STUDIES ON THE GRANULOSIS VIRUS OF *Pericallia ricini* Fabricius (ARCTIIDAE: LEPIDOPTERA)

By

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THESIS

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DECLARATION

I hereby declare that this thesis entitled "Studies on the granulosis virus of Pericallia ricini Fabricius (Arctiidae: Lepidoptera) is a bonafide record of research work done by me during the course of research and that this 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.

BABU M. PHILIP

Vellayani,
August 1978.

CERTIFICATE

Certified that this thesis entitled "Studies on the granulosis virus of Pericallia ricini Fabricius (Arctiidae: Lepidoptera)" is a record of research work done independently by Shri. BABU M. PHILIP, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

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INTRODUCTION

INTRODUCTION

The interest in microbial pesticides has expanded because of problems, such as insect resistance to insecticides, emergence of secondary pests, toxic residues and other deleterious consequences of the use of broad-spectrum chemical insecticides. The organisms that cause diseases in insects are viruses, bacteria, fungi, protozoa, rickettsiae and nematodes. Of these viruses are the most exciting and promising group of pathogenic microorganisms under consideration for use in biological insect pest suppression (Anonymous, 1973).

The field tests with viruses have been conducted mainly with the nuclear polyhedrosis viruses, to a lesser extent with the granulosis viruses and to a limited extent with the cytoplasmic polyhedrosis viruses and the non-inclusion viruses. There are several cases of successful use of insect viruses, especially the nuclear polyhedrosis and granulosis in the control of insect pests (Balch and Bird, 1944; Thompson and Steinhaus, 1950; Bird, 1953; Hall, 1957; Kelsey, 1957; McEwen and Harvey, 1958; Abul-Nasr, 1959; Ossowski, 1959; Tanada and Reiner, 1962; Ignoffo et al., 1965). Commercial formulations containing nuclear polyhedrosis virus of Heliothis are now being used on a

large scale in U.S.A. Several other products containing different viruses are also available now for field trials. A desirable attribute of insect viruses is that they pinpoint the insect pests and leave the beneficial insects unharmed. Further more, in many cases these viruses are passed transoverially to the offspring and so it is possible to destroy generations of insects yet unborn, a thing no chemical insecticides can do.

Granuloses represent a distinct group of insect virus diseases which, like the polyhedroses, are characterized by a special type of virus inclusion bodies which under the light microscope, appear as minute "granules" with dimensions of approximately 300 to 500 mμ. It was for this reason that diseases of this group were called "granuloses" by Steinhaus (1949).

In India studies on insect viruses in general and granulosis in particular are rather limited. During the last decade a few granuloses have been reported upon, but none of them have been intensively studied. These include those from Cnaphalocrocis medinalis (Jacob et al., 1971) Pericallia ricini (Jacob et al., 1972) and Diacrisia obliqua and Spodoptera litura (Rattu et al., 1971).

The black headed hairy caterpillar, Pericallia ricini Fab., is a polyphagous pest feeding on a variety of crop

plants like castor, cotton, banana, cucurbits, sunflower, field beans and gingelly. Though the occurrence of a granulosis in this insect was recorded by Jacob et al. (1972) no detailed studies have so far been made on this and the much needed basic information for judging the suitability of this pathogen for field application is lacking. The present investigations were hence taken up with the objective of gathering detailed information on the following aspects:

1. Symptomatology
2. Infectivity of the pathogen to different larval instars
3. Effect of treating the egg masses of P. ricini with the granulosis virus
4. Histopathology
5. Effect of virus infection on the length and weight of larvae
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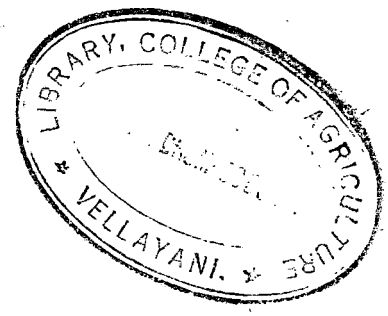
REVIEW OF LITERATURE



REVIEW OF LITERATURE

Early history

The first record of this type of insect virus disease was made by Paillot (1926), who described what was probably a granulosis in the caterpillars of the large white butterfly, Pieris brassicae and he called it as "pseudograsserie". Paillot in 1934 discovered a similar disease in the larvae of the cutworm Agrotis (Euxoa) segetum. Later he (Paillot, 1935, 1936, 1937) described two more diseases in the same host and because he thought he could discern differences, he gave them the names pseudograsserie 1, 2 and 3.

The disease was rediscovered by Steinhaus (1947) in the variegated cutworm, Peridroma margaritosa Haworth. In 1948 Bergold described a similar disease in the pine shoot roller, Choristoneura murinana Hübner, and demonstrated the virus nature of the causative agent with the electron microscope.

Systematic position of granulosis virus

The characterisation and nomenclature of insect viruses are all matters of active research and debate. The insect viruses were earlier classified on the basis

of the presence or absence of inclusion bodies, their shape and characteristics of virions such as shape, outline and symmetry, the type of the nucleic acid, site of viral replication as well as symptomatology of the disease. Based on this granulosis viruses were grouped under two genera viz., Steinhausiavirus (viral replication in nucleus) and Bergoldiavirus (viral replication in cytoplasm and nucleus) (Ignoffo, 1968). At the present time, a system being worked out by the International Committee for the Nomenclature of Viruses seems to be favoured by all. This system is based mainly on the properties of the virus particle. According to this, the granulosis and nuclear polyhedrosis viruses are grouped under the genus Baculovirus (David, 1975).

Distribution and host range

Ignoffo (1968) estimated that granulosis viruses represented about 14 per cent of all arthropod viruses. So far the granuloses virus(es) have been reported only in larvae and occasionally in pupae of lepidoptera (Ignoffo, 1968; David, 1975).

Symptomatology

The external symptoms of granulosis in lepidopterous larvae are somewhat variable. The common symptoms exhibited by lepidopterous larvae infected with granulosis virus as reviewed by Huger (1963) and Smith (1967) may be summarised

as follows: The first indication of infection often is the loss of appetite; later on the larvae ceases feeding. Colour may change from its usual shade to pale white or milky yellow due to the development of vast number of capsules in the affected tissues, the integument often being mottled. The change in colour usually is accompanied by progressive weakening, sluggishness, and flaccidity of the larvae. In addition, they become increasingly less responsive to stimuli. If the disease has progressed far enough, the blood generally is turbid and milky owing to the presence of large number of capsules derived from disintegrated infected tissues. The cuticle becomes fragile and breaks if it is affected liberating the liquefied body contents containing millions of capsules. In the late stages of the disease, larvae show a tendency to seek the highest point available and remain suspended from there by their caudal or abdominal prolegs sometimes in the form of an inverted 'V'. The incubation period of the disease may vary from 3 to 25 days.

Falcon et al. (1967) reported that larvae of corn earworm, Heliothis zea did not exhibit readily distinguishable signs and symptoms during the period of lethal infection. The only noticeable effect was a gradual reduction in body size prior to death. After death the

larvae shrivelled and dried up. Upon dissection a marked reduction in the amount of fat body was evident. Kashkarova and Akramova (1975) observed that in the granulosis of the cutworm caterpillars, Agrotis segetum Schiff., emergence of adults from surviving pupae was delayed by two days and about 3.4 per cent of the adults were deformed. Further, the fertility of female cutworms was decreased by 250 per cent. Jacob et al. (1975) found that the larvae of the rice leaf roller Cnaphalocrocis medinalis first turned pale and then milky white, the body became distended and the cuticle easily ruptured, releasing a milky white fluid.

Shape, size and physicochemical properties of capsules

The shape of the capsules has been described variously as oval, ellipsoidal, ovoid or egg shaped (Smith, 1967). Lower (1954) in a study of the granulosis affecting Peracctonia swingii Wester, found the capsules to be "subovoid" elongate bodies with more or less parallel sides. Besides normal capsules some aberrant long virus inclusions and also bizarre, angled, and branched elements have also been observed in the infected tissues (Bergold, 1948; Steinhaus et al., 1949; Hughes and Thompson, 1951; Tanada, 1953, 1959). The mean size of capsules as given by Huger (1963) ranges between 300 and 511 mμ in length

and 119 and 350 mμ in width.

Oho et al. (1974) reported that the virus capsules of Adoxophyes orana Fischervan Roslerstam were ellipsoidal and averaged 361 ± 3 mμ in length and 206 ± 3 mμ in width.

Electron microscopic studies by Asayama and Inagaki (1975) proved that in Plutella xylostella the inclusion bodies were polymorphic in the malpighian tubules while those in the fat bodies were oval.

The substructure of the granulosis virus of Estigmene acraea (Darray) was analysed by Wilt et al. (1976). Ovoid capsules of various sizes and morphologies were observed. High magnification revealed the presence of mid lateral depression surrounding a protruberance.

Smith (1967) described the physicochemical properties of the capsule. The capsules are completely insoluble in water or alcohol and resistant to enzymic action during the decomposition of the host larvae. They are readily soluble in weak alkalies or strong acids.

Chemical composition of capsules

Wellington (1951, 1954) studied the amino acid composition of the virus and the inclusion body protein from a granulosis disease of Choristoneura murinana (Hb.) and found the following amino acids in the acid hydrolysates

of both virus and capsular protein:- cysteic acid, aspartic acid, glutamic acid, serine, theonine, alanine, tyrosine, methionine, histidine, lysine, arginine, proline, valine, leucine, isoleucine, phenylalanine and glycine.

Bergold (1963) reported that the capsules of Chorlstoneura murinana had a density of 1.279 and when dissolved in weak alkali separated into two components, the main molecules having a sedimentation constant, S_{20} of 11.8 and a molecular weight of 300,000, the same values for the split component being, S_{20} of 3.45 and 60,000 respectively.

Nature of virions

The naked virus particles of Bergoldia virulenta measured 42 x 268 mμ (Bergold, 1953). In granulosis virus preparations, long filaments or virus rods arranged in chain like fashion had been observed by several authors (Steinhaus, et al., 1949; Steinhaus and Marsh, 1960; Schmidt and Phillips, 1958; Smith et al., 1964).

As described by Smith (1967), the capsules contain a short rod, rather thick and slightly curved which is surrounded by an intimate and outer membranes. Huger (1963) summarised the average measurements for granulosis viruses as a whole as having a width ranging from 36 to 80 mμ and a length from 245 to 411 mμ.

Sidor and Krstić (1969) observed that the virus rods of the granulosis of Pygaera anastomosis (L.) were situated in the middle of the capsule and were slightly curved at either end. It consisted of an outer and inner membranes and an inner helical structure.

Arnott and Smith (1969) studied the long branched rods associated with granulosis virus infections in several genera (Plodia, Pieris and Melonchyo). When present, the rods were smaller in diameter than the virus rods and possessed in common with the capsules an epicapsular layer. The branched rods were found attached to capsule-like bodies also possessing an epicapsular layer at the point of junction; this layer was continuous from the rod to the capsule-like bodies.

Studies on the infectious elements of a granulosis virus of Cydia pomonella (L.) by Barefield and Stairs (1970) showed an infectious form smaller than the rod shaped virus particles. This component caused typical granulosis when fed to first instar larvae. Thus it contained the genetic information necessary to cause the development of rod shaped virus particles and mature capsules.

The fine structure of granulosis virus particles isolated from Dendrolaimus angerans (Btlr.) and Agrotis segetum (Schiff.) were studied by Shvedchikova and Tarasevich (1971) and found that alkaline treatment of a suspension of the virus particles destroyed the particles except for their membranes, which were of two types namely intimate and developmental.

Shternshis et al. (1975) separated the granules into five fractions. It was found that the fractions of the capsules of Agrotis segetum with the mean floating density had the greatest virulence and ensured maximum production of virus inclusion for caterpillar infection. The fifth fraction of the granules with the greatest floating density was notable for low content of histidine, glycine, and alanine and possessed the lowest biological activity.

Histopathology

Hughes and Thompson (1951) reported that the diseased fat cells of the omnivorous looper, Sabulodes caberata Guenee appeared more opaque than normal cells and had a brownish colouration when seen in a fresh unstained preparation. The first noticeable evidence of the disease was an enlargement of the nuclei of the infected fat cells. At the same time the entire cell increased in size. In the final stage of the process, it was no longer possible to distinguish between nucleus and cytoplasm.

Martignoni (1957) and Wittig (1959) reported that the first visible reaction after infection might be mitotic proliferation of the fat body cells, described by Paillot (1934, 1935, 1936) as "proliferation cellulaire".

According to Hughes and Thompson (1951), Wittig and Franz (1957), Wittig (1959), Bird (1959) and Huger (1960) the characteristic feature observed was the development of an intensively stainable net work in the highly hyper-trophied nuclear area as well as in the cytoplasm. Huger (1960) suggested that the network might serve as a virogenic stroma. Hamm and Paschke (1963) observed that in the granulosis of Trichoplusia ni the nuclear changes took place early in the infection, followed by the development of a Feulgen-positive net work in the cell, assumed to represent a "virogenetic stroma" as described by Huger and Kreig (1961).

According to Tanada and Leutenegger (1963), the granulosis of the codling moth, Carpocapsa pomonella, was a polyorganotropic disease and produced pathologies in the fat body, hypodermis, tracheal matrix and malpighian tubules. The occurrence of infection in the malpighian tubules suggested that the virus might be excreted through this organ. Laboratory tests indicated this possibility, but faecal contamination was not conclusively established.

Hamm in 1963 studied the histopathology of the granulosis of the fall army worm, Spodoptera frugiperda and found that the virus attacked only the fat body and caused a proliferation of cells.

Meynadier et al. (1969) observed that the virus developed in the nuclear region of the fat body, hypodermal and tracheal cells of the larvae of Diataraxia oleraceae (L.).

Histopathological and histochemical investigations of the granulosis of Carpocapsa pomonella by Wager and Bonz (1971) defined and characterized ten pathogenic stages, based on observations on infected fat body. Cytopathological changes began in stage 2 at 12 to 24 hours after infection, mature capsules were present in large numbers in stage 8, 66 to 72 hours later, and complete disintegration of the cells (stage 10) occurred in 120 to 168 hours after infection.

Hunter et al. (1973) found that in the granulosis of Plodia interpunctella (Hb.) the infection was confined to the cells of epidermis, fat body, tracheal matrix, and to a lesser degree in muscle sheath.

Asayama et al. (1975) studied the ultra-structural changes of cell organelles in diamond black moth Plutella xylostella larvae infected with granulosis virus. The endoplasmic reticulam showed multilayered and whorl shaped figures. Mitochondria changed into balloon shaped structures

with fragmented cristae. These abnormal structures disappeared into cytoplasmic matrix at the advanced stage of the infection. Agglomerated glycogen granules and large clumps of lipids were seen in the infected cytoplasm.

Asayama (1975) observed the development of tubular structures in the fat body cells of P. xylostella infected with granulosis virus. The tubular structures appeared at the same time the inclusion body protein appeared and it was suggested that these structures might result from protein synthesis which took place with virus infection.

Light microscopic studies by Hunter et al. (1975) showed infection in the hypodermis, fat and malpighian tubules of potato tuber moth, Phthorimaea operculella larvae. Unlike many granulosis viruses this did not infect the tracheae.

Larval age and susceptibility to granulosis infection

In general, young larvae were found to be more susceptible to granulosis than old ones (Lower, 1954; Tanada, 1953, 1955, 1956, 1959; Martignoni, 1957; Schmidt and Philips, 1958; Schmidt, 1959; Wittig, 1959; Sager, 1960).

Tanada (1956) reported that in peroral infection experiments with Pseudaletia unipuncta, the average mortality

of the highly susceptible first and second instar larvae was 80.8 and 92.7 per cent respectively compared to 23.4 per cent in the considerably resistant sixth larval instar. But Smith et al. (1956) reported that in Harrisina brillians third instar larvae were more susceptible to the granulosis than the first instar.

Sheppard et al. (1977) assayed the susceptibility of the first and fifth instar larvae of the codling moth, Laspeyresia pomonella to its granulosis virus and found that it was pathogenic for both larval instars. The LD_{50} values for first and fifth instar larvae were 5 and 49 capsules per larvae respectively. Fifth instar larvae were more variable in their response to virus than first instar larvae. Using probit method it was calculated that one capsule could cause death in 25 per cent of both larval instars but 1573 capsules were required to cause 100 per cent mortality of fifth instar larvae as compared to 12 capsules for first instar larvae.

Cross infectivity

The specificity of insect viruses has been extensively reviewed by Ignoffo (1973) and he has concluded that granulosis viruses are the most specific of all insect viruses. However a few instances of cross transmission of granulosis viruses have been reported.

Granulosis virus of Pieris brassicae was successfully transmitted to P. rapae and P. napi (Kelsey, 1958; Smith, 1959, 1960). Studies by Fukuhara et al. (1969) showed that the granulosis of Hyphantria cunea was not infective for larvae of Bombyx mori (L.).

Laboratory studies by Lipa and Ziemnicka (1972) showed that first, second and third instar larvae of Agrotis exclamationis (L.), A. segetum, Heliothis armigera, H. zea and Discestra trifolii were susceptible to infection with a granulosis virus isolated from larvae of A. segetum. Larvae of Noctua fimbriata and N. pronuba were resistant to infection, but died as a result of cytoplasmic polyhedrosis that had apparently been activated by the presence of the granulosis virus. Hunter and Hoffmann (1972) showed that Plodia interpunctella to be moderately susceptible to a similar virus isolated from Ephestia cautella. Capsules in cross infected larvae of P. interpunctella were generally abnormal in form, some containing upto 13 virions.

Hereditary transmission

Smirnoff (1960) found that once the virus of sawfly, Neodiprion swainei had been introduced, either by spraying virus or disseminating infected cocoons, the infection was maintained in a sawfly population from one generation to another by the transmission of the virus through the eggs.

Schmid (1974) demonstrated transovarial transmission of the granulosis virus of larch bud moth Zeiraphera diniana. Further incidence of the disease increased in groups under nutritional stress.

Studies by Etzel and Falcon (1976) did not get any conclusive evidence for the transovum transmission of the granulosis virus of Carpocapsa pomonella. However circumstantial evidence supported the hypothesis of transovum transmission.

Tests conducted by David and Taylor (1976) with a virus free stock of Pieris brassicae and a particular strain of granulosis virus failed to demonstrate transmission of the virus transovarially. There was no evidence that the virus could enter the micropyle of the egg as an external passenger on the spermatozoa.

Effect of environmental factors on the virus and disease development

Kelsey (1958) achieved 100 per cent mortality of Pieris rapae in laboratory infection experiments with capsules of P. brassicae kept at ordinary room temperature.

Vago et al. (1961) reported no loss of infectivity for capsules of the granulosis of P. brassicae when pressed with inert substances into tablets of 200 mg even after storage for 5 years.

The effect of heat, cold and prolonged storage on the virulence of a granulosis virus of Pieris brassicae was investigated by David and Gardiner (1967 b). The result showed that the purified virus was inactivated by 10 minutes at 70°C, 60 minutes at 65°C or 24 hours at 60°C. It was not entirely inactivated by 5 days at 50°C or by 20 days at 40°C although even 10 days at the latter temperature significantly decreased its activity. The virus also withstood storage for 6 months at 20°C and appeared not to be significantly affected by it. During prolonged storage (upto 4 years) the crude virus, in dry film was much less stable when kept in light than in darkness. It was concluded that at naturally occurring temperatures the virus was relatively stable and, considering temperature alone, it could persist from one season to the next.

Hunter and Hartshall (1971) reported that the respiratory quotient of both infected and control larvae of Plodia interpunctella (Hb.) varied with temperature, the highest value being obtained with larvae kept at 32°C and the lowest with larvae at 22°C. Larvae exposed to the virus respired at a lower rate than uninfected individuals.

The stability of a granulosis virus of Pieris brassicae (L.) when in the form of dry deposits of the intact capsules were investigated by David et al. (1971 a). It was

found that in the dry form, the virus lost a significant amount of activity in two days at 20°C and that the rate of loss of activity increased with temperature over a range extending from below 0 to 40°C. Varying the relative humidity of the air to which the virus was exposed from near 1 to 87 per cent, however, had no effect on activity. When both dry deposits and an aqueous suspension of the virus were kept for 7 days at temperature ranging upto 30°C, the resulting activity was lower in the virus stored in the dry form than in those stored in suspension. In further tests with dry deposits of the virus on cabbage leaves, it was found that viral activity diminished after four days exposure of the leaves in the laboratory.

Further studies by David et al. (1971 b) showed that on dry films of intact capsules of a granulosis virus of P. brassicae, the haemolymph solids were found to exert a significant stabilising action, while gelatin was significantly less effective and glucose ineffective. The protective action of haemolymph occurred with film deposited on the lower surface of cabbage leaves as well as those on glass and was considered probably to have been due to the protein present. This explanation could also account for the high level of stability of insect viruses in dried film of crude preparations kept in darkness.

Various experimental factors affecting the infection of Apamea anceps (Schiff.) and Agrotis segetum (Schiff.) with granulosis virus were studied by Shekhurina (1977). The result showed that most important factors were temperature, population density and hunger. In the case of A. segetum temperature and humidity were both important.

Persistence in the field

David and Gardiner (1967 a) found that the granulosis virus of Pieris brassicae (L.) was very stable in garden soil and sand and showed little deterioration after two years. The virus could not be readily washed out of the soil or sand. Even when water equivalent to 48 inch rain was passed through the samples, much of the virus remained in the top layer, though some was carried away in the percolating water.

David et al. (1968) observed that when highly purified preparation of the granulosis virus of Pieris brassicae (L.) was exposed to direct sunlight on the upper surface of cabbage leaves the virus was rapidly inactivated even with an exposure of 3 hours. Total inactivation of the virus occurred between 12 and 19 hours.

Harcourt and Cass (1969) found that the granulosis virus of Pieris rapae (L.) could persist in the environment

from one season to the next. They found that soil collected from the most highly infested localities and stored in the dark at 4°C was still highly infective some 26 months later.

Gitay and Polson (1971) reported that intact inclusion bodies of a granulosis virus apparently identical with the virus of Heliothis armigera were found in the droppings of the cattle erget, Arboela ibis which feed on the larvae, and it was suggested that the stability of the virus in the digestive tract of the erget would favour dissemination of the pathogen.

Crawford and Kalmakoff (1977) reported that the virus affecting larvae of Wiseana sp. would maintain itself from one year to the next at a level ensuring high larval mortality provided the stock movement spreads the virus over the soil surface.

MATERIALS AND METHODS

MATERIALS AND METHODS

Mass rearing of caterpillars of Pericallia ricini.

Pneumatic glass troughs (12" x 5"), glass battery jars (8" x 4"), specimen tubes (3" x 1", 6" x 1"), plastic containers (150 ml) and hurricane chimneys were used for rearing the larvae. The larger glassware and chimneys were sterilized by keeping them in 0.5 per cent sodium hypochlorite solution for one day (Wittig, 1963). They were then washed in running tap water and air dried. Smaller glass jars and tubes were sterilized in a hot air oven at 180°C for 3 hours.

The original culture was started from a single egg mass collected from the field. The eggs (one day old) were surface sterilized by immersing them in 10 per cent formalin for 90 minutes (Thompson and Steinhaus, 1950). They were then washed several times in distilled water and the moisture removed by air drying. The sterilized eggs were kept in clean sterilized petri dishes for hatching.

On the day previous to hatching, when the eggs attained a bluish tinge they were transferred to fresh castor leaves (Ricinus communis L.) kept in glass battery jars. The leaves were kept turgid by keeping the tip of petiole dipped in water contained in specimen tubes. The

jars were covered with muslin cloth. The leaves generally remained turgid for 2 to 3 days in this way. Larvae were transferred to fresh leaves after 3 days and reared like this until the fifth day. Thereafter they were transferred to glass troughs in batches of 20 to 25 per trough. A one inch layer of clean and sterilized (autoclaved) sand was provided at the bottom of the trough. Fresh castor leaves were provided every day. The troughs were covered with muslin cloth. Larvae showing signs of bacterial or other infections were removed immediately. The larvae pupated on the leaves, sides of trough and on the cloth covering.

The adults on emergence were enclosed in batches of 3 or 4 pairs in battery jars or chimneys containing one or two castor leaves. Cotton swabs dipped in 10 per cent honey solution were pasted on the sides of chimneys to serve as food for the adults. Egg laying started within few hours of emergence and continued for 3 to 4 days. Fresh leaves were given every day to serve as substrates for egg laying. Bits of castor leaves containing the egg masses were cut out and sterilized as described earlier.

Preparation and storage of primary inoculam

The primary inoculam was obtained from an infected laboratory culture of larvae of P. ricini in the Division of Entomology, College of Agriculture, Vellayani in 1976.

It was multiplied by feeding contaminated castor leaves to early instar larvae of P. ricini. The capsules were collected as described by Smith (1967). The diseased cadavers were stored in distilled water in large conical flasks and allowed to decay at room temperature for several weeks. The granules which settled as a thin white layer at the bottom were collected and purified by differential centrifugation. The granules were also extracted by maceration of dead larvae in a warring blender and further purification by centrifugation. The purified granules were then suspended in distilled water and stored in refrigerator at 4°C.

Determination of the concentration of capsules

In the absence of an electron microscope, no attempt was made to count the number of granules in the suspension. But at the start of the work itself 3 litres of a suspension containing granules derived from 300 dead larvae were prepared and stored under refrigeration at 4°C. This was used throughout.

Selection of test larvae

In all studies of the granulosis of P. ricini third instar larvae within 6 to 8 hours after their second ecdysis were used. The symptomatology and incubation period were studied in all instars. Normal larvae had six instars and the larval period lasted for 21 to 24 days.

Care was taken to select larvae of approximately the same age and size for each treatment. This was facilitated by keeping correct records of the dates of hatching of each lot.

Inoculation of caterpillars with the virus

The spot feeding technique devised by Jacob (1972) was adopted for all the inoculations. The lamina of a middle aged castor leaf was fixed on a thick card board with pins with the underside of the leaf facing up. Pieces of paper gum tape, one inch square with a circular hole of 6 mm diameter punched in the middle, were pasted over the exposed surface along the periphery of the leaf. Five microlitres of the capsule suspension containing 0.1 per cent teepol as wetting agent was put into each of the circular exposed leaf disc with a micropipette and the suspension was allowed to dry at room temperature. One larva was confined to each inoculated spot by inverting a penicillin vial over the larva. The petiole of the leaf was kept dipped in water contained in a specimen tube. All instars of larvae completed feeding of the treated area in about 4 hours. Only those larvae which had consumed the treated leaf area completely within 4 hours were taken for the tests and the others were discarded. Control larvae were fed similarly except that five microlitres of 0.1 per cent teepol only was used

instead of the virus inoculam. This method was used in all tests except in studies on the incubation period of the sixth instar larvae. As these larvae were too large to be accommodated in the penicillin vials the following method was used. Leaf disc of 6 mm diameter were cut out and each bit was placed over a wet filter paper kept at the bottom of a plastic jar. The leaf discs were then inoculated with the virus suspension and one larva each was released over it.

The larvae which had completely ingested the inoculam were transferred to individual plastic containers and supplied with virus free foliage every day.

Diagnosis of dead larvae

The dead larvae were diagnosed by microscopic examination of squashed preparation of tissues for the presence of the capsules.

Susceptibility of different larval instars to the virus

The susceptibility of first, second, third, fourth, fifth and sixth instar larvae were studied. Twenty five larvae of each instar were fed 5 µl of the virus suspension per larva. Another set of 25 larvae of each instar treated similarly but without virus inoculam served as control.

The experiment was conducted at room temperature

which varied between 25.5°C and 30.5°C. The relative humidity during the period ranged from 85 to 90 per cent. The disease-mortality data obtained were subjected to probit analysis (Finney, 1952).

Effect of treating the egg mass of *P. ricini* with granulosis virus

One day old egg masses were sterilized as described earlier and were divided into 4 batches. Two batches were painted with the virus suspension using a camel's hair brush. The eggs were then air dried and kept in petridishes for hatching. The other two batches treated similarly with 0.1 per cent teepol only to serve as control. Fifty larvae were collected from each batch and reared individually in specimen tubes as described already. Observations were recorded on larval mortality and pupation if any.

Histopathology

Third instar larvae were inoculated with 5 microlitres of capsule suspension per larva as described earlier. At 24 hour intervals upto 192 hours of the treatment, 3 larvae each from the inoculated and control groups were selected at random and used for histopathological preparations.

The larvae were killed in hot alcoholic Bouin's solution and allowed to soak for approximately 10 minutes.

after which the smaller specimens were cut into two and larger ones into three and transferred to cold alcoholic Bouin's fixative for 24 hours (Drake and McEwen, 1959). The fixed specimens were soaked in several changes of 70 per cent ethanol until all the yellow colour disappeared, dehydrated in an ethyl-alcohol-butyl alcohol series and embedded in paraffin according to standard procedure. Longitudinal sections were cut at 6 microns. The sections were stained by an azan staining technique developed by Hamm (1966).

Effect of granulosis on the length and weight of larvae

(a) Weight of the larvae.

These studies were made on third instar larvae of P. ricini. Twenty third instar larvae were inoculated with 5 microlitres each of the virus suspension as outlined earlier. An equal number of larvae were allowed to feed on leaf spots treated with 0.1 per cent teepol only and kept as control. After the leaf spot was eaten completely, each larva was weighed precisely. Both the inoculated and control larvae were released individually in hurricane chimneys and provided with fresh uncontaminated castor leaves. Observations were made on larval weight after every 24 hours.

(b) Length of the larvae.

Twenty 3rd instar larvae were inoculated with the

virus suspension as outlined earlier. Another set of 20 larvae fed with 0.1 per cent teepol only served as control. Control and treated larvae were kept in individual labelled containers and the length of each determined daily. The first measurement was made immediately after the feeding was over. A plastic metric scale was used for the measurement. The larva to be measured was slightly pressed to the scale and the reading taken. The average length of larva in cm was calculated for each interval in both treated and control group. Students' 't' test was done for comparing the difference between means.

Food consumption and growth

These were assessed by working out the consumption index, growth rate, and efficiency of convertion index. The data were collected as described below.

Third instar larvae which had just moulted to this stage were used. Ten larvae were inoculated with 5 microlitres of the virus suspension as described earlier. Another set of 10 larvae fed on leaf spots treated with 5 microlitre of 0.1 per cent teepol served as control.

After the leaf spot was eaten completely, each larva was weighed precisely. Both the inoculated and control larvae were released individually on pre-weighed castor

leaves. Care was taken to select leaves of uniform age and quality. One day after the release, the weight of each larva and that of the leaf left out in each was ascertained. This was repeated until the larvae died or pupated.

To find out the actual loss in weight of the leaves due to normal transpiration, each day a set of 5 leaves were kept in individual chimneys as in the experiment. These leaves were weighed at the start and also after 24 hours. The difference in weight represents the loss due to transpiration. The mean percentage loss due to transpiration was taken for calculating the loss in weight due to transpiration in the treated and control group.

The difference between the weight of the food provided and weight of the food left over was determined. The weight of the food actually ingested by the larva in 24 hours was calculated after deducting the amount of water loss due to transpiration.

The various indices of consumption and utilization were calculated on wet weight basis after Hopkins (1912) and Waldbauer (1964, 1968).

The indices were calculated as described below and students' 't' test was used for comparing the difference between means.

1. Consumption Index

Consumption Index (C.I.) = $\frac{F}{T \times A}$, where

F = Fresh weight of food eaten

T = Duration of feeding period (days)

A = Mean fresh weight of animal during feeding period

In the present studies mean body weight was calculated by summing the body weights determined every 24 hours and dividing by the number of weighings (Soo Hoo and Fraenkel, 1966). Using fresh weight, different consumption indices were calculated.

2. Growth rate or the relative growth rate (G.R.)

Grow rate = $\frac{G}{T \times A}$ where

G = Fresh weight of gain of the animal during feeding

T = Duration of feeding period (days)

A = Mean fresh weight of animal during the feeding period

3. Conversion of ingested food

The gross efficiency or the efficiency of conversion of ingested food to body substances (E.C.I.) was calculated as:

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

or

$$E.C.I. = \frac{G.R}{C.I}$$

Thermal inactivation point of the virus

Five ml of the purified virus suspension was taken in thin walled pyrex glass tube and heated in a water bath maintained at the required temperature for 10 minutes. The heating time of 10 minutes was maintained when the suspension reached the desired temperature. The tubes were cooled immediately. The virus suspension was subjected to heat treatments in the range of 50°C to 100°C.

The heated virus suspension was fed to third instar larvae as described earlier. Ten larvae were used in each treatment. Two sets of larvae (10 each) were kept as control, one set fed with untreated leaves and the other fed with virus not subjected to heat treatment as a check for viral infectivity. Observations on larval mortality, incubation period and pupation were recorded. The room temperature ranged from 25°C to 30°C and the relative humidity from 83 to 92 per cent during this period.

Cross-infectivity

Cross infectivity of the granulosis virus of Pericallia ricini to the following species of lepidoptera were studied.

1. Diacrisia obliqua Walker (Arctiidae)
2. Utetheisa pulchella Linn. (Arctiidae)

3. Spodoptera litura F.(Noctuidae)
4. Euproctis fraterna Moore (Lymantridae)
5. Sylepta derogata F. (Pyraustidae)
6. Spodoptera mauritia Boisduval (Noctuidae)
7. Plusia peponis F. (Noctuidae)

Larvae of S. litura were obtained from a pure laboratory culture and were fed with castor leaves contaminated with the virus inoculam. Larvae of E. fraterna, P. obliqua, S. derogata, S. mauritia, P. peponis and V. pulchella were collected from the field and fed with leaves of castor, cowpea, bhindi, paddy, snakegourd, and sunnhemp respectively which had been contaminated with the virus suspension. After feeding on the treated leaves for one day, the larvae were transferred to fresh untreated leaves and reared until pupation or death.

Effect of weathering on the virulence of granulosis virus of P. ricini

Castor plants were grown in pots and kept exposed to direct sunlight. Convenient numbers of 6 mm diameter discs were marked on leaves. Care was taken to select leaves which were not shaded. Five microlitres of the virus suspension containing 0.1 per cent teepol was placed over each of the marked areas on the leaves. A similar number of discs were treated with five microlitres of 0.1 per cent

teepol only to serve as control. Leaves were plucked out at 0, 12, 24, 48, 72, 96, 120 and 144 hours after inoculation. At each interval the viral activity was assayed by allowing 20 third instar larvae to feed the treated leaf spots as described earlier. At each interval a set of 20 third instar larvae fed on leaf spots and treated with 0.1 per cent teepol were kept to serve as control. Assay of viral activity was conducted at room temperature which ranged from 25 - 29.9°C and the relative humidity of 87 to 90 per cent. Observations were made on larval mortality, incubation period, pupation and adult emergence.

RESULTS

RESULTS

Symptomatology

The external symptoms of granulosis infection in the larvae of Pericallia ricini became evident 3 to 4 days after ingestion of the virus. The infected first, second and third instar larvae assumed a paler colouration compared to the healthy larvae. The later instars did not exhibit any colour change in the early stages of viral infection.

The larvae became lethargic and showed loss of appetite 4 to 5 days after ingestion of the virus. They were less responsive to tactile stimuli. The diseased larvae sometimes discharged a dark brown fluid through the mouth. Many of them were found to wander, trailing behind them a brownish discharge which, on drying, sometimes fastened the larva to the foliage or to other substrates. The larvae exhibited an abnormal feeding behaviour which consisted of "spotty feeding" (Fig. 1a, b). Finally they stopped feeding 2 or 3 days before death. Larvae infected in the early instars appeared smaller in size as the disease progressed (Fig.2).

Upon dissection of a diseased larva the fat body showed an opaque white or porcelain white appearance where as the corresponding tissue of a healthy larva was of faint

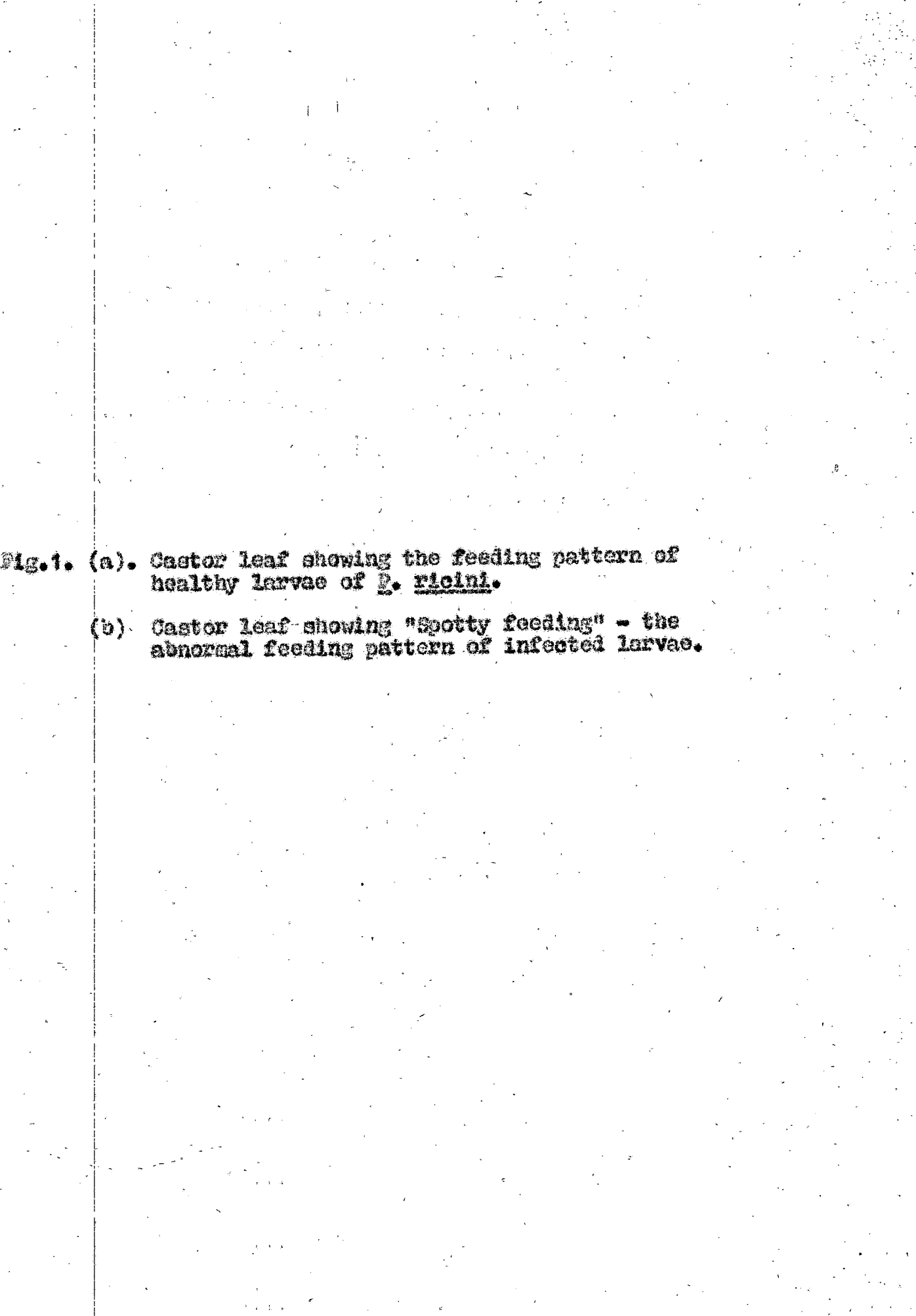


Fig.1. (a). Castor leaf showing the feeding pattern of healthy larvae of P. ricini.

(b) Castor leaf showing "Spotty feeding" - the abnormal feeding pattern of infected larvae.

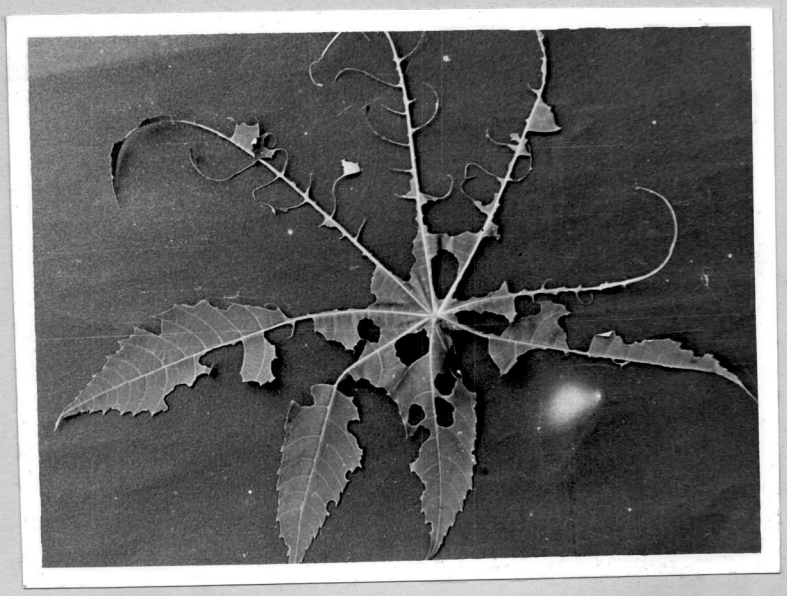


Fig. 1a.

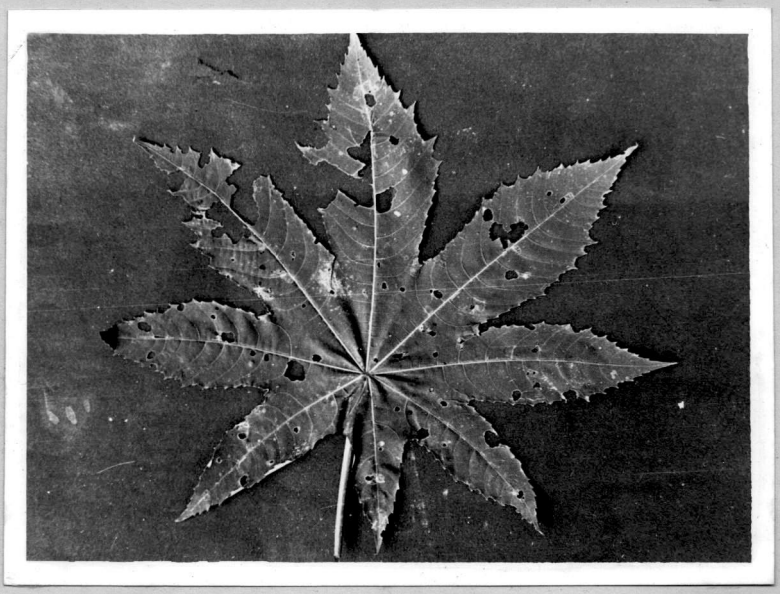


Fig. 1b.

Fig.2. Healthy (H) and Diseased (D) larvae of P. ricini 6 days after ingestion of granules. Note the difference in size.

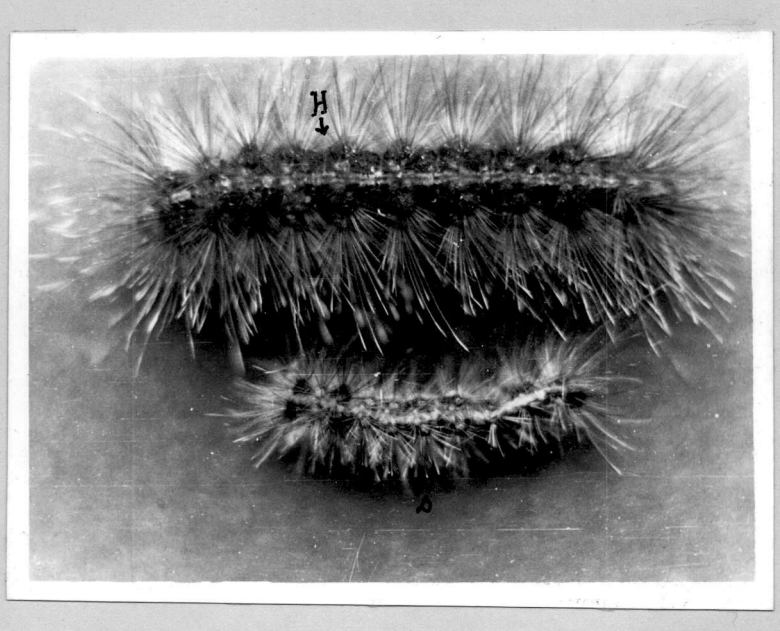


Fig. 2.

straw colour and nearly transparent. Further it was extremely enlarged and thickened compared to the less massive fat body of the healthy larva. In the advanced stages of infection the cuticle was very fragile which ruptured on the slightest pressure liberating the liquefied body contents containing millions of capsules (Fig.3a, b).

The dead or dying late instar larvae exhibited the typical symptom of hanging head downwards in an inverted 'V' position from the top and sides of container (Fig 4). But most of the first, second and third instar larvae were found lying dead on the leaf surface or in the bottom of the container. The cadaver darkened very soon and dried up to a dark scale within 24 to 48 hours of death. No pupal mortality was observed. The incubation period was found to vary from 2 to 14 days.

Susceptibility of different larval instars to the virus.

Data on the incubation period and per cent mortality of different larval instars of P. ricini inoculated with the granulosis virus are presented in Table 1. The incubation period was prolonged from 4.20 days in the first instar to 9.48 days in the fifth instar larvae. The virus infection caused 100 per cent mortality of first, second, third, fourth and fifth instar larvae. Some infected fifth instar larvae were noticed to survive in the diseased condition even after

Fig.3. (a). Healthy larvae of P. ricini

(b). Diseased larvae of P. ricini 9 days after the ingestion of granules. Note the oozing out of the liquefied body contents (arrow).

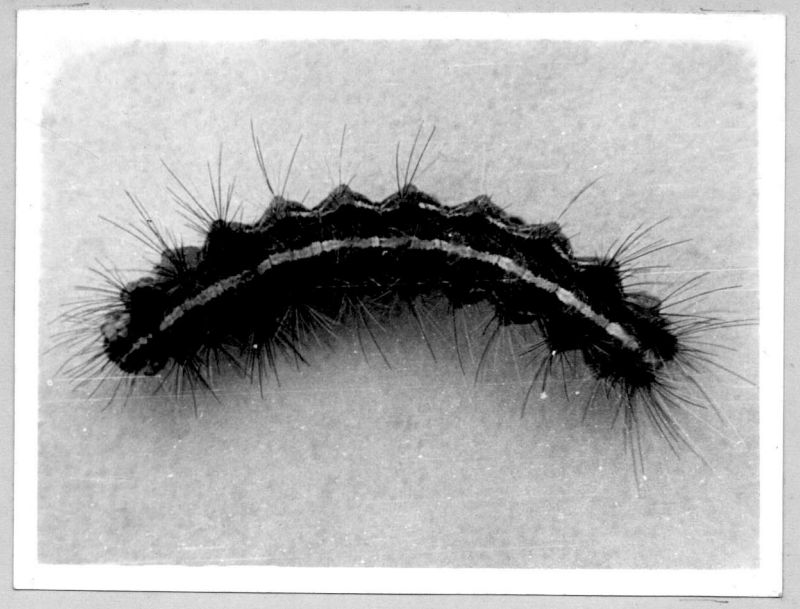


Fig. 3a.

Fig. 3b.

Fig. 4. Larva of P. ricini killed by granulosis, showing the characteristic appearance in the form of an inverted 'V'.

Fig. 4

Table 1. Incubation period and per cent mortality of P. ricini when infected by granulosis virus at different instars

Instar of larvae inoculated	No. of larvae		Time taken for death (days)		No. of larvae dead due to		Per cent mortality due to granulosis	Per cent mortality in control due to	
	Inoculated	Control	Range	Mean	Granulosis	Other causes		Granulosis	Other
First	25	25	2-7	4.20	25	Nil	100	Nil	Nil
Second	25	25	3-9	5.08	25	Nil	100	Nil	Nil
Third	25	25	3-12	7.04	25	Nil	100	Nil	Nil
Fourth	25	25	4-14	9.12	25	Nil	100	Nil	Nil
Fifth	25	25	4-14	9.48	25	Nil	100	Nil	Nil
Sixth	25	25	5-13	9.44	9	Nil	36	Nil	Nil

the control group had emerged as adults. Only 36 per cent of sixth instar larvae were killed by the virus while the rest pupated normally and adults emerged indicating that the last instar larvae are comparatively resistant to the granulosis virus.

The results of probit analysis on LT_{50} values are given in Table 2. Figures 5a - e represent the regression lines for the time mortality of first, second, third, fourth and fifth instar larvae. The LT_{50} value for sixth instar larvae could not be calculated as the mortality that occurred was less than 50 per cent. The LT_{50} values for the first to fifth instars ranged from 3.370 to 8.523 days.

Effect of treating egg masses of P. xicini with granulosis virus on the hatching larva

The results presented in Table 3 show that treating

Table 3. Effect of treating egg masses of P. xicini with granulosis virus on larval mortality

No. of larvae		No. of days after hatching for mortality due to granulosis		Per cent mortality due to granulosis	Per cent mortality due to other causes
		Range	Mean		
Batch A	50	2-5	3.1	90	10
B	50	2-5	3.1	90	10
Control					
A	50	-	-	Nil	Nil
B	50	-	-	Nil	Nil

the egg masses with capsules was as effective as inoculation of the larvae with the virus. There was 90 per cent larval

Table 2. LT_{50} for different larval instars of *P. ricini* inoculated with the granulosis virus

Sl. No.	Instar of the larvae	LT_{50} (days) (1)	Limits of LT_{50} (days)	Regression equation (2)	Heterogenity (3)
1	First	3.370	3.004, 3.784	$y = 4.95x + 2.39$	$\chi^2_4 = 2.04$
2	Second	4.204	3.780, 4.677	$y = 5.59x + 1.51$	$\chi^2_5 = 2.15$
3	Third	5.975	5.448, 6.555	$y = 4.64x + 1.40$	$\chi^2_8 = 3.10$
4	Fourth	8.459	7.577, 8.666	$y = 5.80x - 0.23$	$\chi^2_9 = 7.41$
5	Fifth	8.523	7.941, 9.147	$y = 5.28x + 0.08$	$\chi^2_{13} = 0.93$

1. = LT_{50} = Time required to give 50 per cent mortality of the larvae

2 = Regression equation of probit on log time

3 = In none of the cases the data were found to be heterogenous at $P = 0.05$

LOG TIME-PROBIT MORTALITY RELATION BETWEEN GRANULOSIS VIRUS AND DIFFERENT LARVAL INSTARS OF P. RICINI.

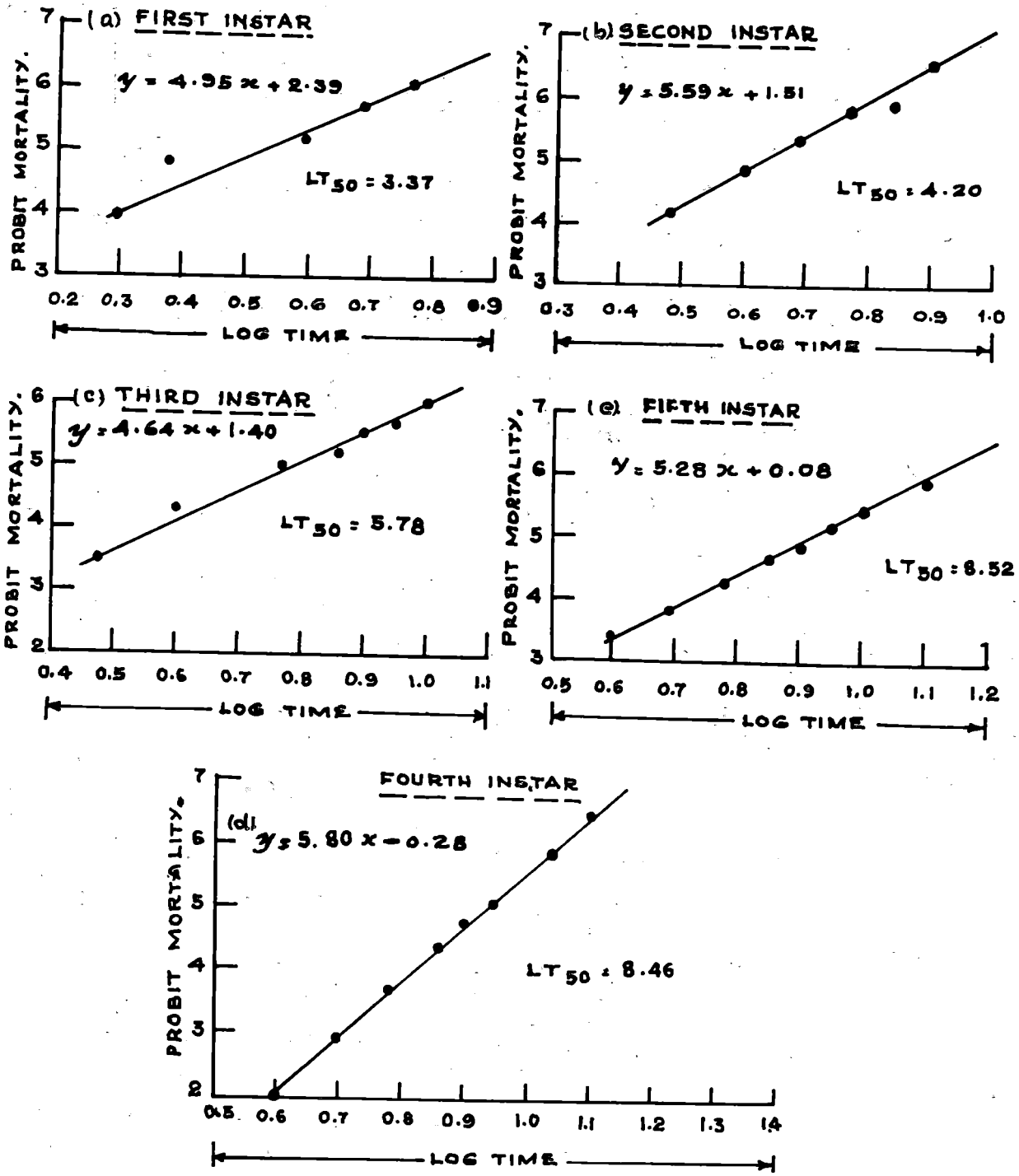


FIG: 5

mortality due to granulosis in the treated group while none died of virus in the untreated group.

Histopathology

Pathological changes were noticed mainly in the adipose tissue and to a limited in hypodermis with scattered infection of tracheal matrix cells. At 24 hours after treatment signs of infection were noticeable in some adipose tissue cells. A few cells showed slight enlargement of the nuclei and the presence of a net work of strands (Fig. 6). Others resembled those of untreated larvae in which the nuclear material appeared as small stained granules.

At 48 hours after treatment the number of fat cells showing signs of infection increased further. The earlier infected cells showed further enlargement of the nuclei accompanied with an increase in size of the whole cell.

By 72 hours the proportion of hypertrophied cells and nuclei in the fat tissue of inoculated larvae increased considerably. The fat vacuoles were reduced in size and number. But no mature inclusion bodies were visible at this stage. All cells within a given tissue were not affected simultaneously. It was quite usual to find an apparently healthy fat cell situated next to one that had a greatly hypertrophied nucleus. At this stage hypodermal cells in certain regions also appeared highly enlarged with

Fig.6. Section through fat body of the larva of *P. picini* 24 hours after ingestion of granulosis virus. Note the enlarged nucleus (arrow).

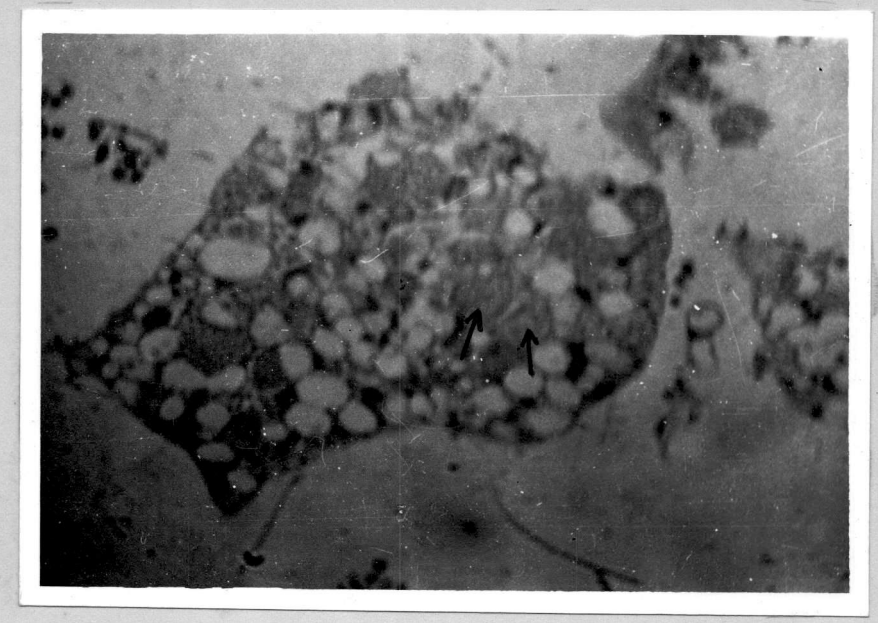


Fig. 6.

hypertrophied nuclei (Fig. 7a, b).

Sections made at 96 hours after inoculation showed that in the infected cell the chromatin net work had spread over the entire cell, the whole cell appearing as a uniform dark staining mass the nucleus being more or less undifferentiated from the cytoplasm many cells at this stage showed the presence of capsular inclusion bodies (Fig. 8). At the same time there remained other cells or lobes of the adipose tissue which were either normal or in the early stages of infection (Fig. 9). Capsules were also observed in some cells of the hypodermis (Fig. 10) and the tracheal epithelium (Fig. 11).

The infection progressed further by 120 and 144 hours after treatment. By 168 hours almost all fat cells except a few in the margin of adipose lobes were found infected (Fig. 12). At 192 hours after the treatment the whole adipose tissue was in a late stage of infection and in many areas it had lost its cellular integrity. Hypodermal cells also were heavily infected and showed signs of disintegration.

Effect of granulosis on length and weight of larvae of P. ricini

(a) Weight of larva.

The results are presented in Table 4 and illustrated in Fig. 13. It may be seen that the healthy larvae increased

Fig. 7.(a). Section through the hypodermis of Healthy larvae of P. ricini.

(b). Section through the hypodermis of the larva of P. ricini 72 hours after ingestion of granulosis. Note the hypertrophied cells and nuclei.

Fig. 7a.

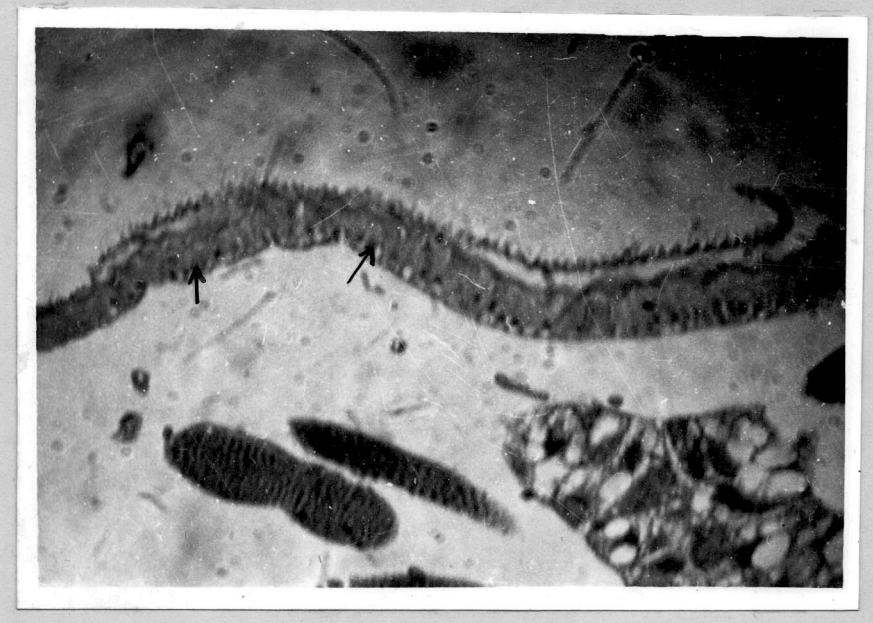


Fig. 7b.

Fig. 8. Section through the hypodermis of the larva of P.ricini 96 hours after ingestion of granulosis virus. Note the presence of capsules in the infected cells.

Fig. 9. Section through the adipose tissues showing normal (N) and infected cells (I).

Fig. 8.

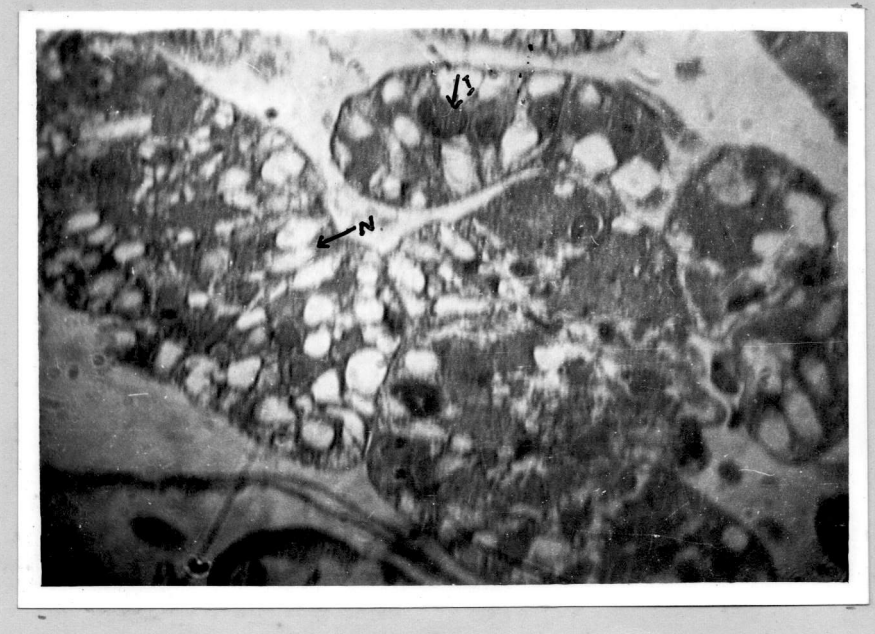


Fig. 9

Fig. 10. Section through the hypodermis of the larva of P. ricini 96 hours after the ingestion of granules. Note the hypertrophied cells containing the capsules.

Fig. 11. Section of the larva of P. ricini 96 hours after the ingestion of granules. Note the infection at tracheal epithelium.

Fig. 10.

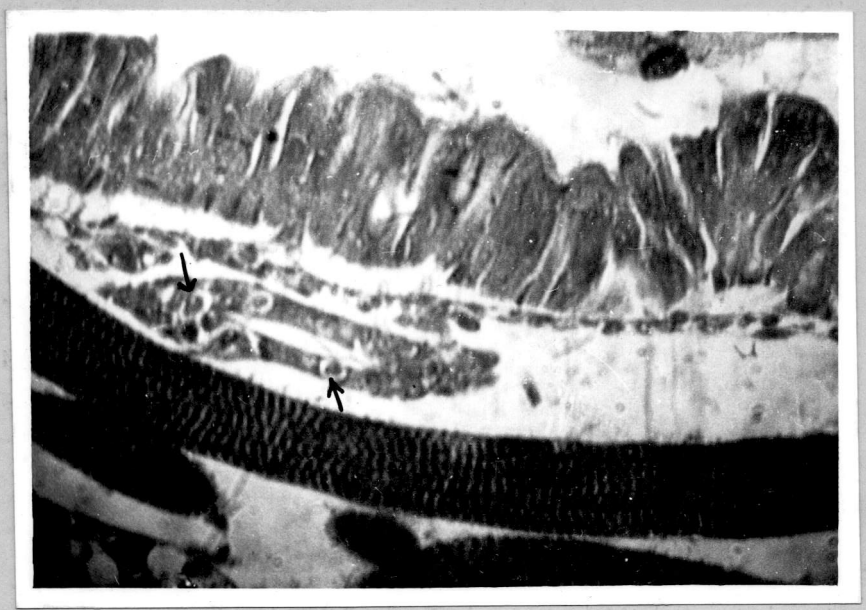


Fig. 11.

Fig.12. Section through the adipose lobes of the larvae of P. ricini 168 hours after the ingestion of granules. Majority of the fat body cells are infected with the granulosis virus (I). A few cells at the margin (II) are uninfected.

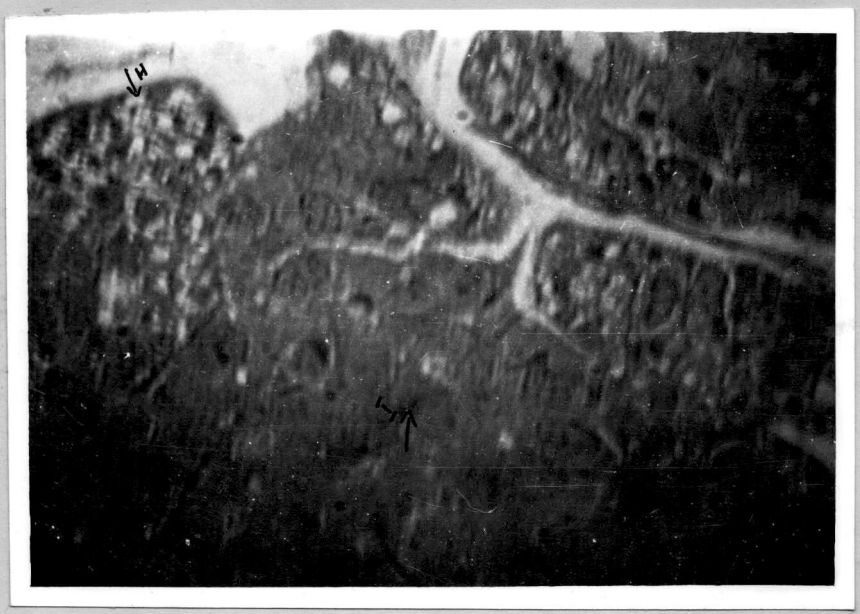


Fig. 12.

Table 4. Mean fresh weight of healthy and granulosis infected larvae of P. ricini

Post inoculation periods in days[1]	Mean fresh weight of larva in grams[2]	
	Healthy ± SE	Granuloses treated ± SE
1	0.029 ± 0.001	0.030 ± 0.0002
2	0.060 ± 0.002	0.050 ± 0.0003
3	0.126 ± 0.006	0.032 ± 0.0025
4	0.184 ± 0.015	0.087 ± 0.005
5	0.317 ± 0.022	0.099 ± 0.005
6	0.427 ± 0.020	0.105 ± 0.004
7	0.506 ± 0.025	0.102 ± 0.004
8	0.661 ± 0.070	0.102 ± 0.005
9	1.107 ± 0.088	0.107 ± 0.011
10	1.376 ± 0.066	0.101 ± 0.005
11	1.516 ± 0.051	0.101 ± 0.005
Mean	0.574	0.095

1. Larvae were inoculated when they were in the early third instar

2. Average of 20 estimations

MEAN FRESH WEIGHT OF HEALTHY AND GRANULOSIS INFECTED LARVAE OF P. RICINI.

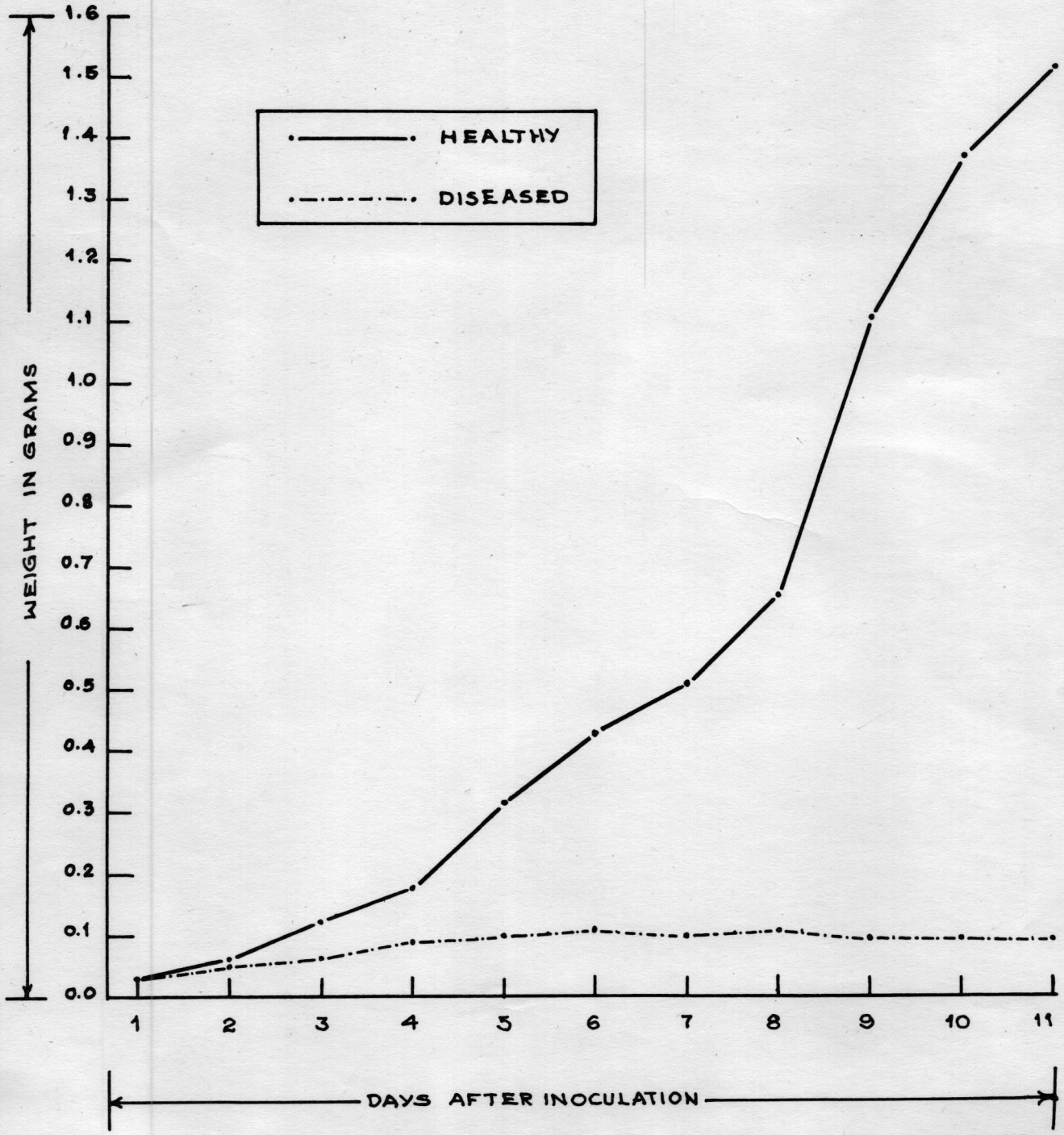


FIG: 13

in wet weight as they advanced in age. Thus it increased from 0.029 g at one day after treatment to 1.516 g after 11 days. But the diseased larvae showed a slow gain in weight until 6 days after treatment. Thereafter the diseased larvae did not show any further increase in weight. A comparison between the wet weight of healthy and diseased larvae at different periods would show that the diseased larvae had significantly lower weight than the healthy ones at all intervals from 3rd day onwards. Statistical analysis by 't' test showed that the mean weight of healthy and diseased larvae were significantly different. Thus at the end of 11 days after inoculation the diseased larvae had a mean weight of only 0.101 g while that of the healthy larva was 1.516 g which is about 15 times that of the diseased ones.

(b) Length of larva .

The data on the mean length of healthy and diseased larvae are presented in Table 5 and illustrated in Fig. 14. It will be observed that the length of healthy larvae increased steadily with advancing age. Thus it increased from 1.13 cm observed at one day after treatment to 5.38 cm after 11 days. The diseased larvae also showed a gradual increase upto 7 days after treatment but at a rate lesser than that in the healthy ones.

Table 5. Mean length of healthy and granulosis infected larvae of P. ricini

Post inoculation period in days[1]	Mean length of larva in cm^2	
	Healthy ± SE	Granulosis treated ± SE
1	1.13 ± 0.034	1.05 ± 0.022
2	1.55 ± 0.022	1.31 ± 0.018
3	2.06 ± 0.016	1.61 ± 0.023
4	2.55 ± 0.017	1.86 ± 0.018
5	3.17 ± 0.021	2.04 ± 0.020
6	3.58 ± 0.025	2.18 ± 0.020
7	3.96 ± 0.022	2.28 ± 0.020
8	4.37 ± 0.037	2.28 ± 0.020
9	4.77 ± 0.037	2.28 ± 0.020
10	5.10 ± 0.037	2.40 ± 0.020
11	5.38 ± 0.037	2.50 ± 0.023
Mean	3.42	1.98

1. Larvae were inoculated when they were in the early third instar

2. Mean of 20 estimations

MEAN LENGTH OF HEALTHY AND GRANULOSIS INFECTED LARVAE OF P. RICINI

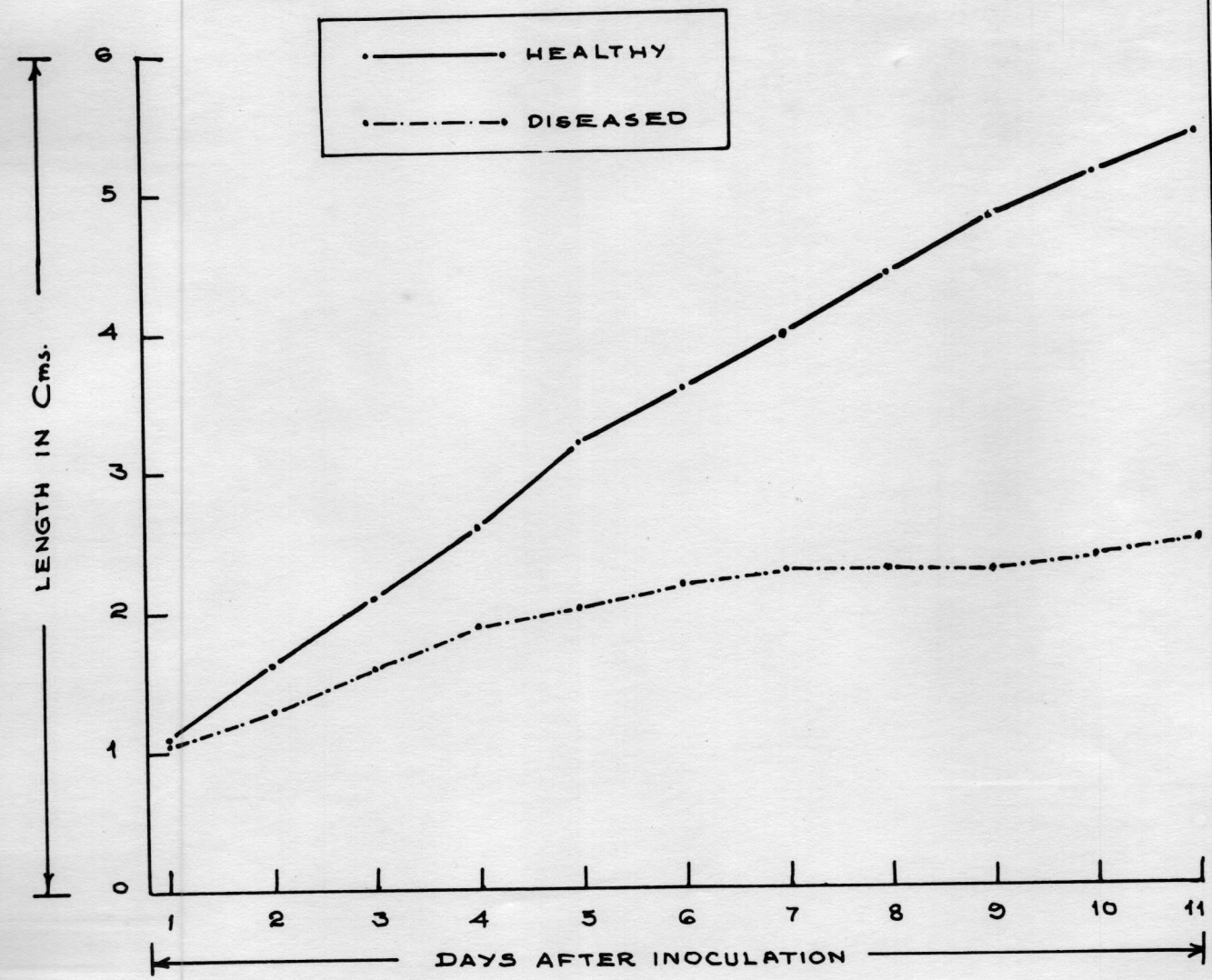


FIG: 14

Comparing the length of healthy and diseased larvae, it is seen that the mean length of diseased larvae was remarkably shorter than the corresponding healthy ones at all intervals from 3rd day onwards. Thus after 11 days of inoculation the diseased larvae had a mean length of only 2.50 cm while that of healthy larvae was 5.38 which is nearly two times that of diseased ones. Analysis of the data by 't' test showed that the mean length of diseased and healthy larvae were significantly different.

Food consumption and growth of larva of *P. ricini* infected by granulosis

The average quantities of castor leaf consumed by healthy and granulosis infected larvae at different intervals after inoculations are summarised in Table 6a. It may be seen that the mean fresh weight of castor leaf consumed by the diseased at all intervals except on the first day were significantly lower than that by the healthy larvae. A slight increase in the amount of food consumption was noticed in the inoculated larvae on the first day. While the quantity of leaf consumption in healthy larvae increased from 0.451 g on the first day to 1.170 g on the tenth day, that of inoculated larvae decreased from 0.558 g to 0.027 g during the same period. The treated larvae died after 10 days while the healthy ones started pupating after 11 days. The food consumption by the healthy larvae on the eleventh day was

Table 6a. Average quantity of castor leaf consumed by healthy and granulosis infected larvae of *P. ricini*

Post inoculation periods in days	Average quantity of leaf consumed/ larva* in g ± S.E.		Per cent increase (+) or decrease (-) over healthy
	Healthy	Diseased	
1	0.451 ± 0.111	0.558 ± 0.097	+23.73
2	0.346 ± 0.052	0.137 ± 0.037	-60.41
3	0.374 ± 0.050	0.202 ± 0.035	-45.99
4	1.121 ± 0.032	0.122 ± 0.022	- 89.12
5	1.011 ± 0.082	0.075 ± 0.014	-92.58
6	0.560 ± 0.086	0.041 ± 0.012	-92.68
7	1.169 ± 0.052	0.084 ± 0.056	-92.81
8	0.861 ± 0.150	0.042 ± 0.013	-95.12
9	0.898 ± 0.098	0.037 ± 0.018	-95.88
10	1.170 ± 0.075	0.027 ± 0.021	-97.69
11	0.277 ± 0.045	-	-
Total	8.238	1.325	-

* Ten larvae each were employed in both inoculated and control groups

Table 6a. Average quantity of castor leaf consumed by healthy and granulosis infected larvae of *P. ricini*

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5	1.011 ± 0.082	0.075 ± 0.014	-92.58
6	0.560 ± 0.086	0.041 ± 0.012	-92.68
7	1.169 ± 0.052	0.084 ± 0.056	-92.81
8	0.861 ± 0.150	0.042 ± 0.015	-95.12
9	0.898 ± 0.098	0.037 ± 0.018	-95.88
10	1.170 ± 0.075	0.027 ± 0.021	-97.69
11	0.277 ± 0.045	-	-
Total	8.238	1.325	-

* Ten larvae each were employed in both inoculated and control groups

lower than that on the tenth day.

Data on the mean fresh weight of food consumed, mean fresh weight of animal during feeding period, mean fresh weight gain of animal during feeding period, consumption index (C.I), growth rate (G.R.) and efficiency of conversion of ingested food (E.C.I.) by healthy and diseased larvae are presented in Table 6b. It will be seen that while the mean fresh weight of food consumed by a diseased larva was very low i.e., 1.201 g during the experimental period, that of healthy larva was 7.434 g recording more than 6-fold increase over the former. The infected larva recorded a very low mean fresh weight of 0.077 g in contrast to 0.470 g of the comparable healthy larva. Further the infected larva showed a very low rate of gain in weight compared to the healthy larva. The mean fresh weight gain of infected and healthy larvae were 0.071 and 1.318 g respectively. Test of significance by 't' test showed that all these differences between diseased and healthy larvae were significant.

The consumption indices were 2.030 and 1.646 for diseased and healthy larvae respectively showing an increased index for the former.

The mean growth rate of diseased larvae was 0.129 whereas that of healthy larvae was 0.297 indicating a considerably lower growth rate of the diseased. Analysis of

Table 6b. Indices on the consumption of food, growth rate and efficiency of conversion of ingested food by healthy and granulosis-infected larvae of *P. ricini*

Fresh weight of food eaten in grams		Duration of feeding period in days		Mean fresh weight of animal during feeding period in grams		Fresh weight gain of animal during feeding period in grams		Consumption index (C.I)		Growth rate (G.R.)		Efficiency of conversion of ingested food (E.C.I)	
Control	Diseased	Control	Diseased	Control	Diseased	Control	Diseased	Control	Diseased	Control	Diseased	Control	Diseased
7.366	1.701	11	9	0.459	0.095	1.380	0.090	1.459	1.989	0.273	0.105	0.187	0.053
7.755	1.510	11	10	0.492	0.081	1.490	0.071	1.433	1.864	0.273	0.093	0.191	0.047
7.370	1.670	11	10	0.692	0.086	1.575	0.072	0.982	1.942	0.210	0.084	0.214	0.043
3.915	0.840	9	8	0.397	0.079	0.851	0.072	1.124	1.329	0.244	0.114	0.217	0.086
7.010	1.330	10	10	0.414	0.082	1.071	0.071	1.693	1.683	0.259	0.097	0.153	0.052
7.344	0.790	9	5	0.395	0.062	1.321	0.061	2.066	2.548	0.372	0.197	0.180	0.077
7.677	0.910	9	7	0.376	0.075	1.091	0.071	2.269	1.733	0.322	0.135	0.142	0.078
8.830	0.918	10	6	0.590	0.082	1.720	0.091	1.497	1.866	0.292	0.165	0.195	0.099
7.029	1.488	9	8	0.410	0.072	1.220	0.062	1.905	2.583	0.331	0.108	0.174	0.042
10.040	0.800	10	5	0.494	0.058	1.470	0.050	2.032	2.759	0.298	0.172	0.147	0.062
Mean													
7.434	1.201	-	-	0.470	0.077	1.318	0.071	1.646	2.030	0.287	0.128	0.180	0.064

the data by students 't' test showed that difference between growth rate of healthy and diseased larvae was significant.

The efficiency of ingested food by diseased larvae was also seen considerably reduced as shown by the indices 0.064 and 0.180 of diseased and healthy larvae respectively. Statistical analysis showed that this difference between the two groups was significant.

Thermal inactivation point of the virus (TIP)

The incubation period, per cent mortality and pupation, when third instar larvae were exposed to granulosis virus subjected to heat treatments at different temperatures within the range of 50° to 100°C are given in Table 7. The results are illustrated in Fig. 15. It will be seen that the granules subjected to heat treatments within the range of 50 to 70°C caused 100 per cent mortality. The larval mortality was reduced progressively as the temperature of treatment was increased recording 80, 50 and 20 per cent mortality after treatment at 80, 90 and 95°C respectively. There was no mortality due to granulosis in the larvae treated with virus heated to 100°C and all the larvae pupated normally. Larvae inoculated with untreated virus caused 100 per cent mortality.

Table 7. Mortality, pupation and adult emergence of third instar larvae inoculated with granulosis/virus subjected to heat treatment for 10 minutes at different temperatures

Temperature	Time taken for death (days)		Per cent mortality due to granulosis	Per cent mortality due to other causes	Per cent pupation	Per cent adult emergence
	Range	Mean				
50°C	4-12	7.5	100	Nil	Nil	Nil
60°C	4-12	7.6	100	Nil	Nil	Nil
70°C	5-13	8.9	100	Nil	Nil	Nil
80°C	4-11	7.1	80	Nil	20	20
90°C	5-10	7.2	50	Nil	50	50
95°C	5-11	7.3	20	Nil	80	80
100°C	-	-	Nil	Nil	100	100
Control - Active virus	4-12	7.6	100	Nil	Nil	Nil
Control - Without virus	-	-	-	-	100	100

10 larvae were used for each assay

THERMAL INACTIVATION POINT OF GRANULOSIS VIRUS OF P. RICINI

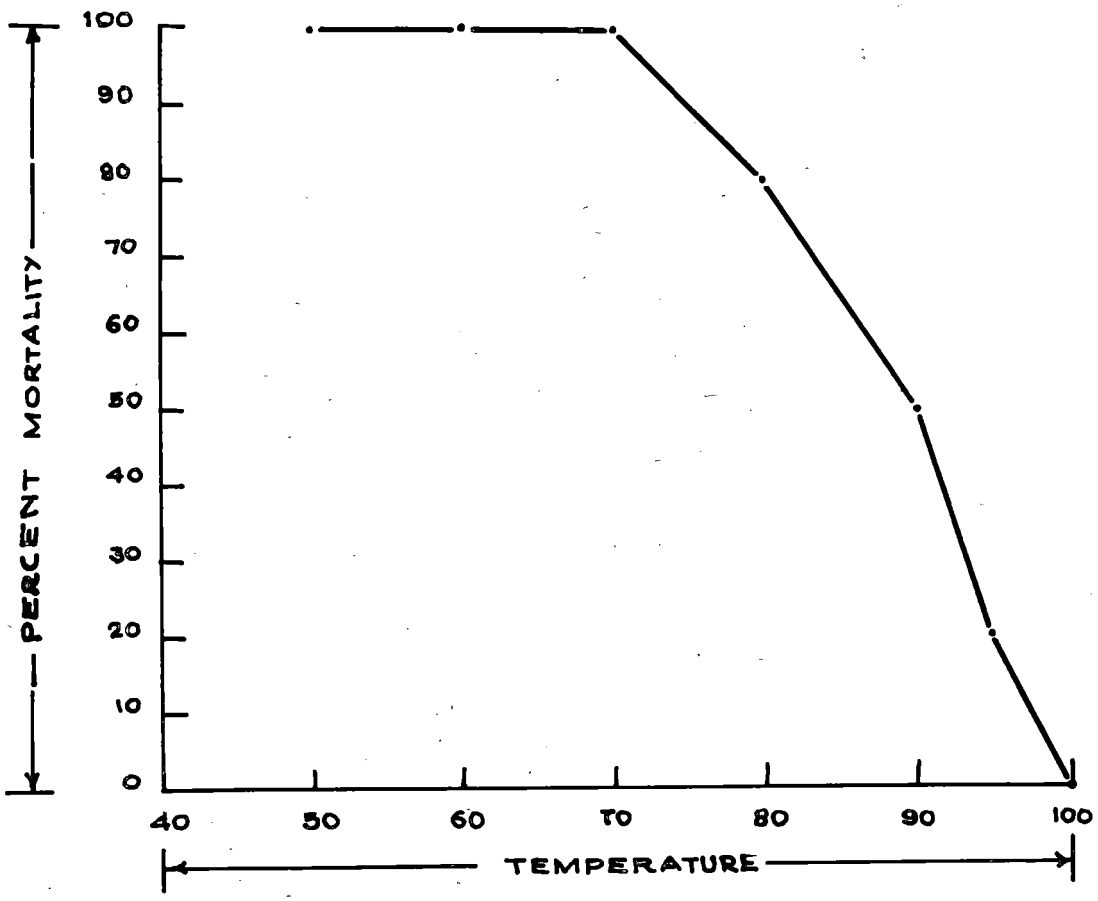

FIG: 15

Cross infectivity of the virus to other species of caterpillars

Results represented in Table 8 show that the granulosis virus of P. ricini was not infective to the larvae of P. obliqua, U. pulchella, S. litura, E. fraterna, S. derogata, S. mauritia, P. peponis. There was no detectable symptoms of granulosis and no death occurred due to virus infection in any test insects and they pupated normally except for those died due to other causes.

Effect of weathering on the virulence of granulosis virus of P. ricini

The data on the incubation period, per cent larval mortality and pupation when third instar larvae of P. ricini were inoculated with capsules exposed to field conditions for different periods are recorded in Table 9 and illustrated in Fig.16. It is evident that the infectivity of the virus was unaffected by exposure upto 12 hours but further exposure to environmental conditions reduced its infectivity. However analysis of the data by 't' test showed that there was no significant difference in the larval mortality caused by virus exposed to zero hour and that caused by virus exposed up to 96 hours. The infectivity decreased thereafter and exposure to environmental conditions for 120 hours caused significant reduction in the infectivity of the virus.

Table 9. Cross infectivity of granulosis virus of P. rieini to other species of caterpillars

Test insect	Instar of larvae at inoculation	No. of larvae inoculated	Mortality due to Granulosis	Mortality due to Others	Infectivity
1. Diacrisia obliqua	Third	100	Nil	10	-ve
2. Utethesia pulchella	Second	40	Nil	Nil	-ve
3. Spodoptera litura	Third	100	Nil	5	-ve
4. Earproctis fraterna	"	100	Nil	Nil	-ve
5. Sylepta derogata	Second	40	Nil	Nil	-ve
6. Spodoptera mauritia	Third	50	Nil	10	-ve
7. Plusia peponis	"	50	Nil	Nil	-ve

There was no mortality in control

Table 9. Effect of weathering on pathogenicity of granulosis virus of P. ricini[1]

	Larval response to virus exposed to field conditions (in hours)								
	No virus (control)	0.0 hrs	12 hours	24 hours	48 hours	72 hours	96 hours	120 hours	144 hours
Number of larva	20	20	20	20	20	20	20	20	20
Per cent mortality	0	100	100	95	90	70	65	10	0
Mean number of days to death	-	6.9	5.5	5.55	6.15	6.64	6.92	10.5	-
Per cent pupation	100	0	0	5	10	30	35	90	100
Per cent adults	100	0	0	5	10	30	35	90	100
LT_{50} (days)[2]	-	5.72	4.70	5.15	5.47	7.59	8.38	-	-

1. Dosage of 5 m/l of granules/larva
2. Time required for 50 per cent mortality from virus

Exposure to field conditions beyond 120 hours caused complete loss of infectivity of the virus as there was no mortality of larvae inoculated with virus exposed for 144 hours. Results of probit analysis on LT_{50} values are given in Table 10. Figures 17a to f represent regression lines for the time mortality of third instar larvae inoculated with virus weathered for 0 to 96 hours. The LT_{50} values also did not show much variation by the expos up to 48 hours but showed an increase beyond that.

Table 10. LT_{50} values for third instar larvae of *P. ricini* inoculated with granulosis virus weathered for different intervals

Hours of weathering	LT_{50} (in days)	Limits of LT_{50} (days)	Regression equation	Heterogenity[1]
12 hours	4.70	(4.283, 5.154)	Y = 6.279x + 0.78	1.105
24 hours	5.15	(4.677, 5.670)	Y = 5.098x + 1.37	1.152
48 hours	5.47	(4.918, 6.086)	Y = 4.978x + 1.52	0.094
72 hours	7.59	(6.757, 8.519)	Y = 3.746x + 1.70	1.130
96 hours	8.38	(7.381, 9.497)	Y = 3.729x + 1.56	0.235
Control - Active virus (0 hours)	5.72	(5.264, 7.004)	Y = 4.431x + 1.65	3.210

1 - In none of the cases the data were found to be heterogenous at P = 0.05

LT_{50} - Time required to give 50 per cent mortality of the larvae

LOG TIME – PROBIT MORTALITY RELATION BETWEEN GRANULOSIS VIRUS EXPOSED TO FIELD CONDITION FOR DIFFERENT PERIODS AND THIRD INSTAR LARVAE OF P. RICINI

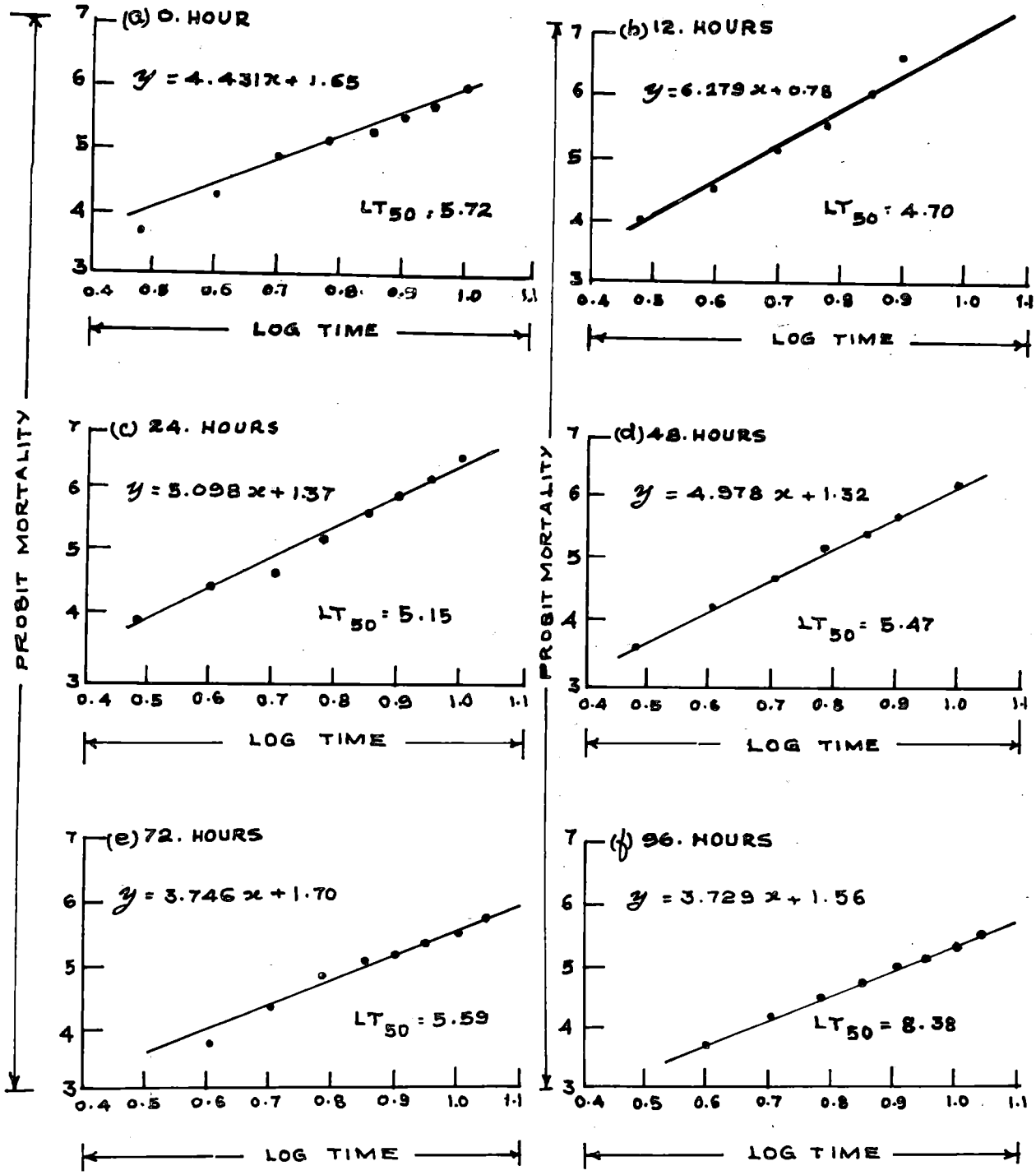

FIG: 17

DISCUSSION

DISCUSSION

The objectives of the present investigations have been to gather more information on the granulosis of Pericallia ricini and to assess the suitability of this virus as a microbial agent for field application. Studies were conducted on the symptomatology, larval susceptibility, effect of treating the egg masses of P. ricini with the virus, histopathology, effect of virus on the length and weight of larvae, effect of virus on the food consumption and growth of the larvae, thermal inactivation point of the virus, cross-infectivity of the virus to other species of lepidoptera and persistence of the virus under field conditions.

The symptoms generally resemble those described for granuloses of other lepidopterous larvae as reviewed by Huger (1963) and Smith (1967). The infected larvae, especially the first, second and third instars, assumed a paler colouration than normal larvae. Tanada (1953) reported that in the case of Pieris rapae, as the infection advanced, the larva became progressively paler than normal ones and appeared greenish to milky yellow on the dorsal and lateral surfaces, while the ventral surface was almost white. According to Wilson (1960) the change in colour

might depend, in part, on the larval age at the time of infection. No mortality at pupal stage was observed in the present studies. But in Pieris rapae mortality at prepupal and pupal stages were noticed by Tanada (1953). The spotty feeding of the leaf observed in the present study agrees with that reported by Smith et al. (1956) in the case of granulosis of Harrisaina brilliana B and McD.

Observations made on the infectivity of the virus to the different larval instars of P. ricini revealed that it caused 100 per cent mortality of the first five instars. However the LT_{50} values calculated show that it increased from 3.370 for the first instar larvae to 8.523 for the fifth instar. There was only 36 per cent mortality in the sixth instar larvae and the mean time taken for death was comparatively longer. These observations indicate that P. ricini develop an increasing resistance as they advance in age. In general young larvae have been found to be more susceptible to granulosis than old ones (Lower, 1954; Tanada, 1953; 1955; 1956a; 1959; Martignoni, 1957; Schmidt and Philips, 1958; Schmidt, 1959; Wittig, 1959; Sager, 1960). Similar observations have been made in the case of several nuclear polyhedroses also (Tanada, 1956; Stairs, 1965; Doane, 1967; Jacob and Subramanian, 1972).

It was observed in the present studies that treating the egg masses of P. ricini with granulosis virus suspension in the laboratory caused 90 per cent mortality of the emerging larvae. These observations indicate the effectiveness of the virus in controlling the pest, when sprayed on egg masses in the field. It is possible that the larvae ingest the virus along with portions of egg shells, which are generally eaten by them on hatching. A more or less similar observation was made by Smirnoff (1961) from field trials on the effectiveness of a nuclear polyhedrosis of the jack pine saw fly, Neodiprion swainei.

Martignoni (1957) proposed the terms monoorganotropic and polyorganotropic diseases for granulosis infections in insects on the basis of the number of organs attacked by the virus. Huger (1963) listed 9 out of 17 insect species in which the granulosis had been reported to be polyorganotropic. In most of these cases the organs most frequently attached were the fat body and hypodermis. Granulosis in P. ricini is a polyorganotropic disease in which the fat body, hypodermis and tracheal matrix cells exhibit pathologies and are infected by the virus. Though infection in other tissues were not observed under the light microscope, the possibility exists

that some of them may also be infected especially at a late stage of infection. In general, as pointed out by Huger (1963) the fat body is usually the first organ to show pathology in granulosis. This is also the situation in P. ricini.

In the case of Euxoa segetum (Schiffermuller) (Paillot, 1934), Pseudaletia unipuncta (Haworth) (Tanada, 1959) and Trichoplusia ni (Hubner) (Hamm and Paschke, 1963) a distinct proliferation of cells of the fat body were observed. According to Huger (1963) the first sign of infection develops in the fat body where the cells begin to proliferate leading to more voluminous fat lobes referred to by Paillot (1934, 1935, 1936) as "Proliferation cellulaires". The proliferation of cells of the diseased fat body does not always occur as is evidenced in the granulosis of P. ricini. Hughes and Thompson (1951) also did not observe such proliferation of fat cells in a granulosis of Sabulodes caberata Guenée.

The most characteristic feature in the histopathology of granuloses is the development of an intensively staining net work in the nuclear area as well as the cytoplasm (Hughes and Thompson, 1951; Wittig, 1959; Huger, 1960; Hamm and Paschke, 1963). Finally the nuclear membrane

breaks down and the constituents of nucleus and cytoplasm intermingle. Huger (1960) suggested that this net work might serve as a virogenic stroma. In the case of granulosis of P. ricini also it was observed that the chromatin material of the nucleus formed a net work which subsequently spread throughout the cell. It is believed that this might correspond to the virogenic stroma. From the present studies it is concluded that the formation of granular inclusions takes place sometimes after the evolution of stroma and that virogenesis in the granulosis of P. ricini is comparable to that in the similar instances referred above.

The results of the experiment to study the effect of virus infection on the weight of larvae of P. ricini showed that there existed much difference in the mean weight of healthy and diseased larvae. Similar observations were also made in the case of nuclear polyhedrosis infected larvae of Ceramica picta (Adams et al., 1968) and Spodoptera mauritia (Jacob and Subramanian, 1972). But no previous reports are available on the effect of granulosis infection on the larval weight. It was also observed that in the diseased larvae there was considerable reduction in length. Food consumption experiments (Table 6a) showed that the diseased larvae fed very little compared to the healthy ones from 3 to 4 days of infection which might cause

a retardation in growth and consequent reduction in size and weight. Jacob (1972) attributed such a reduction in the size and weight of tobacco caterpillar, Spodoptera litura infected with NPV to the depletion of fat and carbohydrates. The observations on mean fresh weight and length would clearly show that the virus infection has a tremendous retarding effect on growth rate of the host larvae and this become more apparent after the initial signs of infection are observed externally.

The observation on the leaf consumption of healthy and granuloses infected larvae revealed that virus infection caused a retardation of feeding from second day of infection onwards. Though loss of appetite and cessation of feeding have been reported as common symptoms associated with granuloses infections (Tanada, 1953; Huger, 1963), no quantitative data are available on this aspect. Jacob and Subramaniam (1974) observed such a phenomenon in the larvae of Spodoptera litura infected with NPV and they suggested that the virus might have overcome the animal systems in the course of infection causing a general physiological debility which indirectly would have been responsible for the retardation and cessation of feeding of the larvae. It was also seen that infected larvae consumed only one sixth of the quantity of leaf consumed by the healthy larvae

during the period of 11 days. These observations indicate that though it takes 5 to 12 days for an infected third instar larva to die, the level of damage caused to the crop by them would be considerably lower than that by comparable healthy larva. This is an important consideration in utilising this pathogen in the biological control of *E. gigini*.

Data on the quantitative variation in the food consumed by virus infected insects compared to healthy ones have been reported by few workers (Jacob and Subramaniam, 1974). However their data do not provide an overall understanding of utilisation of food by diseased animal and its growth rate. Hence an attempt was made in the present investigations to assess quantitatively the difference in the consumption of food, growth rate and in the utilisation of ingested food between those of healthy and diseased caterpillars.

The mean consumption index calculated for diseased larvae was found to be higher than that for comparable healthy larvae. This may appear incongruous that a diseased larva has a high consumption efficiency. It is only an apparent phenomenon caused by the lower mean fresh weight of the infected larva and its shorter duration of feeding. In the previous experiment it was found that the absolute quantity of leaf fed by diseased larvae was considerably lower than that of the healthy larvae. The significance of

this finding is further explainable based on the indices of growth rate and efficiency of conversion of ingested food. It was observed that the relative growth rate (G.R.) and the efficiency of conversion of ingested food (E.C.I.) were significantly lower in the diseased larvae than those for the healthy larvae. In this context it should be noted that it is not the rate of consumption, but the efficiency of conversion of ingested food that ultimately decides the growth rate of the animal. In all the ten larvae under experiment the E.C.I. values were significantly lower than that for the corresponding healthy ones and same is the case with growth rate. Thus, eventhough the rate of food intake relative to the mean weight of the animal during the feeding period was higher in diseased larvae, their efficiency for conversion of ingested food was considerably lower which was reflected in the relative growth rate also. According to Waldbauer (1968) the E.C.I. indices vary with both the digestibility of the food and the proportional amount of the digestible portion of that food which are, on the one hand converted to the body substance and on the other hand metabolised for energy to maintain life. In the present studies the factor of digestibility of the food was kept constant as far as possible since both the healthy and diseased larvae were provided castor leaves of uniform age and quality. Thus it

is apparent that in the present instance the functions of conversion of digestible portion to body substance and/or the metabolisation of the digestible portion for energy to maintain life are the factors subjected to variation in the infected animal. In the virus infected larvae, as revealed by the histopathological studies, the adipose tissue which is the main centre of metabolism in the insect is also the major tissue affected by the virus. Thus, with a weakened adipose tissue system the infected animal may have a reduced efficiency of conversion of ingested food and resultant lower growth rate.

It is also evident from the present studies that thermal inactivation point of the granulosis virus of P. ricini, when heated for 10 minutes, lie between 95 and 100°C. But Tanada (1959) found that the granulosis virus of Pseudaletia unipuncta was inactivated when heated at 75°C for 10 minutes or at 70°C for 40 minutes. Comparatively high thermal inactivation point between 90 to 95°C had also been reported for nuclear polyhedrosis viruses of Spodoptera litura (Pawar and Ramakrishnan, 1971), Spodoptera mauritia (Lathika and Jacob, 1974) and Pericallia ricini (Nair and Jacob, 1975). The present observation exceeds the general limits of 80°C reported for other inclusion viruses (Bergold, 1953; Aizawa, 1963; Huger, 1963). Steirner and Bullock (1968) found that the nuclear

polyhedrosis virus of Heliothis was more heat tolerant, the virus withstanding exposure to 60°C for 2 hours without loss of infectivity and retained some infectivity even after 30 minutes at 93.3°C. But it was completely inactivated at 93.3°C in one hour. The present finding indicate that the granulosis virus of P. ricini is comparatively more thermostable but it was less heat tolerant than Heliothis virus.

The present observations on cross-infectivity of the granulosis virus of P. ricini shows a high degree of host specificity which is a general characteristic of insect viruses though there are exceptions especially with the nuclear polyhedrosis. The situation as regards the inter-transmissibility of the granulosis viruses is less clear. According to Huger (1963) they possess a high degree of host specificity. Ignoffo (1973) concluded that granulosis virus are the most specific of all the insect viruses. However in the case of Pieride, Pieris brassicae, P. rapae and P. napi one granulosis virus appeared equally infectious for all three species (Smith, 1959, 1960). Attempts by Smith et al. (1964) to infect various species of cutworms (Noctuidae) with a granulosis virus from other species of cutworm were unsuccessful. On the other hand the same granulosis virus infected larvae of P. rapae with ease. The specificity of granulosis virus of P. ricini can be

definitely concluded only after testing its infectivity to a wide range of host species of related and non-related groups.

The observation on the effect of exposure of the virus to field conditions on its infectivity to third instar larvae of E. ricini showed that it could withstand exposure for 96 hours without much loss of infectivity but was almost non-infective after 120 hours. Lathika and Jacob (1973) found that nuclear polyhedrosis of Spodoptera litura lost most of the viral activity after 48 hours of exposure and was almost non-infective after 96 hours. Hirt et al. (1960) and Turner and Kaplan (1965) suggested that sunlight-UV, although not directly responsible, might indirectly inactivate or catalyse reactions which result in viral instability and loss of infectivity. Bullock (1967) found that nuclear polyhedrosis virus of Heliothis lost most of its viral activity after one day and only a slight activity persisted on the second day, when applied on cotton foliage. David et al. (1969) observed that granulosis virus of E. brassicae exposed to direct sunlight was rapidly inactivated with an exposure of 3 hours. Total inactivation of the virus occurred between 12 and 19 hours. The infectivity of nuclear polyhedrosis of Lambdina fiscellaria somniaria was reduced considerably after exposure for 5 hours and

only 11 per cent persisted after 35 hours (Morris, 1971). The present observation indicate that granulosis virus of *P. xylini* is more tolerant to field conditions than the previously reported ones.

SUMMARY

SUMMARY

A detailed study on the granulosis of the black hairy caterpillar, Pericallia ricini (Arctiidae) was conducted.

The virus infected larvae exhibited all the typical symptoms of granulosis infection as reported from other lepidopterous larvae. The infected larvae showed loss of appetite and became sluggish in 4 to 5 days after the ingestion of the virus. In the advanced stages of the disease the cuticle was very fragile which ruptured easily liberating the liquefied body contents. The dead or dying larvae showed the characteristic symptom of hanging head downwards in an inverted 'V' position from the top and sides of containers. Death occurred in 2 to 14 days.

The virtue caused 100 per cent mortality of first, second, third, fourth and fifth instar larvae. But the incubation period and LT_{50} values increased as the larvae advanced in age. Further, the per cent mortality in sixth instar larvae was considerably low. These observations indicate an increase in the resistance of the larvae with age.

Treating the egg masses of E. ricini with the granulosis virus suspension resulted in almost complete mortality of emerging larvae in 2 to 4 days.

Histopathological studies revealed that adipose tissues were the major site of virus infection. Moderate infection was observed in hypodermis and tracheal matrix cells. At 24 hours after treatment signs of infection were noticed in some adipose tissue cells. By 72 hours some cells of hypodermis also appeared highly enlarged in size with hypertrophied nuclei. By 96 hours the infection had greatly advanced and capsules were evident in many fat body cells and in a few cells of the tracheal matrix and hypodermis. The infection progressed further by 120, 144 and 168 hours after treatment. At 192 hours after treatment the whole adipose tissues were in a late stage of infection and in many areas it had lost the cellular integrity. Heavy infection was noticed in hypodermal cells in many regions.

Compared to healthy larvae the infected ones recorded low fresh weight at all intervals from third day of infection. The length of healthy larvae increased steadily with advancing age but that of the diseased larva increased only slowly. Mean length of granulosis infected larvae was found to be remarkably shorter than the corresponding

healthy ones at all intervals from 3rd day onwards.

Feeding was at a slower rate in diseased larvae from the second day onwards and it ceased on the tenth day. The mean fresh weight of castor leaves consumed by diseased larva was significantly lower than that of healthy ones. The granulosis infected larvae recorded a very low rate of gain in weight.

The consumption indices calculated for diseased larvae were found to be higher than those of healthy larvae. It was observed that the relative growth rate (G.R.) and efficiency of conversion of ingested food (E.C.I.) by diseased larvae were considerably lower than those of comparable healthy ones.

A comparatively high thermal inactivation point between 95 to 100°C was observed for the virus when heated for 10 minutes.

The virus was not infective to larvae of Diacrisia obliqua, Utetheisa pulchella (Arctiidae), Spodoptera litura, Spodoptera mauritia, Plusia peponis (Noctuidae), Euproctis fraterna (Lymantriidae) and Sylepta derogata (Pyraustidae).

The virus could withstand weathering upto 96 hours without any loss of infectivity. Further weathering caused

a gradual loss of virulence and beyond 120 hours of weathering complete inactivation of the virus occurred. The LT_{50} values for the third instar larvae inoculated with virus exposed to weathering for different intervals showed an increase from 4.70 to 8.38 days.

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*Original not seen

ABSTRACT

The black headed hairy caterpillar, Pericallia ricini Fab., is a polyphagour pest feeding on a wide variety of crops such as castor, cotton, banana, cucurbits, sunflower, field beans, and gingelly. The occurrence of a granulosis in this insect was recorded in 1972, but no detailed studies have so far been made on this disease. A thorough knowledge of the disease including the host pathogen relationships and the physico-chemical properties of the pathogen is essential to judge its suitability in practical pest management programmes. With a view to collect these information the present studies were taken up.

Studies were made in the laboratory and in field cages. Larvae of P. ricini reared in the laboratory on castor leaves were used. Third instar larvae were used in all studies except those on symptomatology and larval susceptibility. A purified suspension of granules derived from 300 dead larvae in 3 litres of distilled water formed the inoculam. All the experiments were conducted at room temperature and humidity.

The infected larvae exhibited all the typical symptoms of granulosis. The incubation period increased

as the larvae advanced in age. Thus LT_{50} values of first to fifth instar larvae ranged from 3.370 to 8.523 days. The sixth instar larvae appeared highly resistant to virus infection.

The egg masses when treated with the virus resulted in almost complete mortality of emerging larvae.

Adipose tissues were the major site of infection. Moderate infection was seen in hypodermis and tracheal matrix. Signs of infection were visible in the adipose tissues 24 hours after inoculation. By 72 hours some cells of hypodermis also appeared enlarged in size with hypertrophied nuclei. By 96 hours post inoculation capsules were evident in many fat cells, a few cells of hypodermis and some cells of tracheal matrix. By 192 hours infection was noticed over the entire fat body, hypodermal cell in many regions and some tracheal cells.

The mean fresh weight and length of diseased larvae were significantly lower than that of healthy ones.

The mean fresh weight of castor leaves consumed by infected larva was significantly lower than that by comparable healthy ones. Infected larvae also showed a slow rate of gain in weight.

The consumption index calculated for the diseased

larvae was found to be higher than that for comparable healthy ones. But the efficiency of conversion of ingested food and relative growth rate were comparatively lower.

The thermal inactivation point of the virus was found to lie between 95 to 100°C.

The granulosis virus of P. ricini was not infective to Diacrisia oblique, Utethesia pulchella, Spodoptera litura, Spodoptera mauritia, Plusia peponis, Euproctis fraterna and Sylepta derogata.

The virus could withstand weathering upto 96 hours without any loss of virulence. But further weathering caused a gradual loss of virulence and beyond 120 hours complete inactivation of the virus occurred. The LT_{50} values were also calculated for different intervals.

The present studies have largely contributed to the information on basic features of the granulosis of P. ricini which in turn help to assess the suitability of the pathogen for field application. The literature on granulosis in insects pertinent to the present studies have been reviewed.