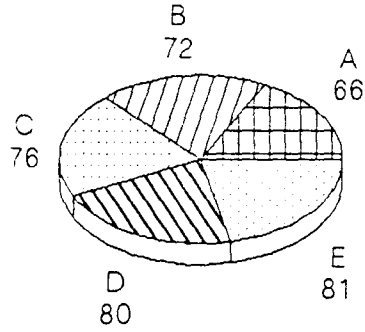

5 days after treatment
B
45
C
53
A
34
D
59
E
63

Fig.12

3 days after treatment



Control

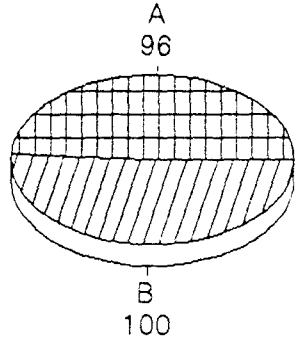


Table 17. Reproductive potential and transmission of *B. oryctes* during mating

	Eggs from virus treated adults surface sterilised				Eggs from virus treated adults surface unsterilised			
	HM x DF	HF x DM	DM x DF	HM x HF	HM x DF	HF x DM	DM x DF	HM x HF
No. of eggs laid	75	81	19	209	74	79	20	219
Percentage hatchabilty	80	82	80	82	81	82	83	82
Percentage of pupal mortality	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Percentage of pupal mortality	NIL	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Adult emergence	100	100	100	100	100	100	100	100

HM - Healthy male DM - Diseased male

HF - Healthy female DF - Diseased female

by the virus, there was significant reduction on the fecundity of the adult beetles. When either of the sexual partners was infected, the number of eggs laid varied from 74 in unsterilised to 81 in surface sterilised and when both the partners were infected, it was only 19 in surface sterilised and 20 in unsterilised. When both the partners were uninfected, the number of eggs laid were 209 in surface sterilised and 219 in unsterilised. There was no difference in the percentage hatchability when the eggs were allowed to hatch with or without sterilisation. There was no incidence of grub or pupal mortality and adult emergence was normal. Histopathology of testis and ovary did not show any change from that of normal.

4.11 Cross infectivity of the virus

Data presented in Table 18 showed that the other important pests of coconut like *Rhyncophorus ferrugineus*, *Leucopholus coneophora* and *Opisina arenosella* and the predator *P. laevicollis* were not susceptible to the virus and the treated larvae pupated normally and emerged.

Table 18. Host-specificity of *B. oryctes*

Insects tested	No. of insects tested	Percentage of larval/pupal mortality	Adult emergence
Rhyncophorus ferrugineus	75	Nil	100
Leucopholus coneophora	75	Nil	100
Opisina arenosella	75	Nil	100
Platymerus laevicollis	75	Nil	100

4.12 Thermal inactivation of virus

The observation from the experiment and the results of its statistical analysis are given in Table 19 and Fig. 13. It was observed that the grub mortality of cent per cent by infection due to unheated baculovirus was gradually reduced to zero level with 4 1/2 hours of heating. With 30 minutes heating of the virus there was no change in the grub mortality rate, but there was a delay by one day for getting complete mortality. On further heating, the mortality was reduced to 97.5 and 92.5 per cent at one hour and 1 1/2 hours respectively. On heating further, mortality rate fell down sharply to 72.5 per cent at two hours and 60 per cent at 2 1/2 hours. Beyond 2 1/2 hours, there was a drastic reduction in the mortality rate to 47.5 per cent at 3 hours, 40 per cent at 3 1/2 hours and 27.5 per cent at 4 hours. The mean time taken for the death of the grub was prolonged from 10.800 to 14.375 days. The mortality of the grub was zero at 4 1/2 hours of exposure to 37°C. The LT_{50} values ranged from 10.090 days with unheated to 15.700 days with virus exposed to

Table 19. Effect of continuous exposure of virus to a constant temperature of 37°C for varying periods.

	Period of exposure (hours) to heat										
	1/2	1	1 1/2	2	2 1/2	3	3 1/2	4	4 1/2	Contol	Unheted virus
Percentage of grub mortality	100	97.5	92.5	72.5	60.0	47.5	40.0	27.5	N o	N o	100
Time taken for death (ranges)	9-14	9-15	10-16	10-15	11-17	12-17	12-17	12-17	M o	M o	9-13
Mean time taken for death	11.1	11.410	12.069	12.486	13.636	13.737	14.363	14.375	r t	r t	10.800
LT_{50} (days)	10.538	10.665	11.888	12.315	15.700	--	--	--	a	a	10.090
Fiducial limits	10.123 10.613	10.390 10.971	11.462 12.236	11.892 12.800	15.339 16.084	--	--	--	l	l	9.880 10.324
Heterogeneity	3.202	1.888	0.804	1.336	0.265	--	--	--	i	i	4.281
Regression equation	3.0406x 5.265	3.144x 4.360	3.245x+ 3.810	3.468x+ 2.944	3.354x 2.178	--	--	--	t y	t y	3.208x+ 5.598

Effect of continuous exposure of *B. oryctes* to a constant temperature of 37°C.

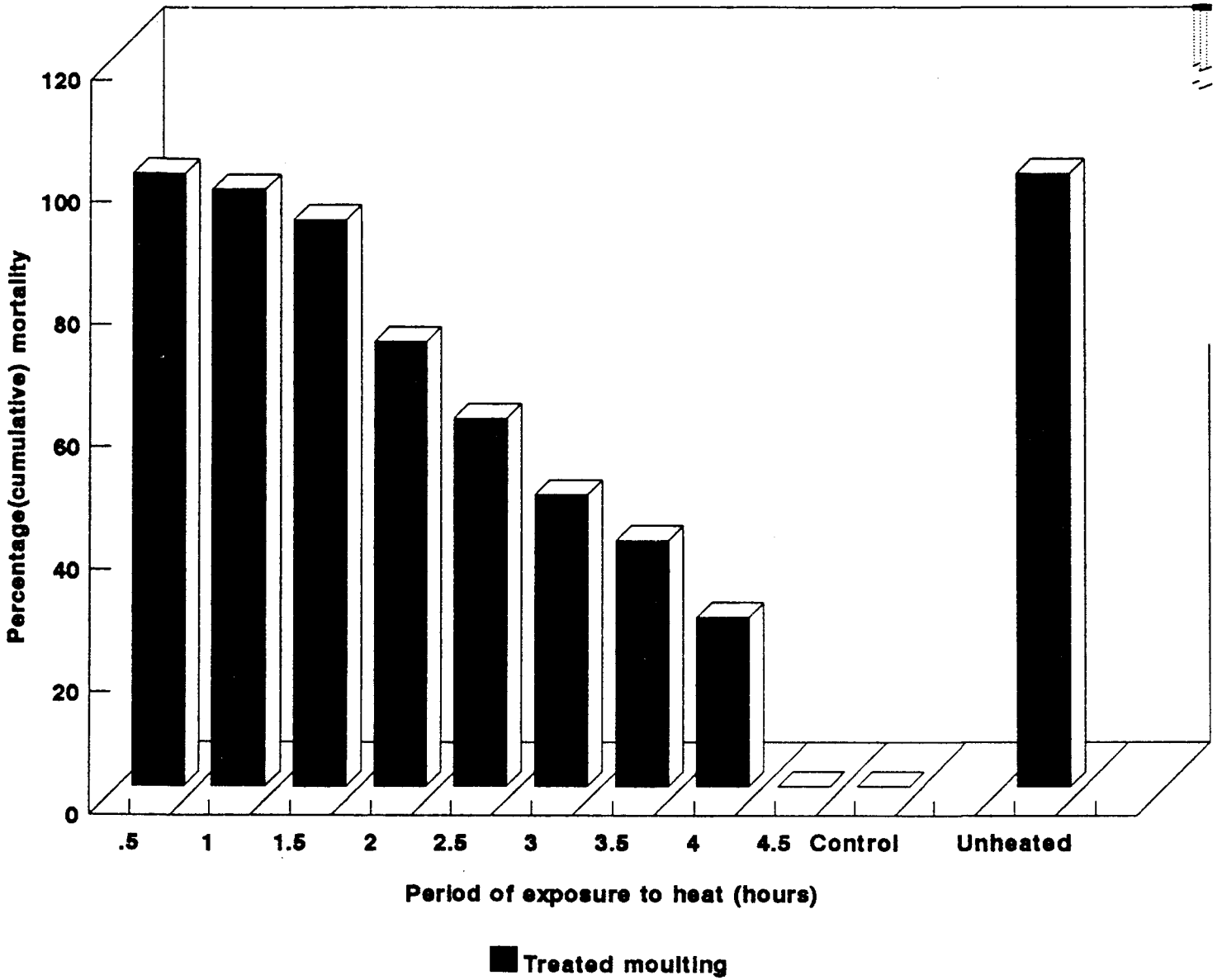

120
100
80
60
40
20
0
Percentage(cumulative) mortality
.5
1
1.5
2
2.5
3
3.5
4
4.5
Control
Unheated
Period of exposure to heat (hours)
Treated moulting

Fig.13

heat treatment for 2 1/2 hours. As the mortality rate was below 50 per cent beyond 2 1/2 hours of treatment of virus the LT_{50} values could not be calculated. Exposure to heat at 37°C for 30 minutes has not produced any change in the infectivity of the virus compared to unheated virus but there was a small delay of LT_{50} from 10.090 to 10.538.

4.13 Dosage requirement in adult equivalent per unit volume of breeding media

The data relating to the experiment is presented in Table 20 and 21. The mortality due to viral infection ranged from 30 to 100 per cent for a dose which ranged from 2 to 10 adults/box. The time taken for death ranged from 2 to 6 weeks. The mean time taken for death ranged from 3.150 to 4.154 weeks. For the different doses, the LT_{50} values ranged from 4.621 to 2.544 weeks for the doses ranging from 4 to 10 adults. The fiducial limits were not seen to be high. In the first two higher doses, cent per cent mortality was obtained, but the time taken for eight adult was two to five weeks and for 10 adults this was two to

Table 20. Effect of different doses of *B. oryctes* infected adults per cowdung pit (30 cm^3)

Dose (No. of adults released	Per cent mortality of grub	Interval (weeks)	Mean time taken for death (weeks)	LT_{50} (weeks)	Fiducial limits	Regression equation	Heterogeneity
2	30	2-5	3.583	-	-	-	-
4	65	2-6	4.154	4.621	4.154 5.157	y=3.065x+ 3.462	0.568
6	90	2-6	3.806	3.313	3.038 3.625	y=3.599x+ 3.847	0.156
8	100	2-5	3.875	2.660	2.495 2.843	y=3.464x+ 6.977	0.708
10	100	2-4	3.150	2.544	2.391 2.713	y=3.637x+ 7.229	1.268

Table 21. Dosage requirements (No. of adults/pit size 30 cm x 30cm x 30 cm) at different interval (weeks) after treatment.

Time (week)	Heterogeneity	Regression equation	LD_{50} (adults)	Fiducial limits
Third	0.544	y=3.307x + 2.301	4.400	3.935 4.996
Fourth	16.405	y=4.043x + 3.632	3.668	3.504 3.844
Fifth	4.518	y=4.338x + 3.712	3.016	2.864 3.176
Sixth	2.224	y=2.889x + 7.601	2.889	1.754 2.032

four weeks. The LT_{50} values for the two doses were 2.660 and 2.544 weeks respectively. In the six adults released, the percentage mortality was high i.e. 90 per cent with the LT_{50} value of 3.313 weeks. There was great reduction in mortality from four adult released doses. In the four adults dose, the mortality was 65 per cent with the LT_{50} value of 4.621 weeks. In two adults dose, the mortality was only 30 per cent. The LT_{50} value for this dose could not be calculated since the mortality was less than 50 per cent.

The LD_{50} values were 4.400, 3.668, 3.016 and 2.889 adults per pit of size 30 cm x 30 cm x 30 cm at 3rd, 4th, 5th and 6th week. The LD_{50} for the second week could not be calculated since the mortality was less than fifty per cent.

4.14 Safety of the virus

4.14.1 Safety to silkworm *Bombyx mori*

Data on the response of third instar grub of *B. mori* to *B. oryctes* infection is presented in Table 22. Mortality due to viral infection was not observed in

Table 22. Effect of oral feeding of *B. oryctes* to mulberry silkworm *B. mori*

Characteristics	Treated	Untreated
Per cent mortality due to virus infection	Nil	Nil
Percentage of larval survival	100	100
Mean pupal period (days)	10.43	10.40
Mean increase in length of larvae	+3.4 cm (2.4 to to 5.8 cm)	+3.45 cm (2.35 to 5.8 cm)
Mean larval weight (g)	0.479	0.476
Mean cocoon weight (g)	1.469	1.479
Per cent of adult emergence	100	100
Mean fecundity	485	472

the treated and untreated ones of *B. mori*. All the larvae pupated normally and recorded complete adult emergence. The mean pupal period for treated and untreated ones were 10.43 and 10.40 respectively. The mean weight of the cocoons were 1.469 and 1.479 g for the virus fed and for the control group respectively. The larvae increased in size by 3.40 cm in treated and 3.45 cm in control and weight of larvae by 0.479 and 0.476 g. The mean number of eggs laid per female in treated and control was 485 and 472 respectively.

4.14.2 Safety to chick embryo

The result of the experiment showed that chick embryo was not susceptible to infection by the *B. oryctes*. None of the chick embryos treated with either the virus or normal saline died during the test period. The embryos were found to be healthy and showed normal appearance and development (Plate 29). Post-mortem examination of the embryo was done after killing the embryo by keeping in refrigerator at the end of the test period. Macroscopic examination of the internal organs also showed no evidence of viral infection.

Plate 29. Chick embryo treated with *B. oryctes*

A - Control

B - Treated


A
B

4.14.3 Safety to white rats and white mice

Data relating to the experiment is shown in table 23 and 24. There was no death in the virus fed animals or in the control. Body weight gain in virus treated white rat and white mice were on par with that of untreated animals. The virus fed animals never showed any rise in body temperature. No difference due to virus treatment was detected in the total blood count, differential count or haemoglobin levels. Postmortem examination of the various organs like heart, lungs, liver, kidney, spleen or thymus did not show any change in size or texture and no focal lesions could be detected on macroscopic examination. The average weight of the organ did not vary.

Table 23. Effect of feeding white mice with *B. oryctes*

Observations	Effect on	
	Treated	Control
Initial mean weight of the animal (g)	18.80	18.78
Final mean weight of the animal (g)	20.68	20.40
Mean weight gain (g)	1.88	1.62
Body temperature (°C)	37.4	37.4
Blood Count		
Haemoglobin (g/100 ml)	13.8	14.2
Lymphocyte (%)	42.0	39.0
Neutrophil (%)	54.0	62.0
Eosinophil (%)	4.0	6.0
Basophil (%)	0.0	1.0
Monocyte (%)	0.0	0.0
Total count (per Cu mm)	4200.0	4300.00
Organ weight (mg)		
Lungs	147.82	146.54
Liver	837.3	832.36
Kidney	272.76	270.46
Spleen	50.7	49.8
Thymus	47.8	46.76

Table 24. Effect of feeding white rat with *B. oryctes*

Observations	Effect on	
	Treated	Control
Initial mean weight of the animal (g)	178.80	177.78
Final mean weight of the animal (g)	182.50	181.50
Mean weight gain (g)	3.70	3.72
Body temperature (°C)	37.5	37.5
Blood Count		
Haemoglobin (g/100 ml)	12.8	13.2
Lymphocyte (%)	38.0	34.0
Neutrophil (%)	56.0	62.0
Eosinophil (%)	4.0	3.0
Basophil (%)	1.0	1.0
Monocyte (%)	1.0	0.0
Total count (per Cu mm)	6000.0	6000.0
Organ weight (mg)		
Lungs	965.28	959.20
Liver	5140.72	5182.30
Kidney	1240.74	1213.20
Spleen	511.40	490.00
Thymus	207.04	196.02

Discussion

DISCUSSION

The Rhinoceros beetle, *Oryctes rhinoceros* is a major pest of coconut palm in all areas where the crop is grown. The *Baculovirus oryctes* discovered in Malaysia by Huger (1966 a and b) was later shown to be a valuable agent for the biological control of the beetle. Experimental release of the baculovirus in Kerala and the Minicoy Islands gave promising results. Adequate basic information on the pattern of the virus infection in the host populations and its safety to non-target organisms has not been generated. The present studies were mainly on the methods of inoculation, symptomatology, gross pathology, patho-physiology, histopathology, methods of transmission, effect of environmental factors on viral activity, dosage requirement for field application and safety to productive insects and higher animals.

5.1 Techniques of inoculation of *O. rhinoceros*

In mass multiplication of the baculovirus on its preferred host, the efficiency of the mode of

inoculation is of considerable importance. In the oral inoculation method, the mortality in adult was found to be 98.66 per cent, whereas in the swim technique, the mortality rate was 74.66 per cent. Lethality occurred in a shorter period, consequent on oral inoculation (Table 1). In this method, the beetle could acquire a definite quantity of viral suspension, while in the swim technique, the consumption of the viral suspension by the beetle varied considerably among the individuals. Some beetles did not feed on the suspension. The observation of Zelazny (1978) corroborates with the present findings.

The oral inoculation technique is preferable for laboratory culture maintenance and experiments while, the swim technique could be adopted for large scale contamination of adults before they are field released. The extent of infection induced by the swim technique could be further improved by keeping populations of the beetle under crowded conditions.

5.2 Storage of virus

Freeze-drying following the Greave's method (Swain, 1972) was found to be an ideal method for long term storage of *B. oryctes* in the laboratory.

5.2.1 Viability study of the freeze dried virus

The freeze dried virus has been found to retain its viability for more than sixteen months. The viability of the virus was found to decline gradually on storage from 100 to 34.16 per cent over a period of sixteen months (Table 2). The retention of activity and potency of the virus for a long period of sixteen months is a definite advantage in biological control of the Rhinoceros beetle, as a stock culture of the pathogen could be kept ready for long periods to be used as and when needed. The conventional method of storage of the virus is to preserve terminally diseased grubs at -20°C in deep freezers. Whenever field applications are necessary, the frozen grubs are thawed in warm water at 40°C, the midgut dissected out and used (Young, 1974; Bedford, 1976; Mohan *et al.*, 1985). The handicap of the conventional storage

technique is that the culture does not remain stable for long period (Zelazny *et al.*, 1985, 1987). Freeze drying method of storage has been found to be superior to other methods, since the virus could be stored for up to sixteen months without losing the viability and infectivity.

5.3 Morphology of the virus

Electron micrography of the midgut epithelial cells showed groups of rod shaped viral particles 210 to 250 nm long and 75 to 105 nm broad, within the nucleus (Plate 5 and 6). In the Kerala isolate of *B. oryctes*, Mohan *et al.* (1983) recorded almost similar finding with the viral size ranging from 215 to 260 nm in length and 77 to 110 nm in breadth, whereas *B. oryctes* isolated from the South Pacific Island, has been reported to have the size range of 200 $\pm$ 10 nm x 86 $\pm$ 15 nm. The different isolates of *B. oryctes* from various parts of the world have an average size of 200 x 70 nm (Huger, 1966 b; Monsarrat *et al.*, 1973 b; Payne, 1974).

Huger (1966 b) described two types of viral particles from the fat body:- rod forms, representing the mature virus very similar in structure to the rod-shaped nuclear polyhedrosis virus and granulosis virus of lepidopterous insects and single and double membraned spherical particles, representing the viral developmental stage of cell infection. During the early phase of cell infection, the nucleus may be overcrowded with spherical forms while lacking rods at all or exhibiting them only in a comparatively low number. In the course of virogenic process more and more rods are established.

Since the smear from the midgut of the highly infected grubs was taken for laboratory evaluation, the virogenic process might have been very active at that stage causing a dominance of rod shaped forms. The virus containing envelope was not like polyhedra of the nuclear polyhedrosis virus (Bedford, 1980). According to Huger (1966 b), *B. oryctes*, the first rod shaped virus demonstrated in a coleopterous insect, is fundamentally different from the nuclear polyhedrosis virus and the granulosis virus, the former being non-

occluded in polyhedra or capsule or microcrystalline proteinaceous material. Clusters of virus rods may be densely packed in two three-dimensional pseudo-crystalline pattern. Certain novel structures were observed from purified preparations of *O. rhinoceros* obtained from faeces of adult beetle by Payne *et al.* (1977). A small proportion of virus particles had a finger-like protuberance at one end as if the viral envelope was distorted by an internal component, while other particles carried a tail like projection which could be seen when the envelope was disrupted or completely removed.

5.4 Symptomatology

5.4.1 Grubs

The symptomatology was studied in detail to establish certain reliable visual diagnostic features of viral infection in the field populations of *O. rhinoceros*. Most of the symptoms resembled the general profile reported in the case of other members of the family baculoviridae (Smith, 1967; Patel *et al.*, 1968;

Ramakrishnan and Tiwari, 1969; Kumar and Jayaraj, 1978; Deshpande, 1981; Bawage, 1982). Visual symptoms in grubs were observed from third to fourth day of infection. The disintegration of the body fat tissue and increase in the haemolymph fluid render the grub glassy and transparent (Plate 8). Increase in turgor in the haemocoele leads to gut herniation (Plate 7) and the grubs develop diarrhoea. Similar symptoms were observed by Huger (1966 b) in infected grubs of *O. rhinoceros*. The infected grubs migrate to the top of the substrate prior to death and this is a typical symptom of almost all the baculoviridae. This movement of the infected grubs in the substrate is helpful to spread the virus in the breeding media. The gut lumen seemed to be devoid of food material and is filled with the mucoid material containing the disintegrated cells and viral particles.

5.4.2 Adults

The infected adults do not show any early symptoms as grubs. In adults, development of diarrhoea and

cessation of feeding are the prominent symptoms. That the infected adults survive as long as thirty days after getting viral infection is an advantage in as much as the host virtually functions as a continuous reservoir for the production and dissemination of the virus through the faecal matter. As the infected adult beetles stop feeding, they do not cause any damage to the coconut palms. Zelazny (1976) also reported that the infected adults function as a prolific viral factory which facilitates continuous dissemination and transmission of the virus. The infected adult beetle voids upto 0.3 mg of virus per day in the faeces and thus act as a reservoir spreading the disease to breeding sites and also to healthy adults when they feed together in the crown (Monsarrat and Veyrunes, 1976). The infected adults rarely lay a few eggs or none at all. The above observations are in tune with the findings recorded by Huger (1966 b), Zelazny (1977 a) as well as Mohan *et al*. (1985). These desirable traits formed the basis for the effective control of the Rhinoceros beetle, wherever the virus has been introduced (Zelazny, 1976).

The malformation of the wing and elytra as described by Monty (1974) was noted only in two beetles (Plate 9).

5.5 Gross pathology

Establishing the stage susceptibility of an insect host to infection by the microbial pathogen is an essential pre-requisite for decision making on the time of application for obtaining adequate level of control. It was hence necessary to determine the relative susceptibility of the life stages of *O. rhinoceros*, with a view to rationalise field control strategies. The optimum dose of the pathogen was determined by the bioassay method using various instars of grubs and adults as test organisms. It was found that all instars of grubs and adults were susceptible to viral infection. In the case of first instar, 39.17 to 100 per cent mortalities were recorded for the different doses tested (10^{-6} to 10^{-1} dilutions of gut equivalent). Cent per cent mortality was recorded for the highest two concentrations (10^{-1} and 10^{-2}) and more than fifty per cent mortality in all dilutions except the

lowest. The LT_{50} values ranged from 7.862 to 12.723 days for the serial dilutions (Table 3). In the case of the second instar grub, the maximum mortality was only 80.83 per cent, with the LT_{50} value of 14.214 days at the highest concentration (Table 4). In the third instar grub, cent per cent mortality was obtained in the highest concentration of 10^{-1} (Table 5). For the adult, in the highest two concentrations (10^{-1} and 10^{-2}) the mortality levels realised were 98.33 and 90.83 per cent, the LT_{50} values being 22.960 and 23.499 days respectively (Table 6).

The mean lethal concentrations of the *B. oryctes* required to kill fifty per cent of adults (LC_{50}) were 0.377×10^{-4}, 14.417×10^{-4}, 0.297×10^{-4} and 1.137×10^{-4} for the first, second and third instar grubs and adults respectively. Taking the LC_{50} value of the first instar as the standard, the concentration required to cause fifty per cent mortality of second and third instar grubs and adults were 38.241, 0.788 and 3.016 times higher respectively (Table 7).

These studies clearly showed that the first instar grub is the most susceptible stage to viral infection. The mean lethal time was the longest in the case of second instar. The LT_{50} value in respect of the second instar grub was twice that of the first instar. The susceptibility to infection and severity were the highest in the first instar followed by third instar and second instar in that descending order. The above results are in conformity with the observations made by Mohan *et al.* (1985) on the Kerala isolate (KI) of *B. oryctes*. Zelazny (1972), working on the Philippine isolate of the virus, reported significant variations in the lethal infection period between instars, nine days for first instar, 13 days for second instar and 18 to 25 days for the third instar grubs when fed with baculovirus killed grubs at 10^{-4} dosage. Zelazny (1973 a) found that the infected adults died within a period of 25 days compared to 70 days of healthy. He found that the third instar grub was the most resistant stage to infection. The strainal variations in the pathogenicity and the differential

susceptibility of the various instars to their infection are quite natural and the present results are explicable on this basis. In Philippines, several strains of the *B. oryctes* were found to infect *O. rhinoceros* and the strains showed variability in respect of their pathogenic effect. Some strains were found to infect more easily than did those strains occurring in the South Pacific region which originated from Malaysia. With respect to the duration of survival of *O. rhinoceros* following inoculation with different Philippine strains, variations were reported (FAO, 1978).

Observations on LC_{50} and LT_{50} values indicated that the second instar grub was less susceptible to *B. oryctes* infection as compared to first and third instars. Since the approximate digestibility and efficiency of conversion of ingested and digested food to body matter have been found to be higher in the second instar (Tables 8 to 10), it can be surmised that the second instar has more efficient defence

mechanisms against infections than the other instars. This may probably be due to the nature of immunological system which confers a good deal of reisistance to the pathogen as compared to other instars. The bioassay studies established that, to kill the first instar and third instar grubs, only a low dosage of the virus is required. The data provide a clear indication about the strong dose and stage dependency in the mortality of grubs belonging to successive instars. This also brings about the need for making suitable variations in the field dosage of the baculovirus in relation to the dominant development stage of grubs at given points of time. Though the second instar grubs and adults require a higher dosage of the virus and take a longer time for mortality, they are also susceptible to the disease. Since the duration of development of the second instar grub and the life expectancy of the adult are prolonged and because they develop diarrhoea on getting the viral infection, these stages continue to disseminate the virus through the contaminated faecal matter.

5.6 Patho-physiology

5.6.1. Consumption index

Diseased grubs in general consumed lesser amount of food material than the healthy ones. The rate of feeding was reduced from second day onwards in all instars. The growth parameters such as consumption index (CI), growth rate (GR), approximate digestibility (AD) and gross efficiency of conversion of ingested (ECI) and digested (ECD) food to the body substance were markedly reduced in diseased as compared to healthy grubs. The weight gain was markedly reduced in diseased than in healthy grubs. The weight gained by the first instar grubs during the course of infection was very low as compared to healthy grubs (Table 8). Greater weight gain in healthy grubs is expected in normal situations as a result of the higher food balance. The weight gained by the first instar grubs is almost eight times higher than the control from second to eighth day, while in the second instar, this was only four times higher than control from second to sixteenth day. In

the third instar grub, the increase in weight during the test period were 0.853 and 0.260 g in healthy and diseased respectively (Table 10). The consumption indices of all instars showed greater reduction in the diseased grubs. The consumption index of first, second and third instar diseased grubs decreased considerably, whereas in the healthy grubs, there was a progressive increase in the consumption indices (Table 8 to 10). The reduction in the weight gain and the general adverse trend in the various physiological parameters are naturally expected due to the physiological debilitation and the consequential cessation of feeding and derangement in the normal process of digestion. Similar results have been noted in other viral infections (Ramakrishnan and Chaudhari, 1976; Nair and Jacob, 1980; Narayanan and Gopalakrishnan, 1988).

5.7 Cyto-pathology

The occluded baculovirus producing apparent symptoms of infection can be easily detected by light microscopy because of the presence of polyhedral

bodies. *B. oryctes* which belongs to the non-occluded baculovirus could be detected only through histological examination of the host cells under the electron microscope. Serological analysis was needed to confirm the identity of the virions of *B. oryctes*. Generally, detection of *B. oryctes* is based on Giemsa stained midgut smear preparation (Purrini, 1989). According to Mohan *et al.* (1983), the smear test involving the midgut is the most reliable and consistant method of diagnosing infection by *B. oryctes*. Studies on the midgut contents of the infected grubs showed desquamated epithelial cells with nuclear and cytoplasmic vacuolation, nuclear hypertrophy and the ring stage nucleus (Plate 10 and 11). Mohan *et al.* (1983) and Purrini (1989) also recorded similar changes in the midgut cells and according to them, the cytopathology was characterised by the hypertrophy of nucleus and disintegration of cytoplasm. Mohan *et al.* (1983) observed a deep circular band along the periphery of the nucleus and a central core of deep dark stained granular net work. The nuclear hypertrophy with vacuolation and formation

of ring stage nucleus suggests that the nucleus is the major site of viral multiplication inside the cell.

Healthy nuclei are smaller in size and purple stained. Zelazny (1978) and Gorick (1980) also recorded that the nuclei are smaller in size (7.5 to 12.5 nm) and purple stained.

The virus was first detected in the nuclei of the cells of larval fat body (Huger, 1966 b). It was later observed that the virus multiplies in nuclei of midgut epithelial cells and the gut eventually fills with disintegrating cells and viral particles (Huger, 1973; Payne, 1974). Though the gut cells have been found to be the site of multiplication of the virus, no attempts have so far been made to study the histological changes occurring in the various parts of the gut due to infection. The symptom of diarrhoea developing in the infected grubs and adult beetles is due to the disintegration of the midgut epithelium. This study revealed that the midgut epithelium is the major site of viral multiplication. As the virus

multiply in the midgut epithelium, the faeces of the infected larvae became highly infectious. The wandering of larvae and associated discharge of diarrhoetic faeces serve to disseminate the virus to other larvae and adults through contaminated excreta. The multiplication of the virus in the midgut epithelium and the consequential development of diarrhoea are of considerable advantage in accelerating the natural spread of the baculovirus among the Rhinoceros beetle. Huger (1966 b), Quiot *et al.* (1973), Monsarrat *et al.* (1973 b) and Smith (1976) observed that *B. oryctes* and all other baculoviridae have the nucleus as the principal site of viral multiplication inside the cell.

5.8 Histopathology

The target tissues of *O. rhinoceros* for infection by the *B. oryctes* were the gut and the fat body. The foregut, midgut and hindgut were examined in detail in respect of the histopathological changes taking place as a result of viral infection. The midgut and fat body have been identified as the

common sites of viral multiplication. The changes in the gut tissues in the present studies were mainly confined to the epithelial layer. The midgut was found to be affected more than the foregut and hindgut, and the foregut was the least affected. The virus was found to multiply both inside the nucleus and cytoplasm. The common changes noted in the gut were in the form of cytoplasmic and nuclear vacuolation with hypertrophy of the nucleus giving rise to the ring stage appearance and the desquamation of the epithelial layer.

5.9 Transmission of virus

To understand the natural dynamics of insect disease, information on the mode of dissemination of the pathogens in the host habitat is essential (Forschler and Young, 1993). The ability of *B. oryctes* to survive very well even at low host densities is considered to be an important advantage (Zelazny *et al.*, 1992). As the virus cannot persist outside its living host (Zelazny, 1972), its survival must be the result of various mechanisms of disease transmission

(Zelazny, 1976). The various routes of transmission namely, transtadial, transovum, transovarial and predator-mediated were studied.

5.9.1 Transovum and Transovarial transmission

Transovum and transovarial transmission studies have shown that the virus is not transmitted by any of these methods. These results are in agreement with the observations made by Zelazny (1976). However, transovum transmission is reported by Mercy and Dulmage (1975) in *Heliothis virescence*, Etzel and Falcon (1976) in *Cydia pomonella*, Nordin (1976) and Kunimi (1982) in *Hyphantria cunea* and Philip (1985) in case of *Opisina arenosella*. Transovum and transovarial transmission were also observed in *Mythimna separata* (Neelgund and Mathad, 1978).

5.9.2 Transtadial transmission

Studies on the transtadial transmission have shown that the disease is transmitted from first to second and from second to the third instar, but not from the third instar to the pupae. The disease also

inhibited the moulting and metamorphosis. Inhibition of metamorphosis was reported to be due to the alteration of the juvenile hormone titre in the haemolymph of diseased larvae (Jacob and Subramaniam, 1974; Subramaniam, 1979; Nair and Jacob, 1980; Subramaniam and Ramakrishanan, 1980). When the early first instar grubs were treated with the virus, cent per cent mortality was observed before moulting started. When late first instar grubs were treated, all the larvae moulted and disease was transmitted to second instar (Table 13). When late second instar grubs were treated, disease was transmitted to third instar (Table 14). The late third instar grubs when treated, moulting started from fifth day onwards in the treated and control groups. In the control, 48 per cent moulting was observed on the fifth day, but in diseased it was only 16.67 per cent (Table 15). The moulted larvae pupated normally and no mortality was observed in pupae and adult due to the virus infection.

The transmission from the first to the third instar through the second instar is a definite

advantage for continuous build up of the inoculum in the larval breeding media and the progression of the disease from one instar to the other. But the observation that the virus is not transmissible from the third instar grub to the adults through the pupal instar is a disadvantage in as much as adults emerging from surviving grubs cannot acquire the virus. Zelazny (1976), and Zelazny and Alfiler (1991) also observed that the virus is not transmitted to adult through pupal stage and that the newly emerged beetles are not carriers until they themselves are infected. This difficulty could be surmounted by inducing artificial contamination of the adults with the baculovirus and their release for bringing about transmission to other adults in the breeding sites or in the feeding sites. The lack of transmission of the virus to the adult through the pupae may probably be due to better immunological status in the quiescent stage mediated by unique endocrinological status.

Baculovirus (nuclear polyhedrosis virus and granulosis virus) cause disease in larval stage of

lepidoptera. Infected larvae typically die prior to pupation but transtadial transmission may occur (Young, 1990). Infection of the late instar larvae may result in death of some individuals as pupae, but the percentage of death is small (Sagar, 1960; Stairs, 1965; Melamed-Madjar and Raccah, 1979; Young and Yearian, 1982; Young, 1990). Burand and Park (1992) observed that in gypsy moth, nuclear polyhedrosis virus replication and disease development was disrupted during pupation.

5.9.3 Transmission through predator

Forschler and Young (1993) listed four principal ways by which insect pathogens are transmitted. Of these, the movement of insect pathogens by such non-host carriers as insect predators and parasites has received particular attention. In this study, when the viral suspension was fed to the predatory reduviid bug *Platymerus laevicollis*, it was found that they did not develop any symptoms of the disease nor did they act as a symptomless carrier. This predator did not prefer the infected rhinoceros for feeding

(Table 16). The nuclear polyhedrosis virus could be detected after five days in the excreta of the predator *Oechalia schellembergii* that had fed on virus killed larvae of *Heliothis punctigera* (Cooper, 1981). Eswaramoorthy and Jayaraj (1987) found that the granulosis virus infecting *Chilo infuscatellus* was safer to the coccinellid predator *Chilocorus nigritus* (F.) and *Pharoscymnus horni* Wsl. The parasitoids *Apanteles telengai*, *Aleiodes gasteratus* and *Campoletis annulata* showed preference to healthy larvae of *Agrotis segetum* as compared to granulosis infected ones (Caballero *et al.*, 1991). Narayanan (1980) observed that the nuclear polyhedrosis virus infection in *Heliothis armigera* did not affect the development of the parasitoid *Eucelatoria* sp. nr. *armigera*

5.10 Reproductive potential and transmission of virus during mating

The reproductive potential of *Oryctes* is found to be significantly impaired due to viral infection. The fecundity was reduced when either or both the mating beetles were infected. When both the partners were

healthy, the number of eggs laid was 209 to 219, but when both the partners were infected there was a sharp decline in fecundity to 19 to 20 (Table 17). Zelazny *et al.* (1989) also found that adult females transmitted the virus more frequently to males than *vice versa*. Zelazny and Alfiler (1991) reported that the extent of baculovirus infection among males and females which co-exist in the larval breeding sites was relatively higher than when the beetles occurred singly. According to Zelazny (1973 a and b), oviposition ceased shortly after the females become infected. Several workers have already reported that the impact of the *B. oryctes* infection of *O. rhinoceros* beetle is far greater on the adult populations which are responsible for the active dissemination of the disease (Huger, 1973; Zelazny, 1973 a and b ; 1976; 1977 a).

According to Zelazny (1977 a), the infected adults stop feeding, fly less frequently, males mate less frequently, females stop egg laying and their premature death occurred after four to six weeks. The occurrence of the disease among adult beetles can,

therefore, influence the host population density and also accelerate the rate of transmission of the inoculum to other beetles which co-inhabit the breeding sites. The release of the infected adults will not cause any further crop loss as they stop feeding.

The percentage of hatching of eggs laid by the female counterpart in diseased pairs was found to be the same as that of the eggs laid by the healthy female counterparts in healthy pairs. Eventhough, there is a drastic reduction in fecundity, the viability of the eggs deposited by the healthy and diseased females was almost the same. The development of larvae and pupae from the progeny of diseased adults did not show any deleterious effects due to viral infection.

Histopathological studies under light microscope did not reveal any change in the ovary or testis. The reduction in egg laying could, therefore, be due to physiological derangement from the impairment of the gonadotropic hormone. Monsarrat *et al.* (1973 a) detected virions in the nucleus and cytoplasm of spermatids, in cells and lumen of accessory glands and

in the ejaculatory canal as well as in the chorionated oocytes and follicle cells.

5.11 Cross-infectivity of the virus

The cross-infectivity studies have clearly established that the virus was not infective to other important pests of coconut such as *Rhyncophorus ferrugineus*, *Leucopholis coneophora*, *Opisina arenosella* and the predator *P. laevicollis*. The virus was not infective to cashew stem and root borer *Ploceederus ferrugineus* (Bakthvatsalam and Sundararaju, 1990). The virus was reported to be infective to other members of the family Scarabaeidae such as *Oryctes nasicornis* (Huger, 1966 b), *O. monoceros* (Julia and Mariau, 1976), *Scapanes australis grossepunctatus* (Bedford, 1973), *Strategus aloeus* (Lomer, 1986), *Papuana uninodes*, *P. huoneri* (Zelazny *et al*., 1988), *Heteronychus arator* and *Costelytra zealandica* (Crawford *et al* ., 1985).

Sherlock (1985) recorded that cytoplasmic polyhedrosis virus of *Noctua pronuba* was infective to *Phlogophora meticulosa* but not to *A. segetum*. The nuclear polyhedrosis virus from *Autographa californica*,

Mamestra brassicae and *Trichoplusia ni* were transmissible to *Spodoptera exigua* (Im *et al.*, 1991). The baculovirus of *Ocnogyna baetica* was not cross transmissible to *A. segetum*, *Mythimna loreyi*, *Spodoptera littoralis* and *S. exigua* (Vargas-Osuna *et al.*, 1994).

A high degree of host specificity is an important requirement of the microbial pathogen as they remain tied up to the particular target species without any adverse impact on other species and the likely derangement of the balance of life in nature. In this context, the *B. Oryctes* is a promising candidate for biocontrol of the Rhinoceros beetle.

5.12 Thermal inactivation of virus

The effect of temperature which affects the viability of the virus was studied to determine the stability of the virus. The present study showed that total larval mortality due to infection by *B. oryctes* gradually got reduced to zero level, with 4 1/2 hours of heating at 37°C. (Table 19). There was

no change in the grub mortality rate after 30 minutes of heating of virus and this treatment recorded cent per cent mortality as in the case of unheated virus. The mortality remained without substantial fall even after one hour (97.5 per cent) and also after 1 1/2 hours (92.5 per cent) of heating at 37°C. Above 50 per cent mortality was recorded up to 2 1/2 hours heating. The infectivity declined rapidly after three hours of heating. The mean time taken for the death of the grub was prolonged from 10.800 to 14.363 days. The LT_{50} value ranged from 10.090 with unheated to 15.700 days with virus exposed to heat treatment for 2 1/2 hours. The mean temperature of the dung heap /pit at 25 cm depth varies from 30-40°C in the summer months in South India and the maximum limit cannot be expected at stable levels continuously for long. In the coconut growing tracts of peninsular India, the prevalent summer temperatures cannot be expected to inactivate the virus to such an extent as to adversely affect its survival and dissemination.

Several authors have already reported that constant exposure to high temperature at 35 to 40°C

adversely affected viral stability and viral multiplication (Bird, 1955; Thomson, 1959; Ignoffo,1966; Nair and Jacob, 1976; Pawar and Ramakrishnan,1979). Laboratory studies indicated that normal field temperature of 10 to 30°C did not adversely inhibit viral activity or viral stability in diseased caterpillars (Ignoffo, 1968). The *B. oryctes* is a non-occluded virus. Hence it cannot withstand high temperature. According to Smith (1967), inclusion viruses could persist longer than non-occlusion viruses because of the proteinaceous crystal that protects them from the unfavourable environment. The *B. oryctes* can be stored indefinitely in deep freezers at - 20°C (Bedford,1976; Young,1974). Crawford and Sheehan (1984) observed that only negligible reduction took place in the infectivity of the virus on storage in sterile tissue culture fluid at 4°C for one year. Zelazny (1974) found that the virus stored as macerated cadavers mixed in saw dust at 26°C reduced viral activity to 0.091 per cent of its initial potency at one week of storage and 0.027 per cent at two weeks.

After one month, viral activity could not be detected. Drying or warming accelerated the rate of inactivation of *B. oryctes*. Mohan *et al.* (1985) detected the thermal inactivation point of *B. oryctes* as 54°C. Zelazny (1972) reported total inactivation due to exposure at 70° C for 10 minutes and exposure at 60°C for 10 minutes reduced viral activity to 15 per cent. According to Mohan *et al.* (1985), the ease with which the disease had spread and established in pest population in the South Pacific Islands showed that the rate of transmission outstripped the rate of inactivation. The infective half-life of the baculovirus was approximately five days and total inactivation of the virus occured on the eighth day in virus-cattle dung mixture (Mohan,1991). Philip (1985) observed that the nuclear polyhedrosis virus of *Opisina arenosella* on exposure to 35°C, showed rapid decline in infectivity beyond 84 hours and complete inactivation at 156 hours of exposure. In *Autographa californica*, viral inactivation did not occur at 37°C (Knittel and Fairbrother, 1987). The viral infection

in the aphid *Rhopalosiphum padi*, *Sitobion avenae* and *Diuraphis noxia* was positively correlated with temperature (Laubscher *et al.*,1992).

5.13 Dosage requirement in adult equivalent per unit volume of breeding media

The studies show that the most economical, effective and simple method of disseminating the infection in *Oryctes* population is to release infected adult beetles. Dispersing widely before death, the beetle spreads the disease in the field populations by contaminating the breeding sites harbouring the larval broods and also to other beetles that frequent the area for oviposition.

In studies on the number of adults required per unit area of breeding sites (pits) for effective transmission of the disease to larvae and adults, the load of two to ten adults per pit of size 30 cm^3 caused mortality ranging from 30 to 100 per cent(Table 20). The LT_{50} values ranged from 2.544 to 4.621 weeks for the doses ranging from four to ten adults. In the two

highest doses (8 and 10 adults), complete mortality was obtained with the LT_{50} value of 2.660 and 2.544 weeks respectively. The percentage mortality in 6 adult released pits was also high (90 per cent) with the LT_{50} value of 3.313 weeks. There was a drastic reduction in the mortality in sites where two adults were released (Table 20). The number of adults required per breeding pit of size 30 cm^3 to kill fifty per cent of grubs at different intervals were 4.400, 3.668, 3.016 and 2.889 adults (Table 21).

Huger (1973) showed that the virus multiplied in the midgut cells of adults and was defecated. That the adults are responsible for the spread and transmission of the disease has already been established. Farm yard manure is the most favourable breeding medium and any such materials kept in coconut growing areas in pits or as heaps definitely contain numerous developing grubs (Nirula, 1955). The virus loaded adult population of 4 and 6 per manure pit of size of 30 cm^3 is well within realisable limits and this finding brings into

focus the optimal loads of infected adult populations in coconut gardens to ensure a reasonably high rate of host mortality ranging from 65 to 90 per cent.

5.14 Safety of the virus

5.14.1 Safety to silkworm *Bombyx mori*

Insect virus has great potential for field use because of their specificity and safety to man, demestic animals, beneficial insects, parasites and predators. Ignoffo (1967, 1973) reported that absolute safety of a microbial insecticide is a goal that we should strive for, though it cannot be fully attained. The present study showed that *B. oryctes* is absolutely safe to all stages of the mulberry silkworm *B. mori*. The mean fecundity of the treated and untreated adults were almost similar, it being 485 and 472 in the treated and untreated respectively (Table 22). Such safety evaluation work had not been reported on silkworm *B. mori* earlier. But similar observations were made on *B. mori* with other baculoviruses like nuclear polyhedrosis virus of *H. armigera*

(Narayanan,1980; Bijjur *et al.*, 1991), commercial preparation of the virus (Elcar and Virion/H) (Padhi and Maramorosch, 1983) and nuclear polyhedrosis virus of *O. arenosella* (Philip,1985). The present study showed that the use of virus as a control agent against *O. rhinoceros* will not cause any hazards to sericulture.

5.14.2 Safety to chick embryo

The effect of the virus on developing chick embryo was studied by administering the virus through different routes and no adverse effects were detected. In the chorioallantoic membrane, allantoic fluid, amniotic fluid and yolk changes were not noticed due to the virus infection. Embryonic tissues are highly susceptible to infection due to the low levels of antibodies in the tissues and a large number of actively dividing cells. That the embryos did not show any adverse effects due to virus infections is, therefore, a pointer to the substantial level of safety of the baculovirus. The poultry birds often feed on the Rhinoceros grubs which are found in manure pits in

the homesteads and the present finding that the baculovirus is safer to the chicks is of considerable relevance from the point of safety to poultry and the avian fauna. The saftey aspects of *B. oryctes* to chick embryo are reported for the first time in the present study.

5.14.3 Safety to white rats and white mice

The effect of the virus on higher animals were studied by treating white rat and white mice with higher concentration of the virus. The animals did not show any signs of infection like rise in body temperature, loss of weight, refusal of feed, loss of activity or change in blood profile after treatment with virus as compared to that of untreated animals. The animals were observed for a period of 21 days. Postmortem examination did not show any increase in size of the organ or any focal lesions (Table 23 and 24) Histological studies of the organ showed no evidence of infection. Studies conducted by FAO (1978) in France and by Gourreau *et al.* (1979) established the safety of the virus to human cells,pig

cells,mouse,hamster,fish,calf and other vertebrates. The present studies also revealed that the virus was highly safer to higher animals also.

The *B. oryctes* infecting *O. rhinoceros* has been found to be a promising microbial insecticide for the control of the Rhinoceros beetle. The virus was found to be highly infective to the grubs and adults. Another factor that contribute to the efficiency of the virus as a biocontrol agent is the efficient mode of transmission by the infected adults through virus contaminated excreta which in turn contaminates the breeding medium from which the grubs acquire the pathogen by feeding.

For biocontrol, laboratory reared adult beetles of *O. rhinoceros* could be infected artificially, released for the dissemination of the pathogen among natural populations and thus to bring about enzootics for population reduction. The infected adults do not feed on palms and hence their release for biocontrol will not cause any crop damage.

The virus can be stored for a relatively longer period by freeze drying method in ampoules, to be used for field application whenever required.

Summary

SUMMARY

Studies were carried out to evaluate the efficiency of *Baculovirus oryctes* as a biocontrol agent of the Rhinoceros beetle, *Oryctes rhinoceros* in terms of their patho-physiology, histopathology, mode of transmission and cross-infectivity to other important pests of coconut. The effect of physical factors on the virulence of the virus, safety to the non-target organisms and dosage requirement of the inoculum per unit volume of larval breeding medium were other important areas for the present investigation.

Freeze drying method was used in storing the virus in ampoules and the freeze dried viral particles retained their viability for more than sixteen months.

Among the two methods of inoculation, oral inoculation has been found to be more effective than the swim technique.

The electron micrography showed that the virus is rod shaped measuring 210 to 250 nm in length and 75 to 105 nm in breadth.

For studying the symptomatology of the disease, grubs and adults were exposed to the viral infection. The symptoms noted in grubs were refusal of feed and lethargy. The infected grubs migrated to the top of the medium. The fat body disintegrated and grubs appeared translucent especially in the abdominal region. The infected grubs developed diarrhoea and as a consequence, extroversion of the rectum took place. Death started on seventh day in first instar, twelth day in second instar and ninth day in third instar.

The infected adults do not show much external symptoms, but they refused to take food and developed diarrhoea. Death occurred in twenty to thirty days.

To find out the optimum dose of the pathogen and the more susceptible stage of the pest, bioassay of the viral activity was done using different larval instars and adults of *O. rhinoceros*. All larval instars and adults were found to be susceptible to infection. Among the larval instars the first instar grub was found to be the most susceptible followed by the third and the second in that descending order. In the first instar

grub, the LT_{50} value ranged from 7.862 to 12.723 days for the serial dilutions (10^{-1} to 10^{-6}). In the case of second instar, 80.83 per cent mortality with the LT_{50} value of 14.214 days was obtained in the highest concentration. In the third instar grub the LT_{50} values varied from 10.752 to 13.010 days for the dilutions ranging from 10^{-1} to 10^{-4}.

For the adult, the LT_{50} values were 23.797 and 24.532 days in the highest two dilutions (10^{-1} and 10^{-2}). The LC_{50} values to first, second and third instar grubs and adults were 0.377×10^{-4}, 14.417×10^{-4}, 0.297×10^{-4} and 1.137×10^{-4} gut eqivalents.

Diseased grubs consumed lesser amount of food than healthy ones and the feeding was reduced from second day onwards in all instars. The growth parameters such as consumption index (CI), growth rate (GR), approximate digestibility (AD), gross efficiency of conversion of ingested (ECI) and digested (ECD) food to the body substance were reduced in diseased grubs than in healthy individuals.

Cytopathological study of the midgut contents revealed the presence of clumps of cells with highly disintergated cytoplasm and purple stained hypertrophied nuclei. The infected nuclei contained pink circular bands along the periphery of the nucleus called the ring stage nucleus. In the advanced stage of infection, masses of hypertrophied nuclei with very little cytoplasm were detected.

The histopathological studies of the infected grubs showed that the midgut epithelium was the major site of viral multiplication, followed by the hindgut and foregut. The changes observed were cytoplasmic and nuclear vacuolation, disruption of epithelial lining and desquamation of the cells.

In transmission studies, transovum and transovarial transmission were not observed, but transtadial transmission was found to be the important mode of transmission from the first to second and from second to third instar grubs. However, from the third instar grubs, the virus was not transmitted to pupae.

Viral infection was not observed in the pupae. The reduviid predator *Platymerus laevicollis* did not trasmit the disease among the adult preys.

The reproductive potential of the Rhinoceros beetle was impaired due to viral infection. The fecundity was reduced drastically when both male and female partners were infected (19 to 20 eggs) as compared to healthy (209 to 219). But the hatching of eggs laid by the infected and healthy beetles was not affected.

Cross-transmission studies of the virus showed that the other important pests of coconut such as *Rhyncophorus ferrugineus*, *Leucopholus coneophora*, *Opisina arenoselia* and the predator *Platymerus levicollis* were refractile to the disease.

The viability of the virus was gradually reduced to zero level with 4 1/2 hours of heating at 37°C. Cent per cent mortality was obtained up to 0.5 hours of heating at 37°C and thereafter, the mortality declined to 97.5, 92.5, 72.5, 60.0, 40.0, 27.5 and zero at half an hour interval of heating at 37°C for 4 1/2 hours.

The number of adults required for effective transmission of the virus and a reasonable degree of control (90 per cent of the grubs) in the breeding pits of size 30 cm^3 was found to be six with the LT_{50} value of 3.313 weeks. Below the load of six adults the mortality showed decline.

The effect the virus on silkworm was studied and it was found that the virus did not cause any harmful effect on the growth, development and survival of the different stages of *Bombyx mori*. The effect of virus on developing chick embryo was studied. The virus was administered through four different routes namely, chorio-allantoic membrane, allantoic cavity, amniotic cavity and yolk sac. The result showed that the virus has no effect on the development of the embryo or its growing tissues.

In studies on the effect of virus on higher animals (white rat and white mice), it was found that the animals did not show any symptoms of infection like increase in temperature, loss of weight, decrease in

amount of food intake, loss of activity and changes in blood profile when compared to control. Postmortem examination of these animals revealed no changes in the internal organs.

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* Originals not seen

Abstract

The Baculovirus oryctes has been recognized as one of the most promising pathogens against oryctes rhinoceros. Laboratory studies were conducted to evaluate the virus in respect of its patho-physiology, histopathology, mode of transmission, cross-infectivity, effect of physical factors on the virulence of the virus and safety to non –target organisms.

The oral inoculation technique has been found to be more effective than the swim method. For long term storage of the virus, the freeze drying method was found to be ideal as it retained viability for more than sixteen months.

The viral particles have been found to be bacilliform , measuring 210 to 250nm in length and 75 to 105 nm in width. The main symptoms observed in grubs due to viral infection are loss of appetite, lethargy, pallor,

migration to the top of the breeding medium, disintegration of the fat bodies and development of diarrhoea. Adults did not show any pronounced external symptoms. Infected adults refused to take food and they eventually developed diarrhoea.

All instars of grubs and adults were susceptible to the viral infection. The susceptibility to infection was maximum in the first instar followed by third instar, second instar and the adults in that order. The mean lethal concentration of the virus required to cause fifty per cent mortalities were 0.377 $\times 10^{-4}$, 14.417 $\times 10^{-4}$, 0.297 $\times 10^{-4}$ and 1.137 $\times 10^{-4}$ gut equivalent for the first , second and third instar grubs and adults respectively.

The cytology of the midgut contents showed desquamated epithelial cells with nuclear and cytoplasmic vacuolation, nuclear hypertrophy and ring stage nucleus. The midgut and fat bodies have been identified as the major sites of viral multiplication.

Transovum and transovarial transmission studies have shown that the virus was not transmitted by any

of these methods. The studies on transtadial transmission established that the disease was transmitted from first to second and from second to third instar, but not from the third instar to the pupae. The predator *Platymerus laevicollis* did not transmit the disease.

The reproductive potential of *Oryctes* was found to be significantly impaired due to viral infection. When both the mating partners were infected, the number of eggs laid was only 19 to 20 as compared to the out put of 209 to 219 in the healthy pairs.

The cross-infectivity studies have clearly established that the virus was not infective to other important pests of coconut such as *Rhyncophorus ferrugineus, Leucopholis coneophora, Opisina arenosella* and the predator *P. laevicollis*.

In studies on the effect of temperature on the viability of the virus, it was found that above fifty per cent mortality took place on incubation of the

virus for 2 1/2 hours at 37°C and that the viability was totally lost as a result of exposure for 4 1/2 hours.

The number of adults required for release into the breeding pits of size of 30 cm x 30 cm x 30 cm to kill fifty per cent of the *Rhinoceros* grubs at different time intervals were 4.440, 3.668, 3.016 and 2.889.

Safety studies have revealed that the virus was absolutely safe to the silkworm *Bombyx mori*, chick embryo, white mice and white rat.