

**STUDIES ON KENCHU VIRUS DISEASE OF
SILKWORM *Bombyx mori* L.**

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M. Sc. (Agri.)

**DEPARTMENT OF AGRICULTURAL ENTOMOLOGY
UNIVERSITY OF AGRICULTURAL SCIENCES
BANGALORE**

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R. GOVINDAN

M. Sc. (Agri.)

Thesis Submitted to the
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in

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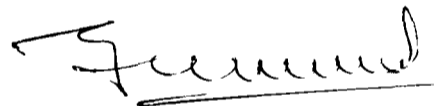
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DEPARTMENT OF AGRICULTURAL ENTOMOLOGY
UNIVERSITY OF AGRICULTURAL SCIENCES
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CERTIFICATE

This is to certify that the thesis entitled "STUDIES ON KENCHU VIRUS DISEASE OF SILKWORM BOMBYX MORI L." submitted in partial fulfilment of DOCTOR OF PHILOSOPHY in AGRICULTURAL ENTOMOLOGY, to the University of Agricultural Sciences, Bangalore, is a record of bona fide research work carried out by Mr.R.GOVINDAN under my guidance and supervision and no part of the thesis has been submitted for the award of any other degree, diploma, associateship, fellowship or other similar titles.



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INTRODUCTION

I. INTRODUCTION

Sericulture is an important agrobased industry providing gainful occupation to farmers and under-employed in rural areas in our country. Natural silk used in the textile industry is produced by sericigenous insects amongst which mulberry silkworm, Bombyx mori L., tasar silkworms, Antheraea spp., muga silkworm, A. assama Westwood and eri silkworm, Samia cynthia ricini Boisduval have been commercially exploited. India is unique in producing all these four types of silk and ranks third in raw silk production among sericultural nations by producing 6895 metric tonnes during 1984-85 (Anonymous, 1986). Out of 5,75,936 villages in India, sericulture was practised in 42,435 villages during 1982-83. The industry provided employment to 44.38 lakh persons (Anonymous, 1984). Karnataka state produces bulk of Indian mulberry raw silk (4059 metric tonnes) (Anonymous, 1986). Kelar, Bangalore, Tumkur, Mysore and Mandya are the major sericultural districts of Karnataka.

Mulberry silkworm cocoon crops are highly unpredictable owing to the fact that the silkworm, B. mori is infected by a number of diseases and infested by pests. On account of the diseases, silkworm cocoon crop production has remained as the most uncertain of all agricultural professions (Mukerjii, 1912). According to Aruga (1971),

most of the damage to sericulture in Japan can be attributed to silkworm diseases rather than unfavourable weather conditions that lead to poor harvest of mulberry. Success in sericulture devolves on several aspects, namely, production of more quantities of nutritious mulberry leaf, breeding of high yielding silkworm breeds and adoption of appropriate silkworm rearing technology including prevention and control of infectious diseases. Hence, the study of silkworm diseases is very important not only to sustain the current level of cocoon production but also to further increase the cocoon yields. Flacherie, grasserie, muscardine and pebrine are the major diseases of silkworm, B. mori, among which flacherie is more important (Dasgupta, 1950; Ghosh, 1962) and considerable cocoon crop losses are caused by this disease.

Flacherie has been considered to be the most serious malady. The term flacherie refers to the flaccid condition of silkworm larvae suffering from dysentery. Flacherie may be caused by several viruses viz., nuclear polyhedral, cytoplasmic polyhedral, infectious flacherie and densonucleosis viruses (Aruga, 1971; Yokoyama, 1963; Aisawa et al., 1964; Watanabe et al., 1976) and different bacteria (Chitra et al., 1975). In Karnataka, several types of flacherie conditions are known based mainly on external symptoms.

According to Shyamala (1978), Kenchu is a widely prevalent flacherie in Karnataka. The etiologic agent of this disease has been proved to be a small, spherical, non-occluded virus and measures 27 ± 2 nm in diameter (Hadimani and Shyamala, 1983). The highly infectious nature of this virus was evident from the fact that only one per oral administration of the virus to first instar silkworms was sufficient to produce disease in all its severity. The Kenchu virus infected silkworms usually produce chain excreta (Plate 1) followed by reddish tinge being imparted to the worm (Plate 2).

Although the involvement of a small non-occluded virus in producing Kenchu was reported (Hadimani and Shyamala, 1983), the identify or otherwise of the virus with other known non-occluded viruses of silkworm such as infectious flacherie virus and densovirus was not established. Hence, as an initial step in the present work, serological studies have been conducted.

In an attempt to minimise the incidence of highly infectious diseases like "Kenchu" in an economically important insect such as the silkworm, B. mori, generation of information on several aspects of the disease is an essential pre-requisite. Management practices, rearing

Plate 1. Kenchu virus infected fifth instar silkworm
showing chain excreta.

Plate 2. Kenchu virus infected silkworms showing
typical "Kenchu" symptoms.



environment, resistance of breeds - all these have their due role in the incidence and severity of expression of the disease. In the present investigations, laboratory studies on the influence of a few factors which are likely to be affected in silkworm rearings, viz., refrigeration of eggs, feeding of leaves of different maturity and starvation have been carried out with special reference to the effect of these factors on Kenchu disease incidence.

Interaction between bacterial and viral infections in insects are of common knowledge. In the present study, the effect of simultaneous infection of Kenchu virus with a bacterial pathogen of the silkworm, Staphylococcus aureus has been investigated.

The objectives of the present investigations are indicated as follows:

1. Testing of different isolates of Kenchu virus against antisera of Japanese infectious flacherie virus and Ina Densonucleosis virus of silkworm, B. mori.
2. Effect of refrigeration of silkworm eggs and Kenchu virus infection in silkworm.
3. Influence of releasing Kenchu virus infected silkworms into healthy populations in different proportions.

4. Influence of nutrition on intensity of Kenchu virus infection.
5. Interaction effect of simultaneous infection with Kenchu virus and bacterium - Staphylococcus aureus in silkworm.

REVIEW OF LITERATURE

II. REVIEW OF LITERATURE

Incidence of diseases and pests is known to be a major constraint in silkworm cocoon crop production. Cocoon crop loss due to diseases was estimated to be over 30 per cent in developing countries of South East-Asia and 10 per cent in advanced countries like Japan, China and Italy (Manavaty, 1965; Yokoyama, 1963). The crop loss due to diseases has been estimated to be about 30 to 40 per cent in India (Janakiraman, 1961).

Studies on a preliminary survey of silk cocoon production conducted by the University of Agricultural Sciences, Bangalore, during 1977-78 in traditional sericultural districts of Karnataka revealed less of crop losses in seed areas compared to commercial cocoon producing areas. The loss was 10 to 15 and 10 per cent, respectively in multi-voltine and bivoltine seed areas. Further, the crop loss due to diseases was low during dry months (January to May) as compared to the rainy months (June to December) (Shyamala *et al.*, 1978).

In India flasherie has been considered to be the most serious malady of silkworm. It is reported to be responsible for 71 per cent of total cocoon crop loss in the country (Sidhu and Singh, 1968) and 20 to 40 per cent in Karnataka

(Chitra et al., 1975) every year. Several types of flacherie conditions, namely, Kenchu (redness of skin), sappe (Paleness of skin), sarahikke (chain excreta) and Karagu (melting of cocoons) were recorded based mainly on external symptoms (Chitra et al., 1975). According to Shyamala (1978), Kenchu is a widely prevalent flacherie condition in Karnataka.

Since Kenchu virus disease of silkworm pertaining to the present objectives of investigation is comparatively little explored, the other literature available on this disease as well as the information in respect of other virus diseases of silkworm and other insects in relation to the current objectives is presented below under separate headings.

2.1 Kenchu virus disease

2.1.1 Causal agent of Kenchu disease

Kenchu disease of silkworm was shown to be caused by a non-occluded, spherical virus measuring 27 ± 2 nm in diameter (Hadimani and Shyamala, 1983). The virus was extracted from whole body extract as well as faecal matter. The incubation period for manifestation of typical symptoms viz., chain excreta and "Kenchu" was found to be 4.5 to 9.0 and 6.0 to 13.75 days, respectively with the artificial infection of different larval instars of silkworm.

Cell-free larval extracts from diseased silkworm larvae were used as stock virus suspensions in the above investigations. The evidence for the involvement of a virus in producing Kenchu disease was obtained by complete elimination of bacteria from the larval extracts by (i) Centrifugation at 18000 rpm for 30 minutes (ii) passing through millipore filter (0.45 μ m) (iii) adding streptopenicillin. The disease was found to show serial transmission. The presence of a spherical, non-occluded virus in the cell-free extracts was confirmed from electron micrographs.

2.1.2 Symptoms of Kenchu virus disease

Several local names viz., Kempu sappe, naschu, Kempu naschu etc., are used for Kenchu disease. The disease is characterised by stunted growth of the worm and pale, reddish appearance of skin. Chain excreta is also observed in most of the worms. Infected early instar worms invariably die. If infection occurs in late larval instars, the worms become flaccid. In case of infection during third, fourth and fifth instars, the result is the formation of comparatively small and flimsy cocoons. The surviving pupae are small, inactive and have deformed wings. Further, the resultant moths are characterised by the presence of sparse scales on the body as well as wings (Hadimani, 1980).

2.1.3 Susceptibility of different instars of silkworm to Kenchu virus

Growth inhibition was observed to be greater when younger larvae were infected compared to older larvae (Hadimani, 1980). The weight reduction on 24th day of infection compared to uninfected controls in first, second, third, fourth and fifth instars of a hybrid - Pure Mysore x NB₄D₂ was 93.96, 85.79, 77.07, 16.19 and 0.46 per cent, respectively. Infection during third instar and onwards resulted in some larvae spinning cocoons and the percentage decrease in cocoon weight was 34.86, 29.72 and 0.06, respectively in case of infection in third, fourth and fifth instars. One hundred per cent mortality was observed when first and second instar worms were infected. Larval mortality corresponding to infection during third, fourth and fifth instars was 95.5, 85.5 and 40.5 per cent respectively, thus revealing that the early instar silkworms are more susceptible to Kenchu virus.

2.1.4 Susceptibility of silkworm breeds to Kenchu virus

The relative susceptibility of two multivoltine breeds (Pure Mysore and Hosa Mysore) and three bivoltine breeds (NB₄D₂, NB₇ and NB₁₈) to Kenchu virus was investigated (Narayanaswamy, 1983). Pure Mysore was the most susceptible breed. The ET₅₀ for Kenchu symptom manifestation was 9 days 12 h, 10 days 18 h, 10 days 5 h, 11 days 7 h and 12 days

for Pure Mysore, Hosa Mysore, NB₄D₂, NB₇ and NB₁₈ respectively, when Kenchu virus stock suspension was administered per os. The weight reduction was more than 50 per cent in Pure Mysore compared to untreated batch even before 8th day with viral dilutions of 10⁻¹ to 10⁻³ and even in lower dilutions the time required for 50 per cent weight reduction ranged from 9.0 to 19.5 days. In case of Hosa Mysore the time required for 50 per cent weight reduction was 9.25 to 25.25 days with different viral dilutions. Weight reduction in NB₇ and NB₁₈ was less marked except at 10⁻¹ and 10⁻⁵ dilutions and weight reduction did not reach 50 per cent with other dilutions. Multivoltine breeds were more susceptible than the bivoltine breeds (Narayanaswamy et al., 1985b).

2.1.5 Epizootiology of Kenchu virus disease

The epizootiological studies on Kenchu virus disease were made by Eswarappa (1983). The virus was found to lose the infectivity on exposure to sunlight. Ten hours exposure to direct sunlight under dry condition and 20 hours exposure under humid condition were sufficient to inactivate the virus.

The virus retained infectivity upto six months of preservation at room temperature (25-30°C), but the virulence decreased gradually (Eswarappa and Shyamala, 1984).

Relative humidity of the atmosphere had a significant effect on virulence. The loss of virulence was quicker at lower humidity levels (10 to 20 per cent) than at higher humidity levels (50 to 90 per cent).

Persistence of Kenchu virus in the silkworm habitat was established as 48 to 56 per cent of worms fed with the washings of rearing room dust, rearing tray and mountage, wherein the Kenchu disease existed in previous rearing, developed the disease. Infected individuals within the population spread the disease to healthy worms. Disease spread throughout the population was noticed with the presence of even 4 per cent of the infected individuals. Presence of 20 per cent of infected individuals was as effective as 40 per cent in spreading the disease throughout the population. Contamination by the rearer due to handling of diseased and healthy worms simultaneously was found to aid in spread of disease as evidenced by 60 to 64 per cent of the worms developing the disease when reared with contamination by handling (Eswarappa, 1983).

When Kenchu virus was orally administered to two common lepidopterous defoliators of mulberry viz., tobacco caterpillar, Spodoptera litura (Fabricius) and the black headed hairy caterpillar, Spilosoma obliqua (Walker), the

virus failed to cross-infect these insects as Kenchu symptoms, mortality, weight reduction and presence of virus in cell free extracts were not observed (Narayanaswamy et al., 1986). Also Kenchu virus was not found to be transmitted transovarially in the silkworm (Narayanaswamy, 1983; Narayanaswamy et al., 1985a).

2.1.6 Effect of disinfectants on Kenchu virus

Surface contamination of silkworm eggs with Kenchu virus that were treated with different disinfectants indicated that bleaching powder was not effective at three concentrations tried. On 26th day, a maximum of 24.57, 24.35 and 22.73 fold increase in larval weight over viral control was observed in case of disinfection with formalin, potassium permanganate and sodium hydroxide, respectively.

Formalin, potassium permanganate and potassium hydroxide proved effective (Radimani, 1980).

2.2 Serological techniques for small non-occluded viruses of silkworm

2.2.1 Infectious flacherie virus

The distribution of infectious flacherie virus in silkworm, B. mori was investigated using fluorescent antibody technique

(Inoue and Ayuzawa, 1971). Specific fluorescence in the midgut appeared at 25 h after virus inoculation and became more intense with the lapse of time.

Inoue (1972) observed through fluorescent antibody technique that the multiplication of infectious flacherie virus occurred in the goblet cells of the midgut epithelium.

Inoue and Ayuzawa (1972a; 1972b) employed fluorescent antibody technique to demonstrate the presence of infectious flacherie virus in the midgut epithelium of the silkworm. Specific fluorescence was observed in the cytoplasm of goblet cells. It was also noticed in the cytoplasm of columnar cells, but the fluorescence was very faint and the number of stained cells were few. Inoue (1974b) studied the multiplication of infectious flacherie virus (IFV) in the susceptible and resistant strains of silkworm, B. mori by means of the fluorescent antibody method, which demonstrated the presence of IFV in the frozen sections of diseased midguts. Fluorescent antibody test revealed that IFV multiplied well in the same degree in larvae of both susceptible and resistant strains. In susceptible larvae the number of cells with fluorescence increased just prior to death while in the resistant larvae

which were alive to pupate, the number of fluorescent cells also increased, but were much reduced temporarily just after every moulting. The temporary reduction of the number of fluorescent cells during IFV infection in resistant larvae indicated that the infected goblet cells were discharged into the gut lumen at every moult and the regenerative cells located in nidi of the midgut developed into new goblet cells for the physiological repair. Therefore, it was inferred that the regenerative ability of the midgut cells might be mainly concerned with the resistance of silkworm larvae to the lethal infection of IFV.

The interaction of silkworm viruses namely, flacherie virus, cytoplasmic polyhedrosis virus and small flacherie virus were investigated in the midgut of silkworm, B. mori by using fluorescent antibody technique (Sato and Inoue, 1978).

Shimizu (1982) developed an enzyme linked immunosorbent assay (ELISA) for detection of the flacherie virus of silkworm. This technique proved to be specific for the flacherie virus and virus protein of 3 ng/ml could be detected. The sensitivity of ELISA was 6000, 17000 and 200 times higher than that of gel immuno-diffusion, ring test and latex agglutination, respectively.

Latex particle agglutination test was used by Shimizu et al. (1983) for detection of flacherie virus of silkworm. This was also claimed to be very effective and sensitive method comparatively.

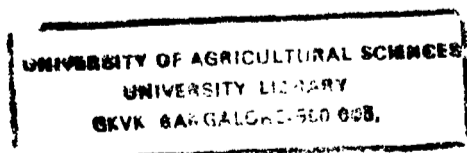
2.2.2 Densonucleosis virus

Immunodiffusion test with the antiserum of purified Ina isolate of silkworm densonucleosis virus (DNV) revealed that it was serologically quite different from the infectious flacherie virus of silkworm (Maeda et al., 1977).

Watanabe and Shimizu (1980) while studying the epizootiology of densonucleosis of silkworm confirmed the presence of virus in the silkworm rearing room dusts by following gel diffusion test.

The Yamanashi isolate of the silkworm densonucleosis virus did not react with the antiserum against the Ina isolate and the small flacherie virus in immunodiffusion tests (Seiki, 1984). These viruses did not appear to share any antigen with the Yamanashi isolate of DNV.

Immunofluorescence observation was successfully employed to study the multiplication and infection of silkworm densonucleosis virus (Sato et al., 1978a; 1978b; Maeda and Watanabe, 1978).



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The enzyme linked counter immunoelectrophoresis was used as a technique for diagnosis of denso-nucleosis virus in silkworm (Wang et al., 1983).

Arakawa and Shimizu (1985) have developed dot immunobinding assay (DIBA) for the detection of denso-nucleosis virus of B. mori. The DIBA (10 ng/ml) was similar to Enzyme linked immunosorbent assay (ELISA, 6 ng/ml) and this assay was found to be 1800, 1000 and 50 times more sensitive than gel immunodiffusion, ring test and latex agglutination.

Double gel diffusion test was employed by Watanabe et al. (1986) to establish the serological characteristics of three denso-nucleosis viruses of B. mori at Japan.

Shimizu and Arakawa (1986) used latex agglutination test for the detection of denso-nucleosis virus and cytoplasmic polyhedrosis virus of the silkworm and found that it was more sensitive than all conventional serological methods but not superior to immunosorbant assay.

2.3 Effect of exposing silkworms in early stages to low temperature on subsequent development of diseases

Literature is available on the effect of refrigeration of silkworm eggs on hatching (Farasimhamurthy, 1943a; 1943b; Devaiah and Thontadarya, 1975). But information on the effect of refrigeration of eggs on development of disease is scanty.

Low temperature may tend to promote the outbreak of diseases in insects.

Aruga and Watanabe (1959) observed that when fifth instar silkworms were exposed to 5°C for 24 hours, the frequency of nuclear polyhedrosis was higher in F_1 hybrid than in the inbred lines. The same tendency was also observed in the induction of cytoplasmic polyhedrosis during spring rearing.

Cold treatment (5°C for 3 to 5 hours) before or after per oral infection with cytoplasmic polyhedrosis virus enhanced the susceptibility of the silkworms to the virus (Aruga et al., 1963).

According to Sidhu and Singh (1968), silkworm larvae refrigerated for 24 hours at 5°C before resuming feeding resulted in 21.25 per cent of grasserie and 18.75 per cent of flacherie diseases and the corresponding figures for unrefrigerated larvae were 8.75 and 10.0 per cent, respectively.

Wu (1983) reported that the ability of silkworm larvae to resist infection of nuclear polyhedrosis was very markedly reduced when they were kept at a low temperature of 5°C for 24 hours. Matsubara et al. (1984) treated

different instars of B. mori at 5°C for 24 hours and studied the mortality rate at different concentrations of NPV. The mortality of infected fifth instar worms was 100 per cent with -log 1 to -log 5 concentration while mortality was nil in unrefrigerated and infected worms with the same concentrations of virus. The mortality of first instar refrigerated infected worms was 20 to 90 per cent while it was 0 to 90 per cent in case of unrefrigerated infected worms. Similarly, in second, third and fourth instars, the mortality of refrigerated infected worms was 10 to 60, 70 to 90 and 100 per cent when compared to 0 per cent in their controls.

2.4 Spread of virus diseases of insects and mites from infected individuals in a healthy population

Disease spread through infected hosts is one of the principal means of dispersal of viruses throughout the host environment. The pathogens are distributed through the faecal matter of infected individuals and also by regurgitation. After the death of infected individuals, their cadavers may form a potential source of pathogens.

Steinhaus and Thompson (1949) observed all the healthy alfalfa caterpillars dying after a few days when they were released together with already polyhedrosis virus-infected individuals in rearing trays.

According to Bird (1953), part of the population of European saw fly, Neodiprion swainei, became infected by consuming nuclear polyhedrosis virus directly and part was secondarily infected by coming in contact with the former.

In granulosis virus infection of caterpillars of Persectania swingii, the healthy larvae became infected by ingesting food contaminated by saliva from diseased larvae (Lower, 1954).

Jacques (1962) found the larvae of Trichoplusia ni treated with nuclear polyhedra transmitting the virus to untreated larvae when they were reared together.

When a single citrus red mite (Panonychus citri) infected with its non-inclusion virus was introduced into a population of 1, 5, 20 and 80 mites caged on a surface area of 45 to 75 cm², the transmission of the disease at 80 mites level was higher than at one and five mites level (Gilmore and Munger, 1964).

Stairs (1968) reported that gregarious feeding habits of larvae of Galleria mellonella and Malacosoma disstria insured the virus from a single diseased individual to reach some of the healthy larvae which, in turn, became diseased thus increasing the inoculum in the population.

Virus infected European red mites, Panonychus ulmi deposited inoculum on the leaves, probably in excreta or oral secretions at feeding sites which was picked up orally by uninfected mites while feeding (Putman, 1970).

Shaw and Beavers (1970) demonstrated the non-inclusion virus infection throughout the population of citrus red mite, P. citri by releasing virus infected individuals into healthy population. Disease development throughout the population was rapid if the population density was high.

Healthy citrus red mites picked up the virus from contaminated surfaces of substrates rather than from within the plant cells. The presence of virus in the faeces of infected mites was also confirmed (Reed et al., 1975).

Selasny (1976) observed that in the population of coconut beetle, Oryctes rhinoceros the baculo virus was transmitted most frequently when the uninfected individuals contacted the virus material excreted by the infected individuals.

The faecal pellets from cytoplasmic polyhedrosis virus infected larvae of fall webworm, Hyphantria cunea

contained large numbers of inclusion bodies suggesting an effective mechanism of spread of the virus to healthy larvae (Boucias, 1979).

2.5 Effect of starvation on the development of viral diseases in insects

Shortage of food may apparently result in the initiation of virus epizootics in insects. Starvation has been identified as one of the stress factors. It may predispose the insects by constitutional weakening to the viral infections and thus aid the increasing incidence of diseases.

According to Jardine (1918), the polyhedral disease of the tea tortrix, Homona coffearia did not appear to spread among the larvae unless there was overcrowding and lack of food.

Larval starvation in B. mori led to reduction in resistance to diseases (Kadohira, 1950).

A virus disease of European saw fly, Gilpinia hercyniae was affected by crowding and shortage of food. Density of sawfly population seemed to be the most important factor determining its control effect, the

percentage of diseased larvae increasing with population, independently of secondary effects of crowding, such as shortage of food (Balch and Bird, 1944).

Edel'Man (1956) attributed the reduction in the outbreak of Lymantria dispar infestation to the physiological weakening of the population associated with forced feeding on unfavourable food plants thereby increasing the incidence of polyhedral disease.

Defoliation by L. dispar was severe over a considerable area and the larvae were deprived of sufficient food thus resulting in heavy infection by a virus disease in that only 10 per cent of them pupated (Szalay-Harzso, 1957).

Wallis (1959) found that due to heavy feeding of larvae of Anisota senatoria, the food supply was rapidly exhausted in summer and they were exposed to intense competition and starvation. Thereby they were forced to feed on unsuitable foliage which resulted in heavy mortality of last instar larvae due to a cytoplasmic polyhedral virus.

Tanada (1961) observed a greater tendency to develop virus epizootics in Mythimna separata due to lack of food when the population increased rather than at low density.

The haemolymph titre of nuclear polyhedrosis of the silkworm, Bombyx mori in starved larvae was determined by Aizawa and Furuta (1962). The level of the virus showed a decrease, an increase and a stationary phase and the titre was nearly the same in starved as in nourished larvae. The number of cytoplasmic polyhedra formed in starved worms was generally less than that in normally fed larvae, and the mean size of polyhedra in starved larvae was smaller than that in larvae that had been fed normally.

Starvation of silkworms resulted in 18 to 75 and 21 to 25 per cent respectively of incidence of grasserie and flacherie (Sidhu and Singh, 1968) as against 8.75 and 10.00 per cent in well fed larvae.

Larvae of B. mori were observed to extend their larval period upto 11.6 days, when they were allowed to feed 4 hr/day as against 6 days required by those allowed to feed 24 hr/day (Muthukrishnan et al., 1978). A decrease in the shell weight with the decreasing feeding rate was observed.

Starvation for fixed durations of 12, 24, 36 and 48 hours from first to fifth instar increased the mortality due to diseases in the silkworm, B. mori subjected to longer period of starvation (Samson et al., 1980).

Incidence of loss due to flacherie was more than nuclear polyhedrosis and irrespective of instars, it was more in repeatedly starved worms. Loss due to nuclear polyhedrosis was significantly high in worms starved after second moult, fourth moult and in worms starved from hatching to fifth instar. Thus, starvation made the worms susceptible to flacherie and grasserie in addition to its adverse effect on growth.

Kawarabata and Funakoshi (1980) studied the polyhedra dissolving activity of silkworm gut juice. Prolonged starvation during active feeding periods caused significant decrease in the polyhedra dissolving activity.

In case of eri silkworm, Samia cynthia ricini Boisduval, the larval period, pupal period, cocoon weight and fecundity per female were 18.99 and 22.50 days, 200.1 \pm 43.7 mg and 199 \pm 28.86 eggs respectively with 18 hours feeding duration per day, while with 24 hours feeding duration the corresponding figures were 17.01 and 20.40 days, 303.1 \pm 46.1 mg and 294 \pm 32.80 eggs (Srivastava et al., 1982-83).

Wu (1985) observed that the resistance of silkworms to nuclear polyhedrosis infection was markedly reduced when they were starved for 24 hours at 25°C.

2.6 Effect of leaf maturity on growth of insects with special reference to silkworm

Succulent mulberry leaves are known to be nutritively superior for successful raising of silkworm cocoon crops of good quality as they contain higher amounts of moisture, protein, starch, total carbohydrates and less of fibre and ash (Anonymous, 1964; 1965).

Quality difference due to maturity among top tender leaves, middle mature leaves and bottom over mature leaves of mulberry have been reported by several workers (Fraisse and Arnoux, 1954; Kafian, 1960; Arai and Ito, 1963; Ito and Arai, 1963; Narayanan et al., 1967). All these workers were in general agreement that top tender or comparatively less mature leaves are nutritively richer and produce better cocoon crops when fed to silkworms. Parpiev (1968) reported that higher moisture content affected both edibility of the mulberry leaves and assimilability of its nutrients in silkworms.

Low dietary moisture has been reported to bring about deleterious effects in phytophagous insects (Waldbauer, 1968).

Cocoon crop resulting from feeding mulberry leaves of different maturities to silkworms were investigated in a series of 11 experiments during the period of

1966-68 in West Bengal by Krishnaswami et al. (1970). More succulent leaves produced on top tender portion of twigs were found to be superior to the leaves below as reflected by the superiority of most of the economic characters like effective rate of rearing, cocoon weight, shell weight etc. Higher moisture content in tender leaves was not harmful to the silkworms. Over-mature leaves were found to be inferior. Feeding of tender leaves even to fifth instar did not produce any harmful effects and there was definite improvement in cocoon and cocoon shell weights. In multivoltine breed-Wistari the effective rate of rearing of 80.0, 75.8 and 66.3 per cent; cocoon weight of 1.03, 0.89 and 0.76 g and shell weight of 0.121, 0.101 and 0.086 g, were observed by feeding tender, medium and coarse leaves, respectively.

Krishnaswami et al. (1971) observed 19.1 g, 19 days, 0.88 g and 0.119 g of larval weight (10 worms), larval period, single cocoon weight and shell weight, respectively in silkworm breed D3C when reared on tender mulberry leaf while on medium and mature leaf they were respectively 19.1 and 18.6 g; 19 and 20 days; 0.89 and 0.72 g and 0.108 and 0.099 g during July-August rearing of 1968-under West Bengal conditions.

According to Rahman and Mandal (1977), different types of mulberry leaves from tree, low cut and bushes had no effect on the weight of silkworms of nistari race indicating that the three leaf types had similar nutritive value for Bombyx mori L.

Decrease in water content affected nitrogen utilization efficiency and lead to poor growth in consumer insects (Scriber, 1978). There was decrease in conversion efficiency with decrease in leaf moisture content for larvae of different butterflies and moths.

In Japan, it was observed that when silkworms were fed mulberry leaves under 30th order, they frequently matured one day later compared with those fed the upper leaves, especially female larvae (Sudo et al., 1979). Significant correlation coefficients were obtained between cocoon weight or cocoon shell weight and the leaf order. The size of the cocoon also became smaller as the leaf order went downwards. The difference in leaf quality derived from leaf order had remarkable effects on the larval growth and cocoon quality. A significant correlation was found between larval body weight after about fourth day of fifth instar and leaf order.

Narayanaprakash et al. (1985) studied the effect of dietary water content on food utilization and silk production in Bombyx mori L. In both the hybrids (NB₁₃ x NB₇ and L x KA), decrease in water content of mulberry leaf affected different energetic parameters. Bivoltine larvae thrived well on tender leaves and the cross breed on mature leaves. Bivoltine larvae fed on fresh, tender leaves spun larger cocoons compared to cross breed larvae which produced heavier cocoons on mature leaves.

According to Yamashita and Yazawa (1980), at each time of growth of mulberry leaf, the aminoacids such as glutamic acid, aspartic acid, alanine occurred at a constant level regardless their ages. On the other hand, asparagine and glutamine accumulated greatly in young leaves at actively growing season. Incorporation of ¹⁴C into total aminoacids in 2nd, 7th and 23rd leaf order was 2.2×10^4 , 12.6×10^4 and 4.9×10^4 dpm per gram leaf, respectively. The content of asparagine was 7.9, 4.9 and 0.1 μ moles per gram of leaf of the above respective leaf orders.

Sudo et al. (1981) compared the quality of mulberry leaves of different nitrogen nutrition for silkworm. A linear regression equation with significant minus correlation between the leaf order and silkworm body weight, cocoon weight or cocoon shell weight existed. There was a

significant negative correlation between nitrogen content in leaf and leaf order. A highly significant correlation was found between nitrogen content in leaf and silkworm body weight, cocoon weight or shell weight.

2.7 Effect of viral infections in late larval instars of insects

According to Vago and Cayrol (1956), the larvae of Plusia gamma that became infected with nuclear polyhedrosis virus when full fed, pupated in some cases but did not give rise to adults.

The resistance to viral infections in Pseudaletia unipuncta was found to increase directly with the age of the caterpillar (Tanada, 1956).

Hafez (1958) investigating on the polyhedrosis of the larvae of Prodenia litura found that when full fed larvae were infected per os, there was 59 to 73 per cent mortality among the resultant pupae as compared with 33 to 36 per cent among uninfected ones. The adults emerging from the survivors were malformed and did not oviposit. Moth survival was found to be low.

Ignoffo (1966) observed maturation immunity to nuclear polyhedrosis virus in both Heliothis zea and H. virescens. The large amount of pupal mortality in

Lymantia dispar observed in the late stage of development in four areas of Connecticut was a polyhedrosis disease. Total moth emergence was less than 50 per cent (Leonard, 1967).

In laboratory tests with a nuclear polyhedrosis virus infecting Heliothis armigera, Atger (1969) observed complete mortality when younger larvae were infected. However, 38 per cent of the older larvae died and the rest pupated, but only half of these gave rise to adults which in turn, laid unfertile eggs. Neelgund and Mathad (1974) also reported maturation immunity to nuclear polyhedrosis virus in P. separata based on decreased mortality, ID_{50} and IT_{50} .

Jacob et al. (1979) observed that the larvae of Nymphula depunctalis infected with nuclear polyhedrosis during later instars were able to complete larval development, but died in pupal stage. The effects of a nuclear polyhedrosis virus on various developmental stages of Spodoptera littoralis were investigated with three concentrations (ranging from $10^2 \times 10^5$ to $10^2 \times 10^8$ /larva) fed to third and fifth instar larvae (Abul-Nasr et al., 1979). The mortality rate was 54 to 100 and 40 to 73 per cent, respectively. The ID_{50} (determined at 20 to 24°C) increased with lower doses and later application of the virus to the larvae. The larvae that survived treatment resulted in pupae of lighter body weight than those developing from

untreated larvae, a higher proportion of deformed adults and females with 22 to 48 per cent reduction in the total number of eggs laid. The rate of mortality in the eggs and larvae that survived virus treatment was higher than that in the untreated females.

Hadimani (1980) observed 40.50 per cent mortality of Pure Mysore x NB₄D₂ worms, when they were infected with Kenchu virus in fifth instar. It resulted in 0.06 per cent reduction in cocoon weight.

According to Subrahmanyam and Ramakrishnan (1981), S. litura infected with nuclear polyhedrosis virus failed to moult more than once after infection. Infected last instar larvae failed to pupate and had prolonged larval stage.

Effect of nuclear polyhedrosis virus infection of Pseudoplusia includens on the surviving pupae and adults on caged soybean plants was studied by Young and Yearian (1982). The mortality in the pupal stage was higher following the treatment in the fifth or sixth larval instar.

2.8 Interactions amongst pathogens of insects including those of silkworm

Multiple infections by different pathogens within an insect occur under laboratory/commercial rearing

conditions. These infections may be simultaneous or may occur one after another. The result of the interaction may be an interference, co-existence or synergism.

Tanada (1959) observed synergism between granulosis virus and nuclear polyhedral virus in the armyworm, Pseudaletia separata.

Enhanced interaction was lacking in the mixed infection of spruce budworm, Choristoneura fumiferana with granulosis virus and nuclear polyhedral virus (Bird, 1959).

Stephens (1959) in her studies with mixed bacterial infections of Pseudomonas aeruginosa and Serratia marcescens in grasshoppers found mortality due to septicemia only, either one of the species predominating in the blood.

According to Shvetsova and Tsai (1962), enhanced interaction was absent when the two noctuid caterpillars viz., Agrotis segetum and Hadena sordida were simultaneously infected with granulosis virus and nuclear polyhedrosis virus.

Aruga et al. (1963) found that when CPV of the pine caterpillar, Dendrolimus spectabilis was fed to first instar larvae one to five days before or simultaneously with wildworm CPV, it interfered with the latter. On the other hand, the

silkworm CPV did not interfere with the infection of pine caterpillar CPV when it was fed one to two days before the latter.

The silkworm CPV enhanced the infection of the alfalfa caterpillar (Colias eurytheme) CPV when the two viruses were fed together to second instar silkworms (Tanada and Chang, 1964).

According to Aruga et al. (1965), when the flacherie virus was fed to silkworm larvae prior to silkworm CPV, it interfered with the infection of the latter. Two applications of flacherie virus were much more effective than a single application in causing the interference. There was no apparent interference when flacherie virus was fed to silkworm larvae after silkworm CPV.

Although there was an increased percentage of mortality of Trichoplusia ni when NPV and granulosis virus (GV) were fed together to the larvae, the increase was not statistically significant from that of the treatment with each virus separately. The increase was being caused by an additive effect of each virus. Neither interference nor synergism was observed between these two viruses (Lowe and Paschke, 1968a).

Lowe and Pašchke (1968b) reported an interference effect in T. ni when NPV was administered 5 to 7 days after GV. The mortality from this treatment was not decreased, but the larvae lived longer than with simultaneous inoculation and no polyhedrosis was evident in infected larvae.

Studies on simultaneous infection of common cockchafer, Melolontha melolontha by Bacillus popilliae, Rickettsiella melolonthae and Vagoid virus melolonthae indicated that the bacterium seemed to inhibit Rickettsia. Virus and Rickettsia were more compatible than bacterium and Rickettsia (Hurpin and Robert, 1968).

Treatment of Pseudaletia unipuncta with NPV alone resulted in only one larva becoming infected with this virus. Addition of Hawaii stock of GV to NPV greatly enhanced the infection by the latter as shown by 14 out of 20 larvae showing infection with NPV alone or together with GV. GV at a concentration below its infection dose did not enhance NPV (Tanada and Hukuhara, 1968).

Amergier et al. (1969) observed synergistic effect between NPV and DNV of Galleria mellonella.

Kodama and Nakasuji (1971) found that a strain of Streptococcus faecalis - S. faecium when fed along with Serratia piscatorum to silkworm larvae facilitated the latter to invade the haemocoel. S. piscatorum by itself was non-pathogenic through per os, but was highly virulent when injected.

When mixtures of GV capsules and nuclear polyhedra were fed to P. unimaculata there was direct increase in number of larvae infected with NPV as the concentration of the capsules increased. A capsule factor, possibly a protein rather than the virus particle occluded within the capsule was responsible for enhancement of infection of NPV. The capsule factor enhanced the infection of NPV even when the larvae were treated under gnotobiotic condition (Tanada and Hukuhara, 1971).

According to Nordin and Maddox (1972), the total larval mortality in H. cunea in treatments involving virus alone, Nosema sp. alone and virus plus Nosema sp. was 97, 21 and 71 per cent, respectively. Combination of virus plus Nosema sp. resulted in a lower mortality.

The interaction between CPV and infectious flacherie virus (IFV) of silkworm, B. mori was investigated by Inoue (1974a). Following the simultaneous infection of

silkworm with two viruses, independent co-existence was observed in that CPV multiplied in columnar cells and IFV multiplied in goblet cells. However, the period of lethal infection was shorter on larvae subjected to double virus infection than on those infected with each virus separately.

Kurisu (1974) studied the lethal effect of secondary bacterial infection on silkworm larvae infected with IFV. The mortality in the mixed infection with IFV and bacteria invading simultaneously was the highest. But in the natural double infection, virus infection happened primarily and secondary bacterial infection occurred through mulberry leaves per os and the lethal effect was moderated. In the case of silkworm reared under aseptic condition when the virus invasion was not accompanied by bacterial infection the mortality of the gnotobiotic larvae became lower. When the gnotobiotic larvae were subjected to bacterial contamination, the mortality of the larvae increased rapidly. Thus the silkworm mortality in viral flacherie is considered to be caused by the secondary bacteriosis which had been predisposed by the primary virus infection.

Double infection with the viruses each of IFV, CPV and SFV in the midgut of silkworm was investigated by Sato

and Inoue (1978). The flacherie virus (FV) multiplied in goblet cells and CPV in columnar cells of midgut epithelium. The number of virus-infected cells in the midgut infected doubly with flacherie virus and small flacherie virus (SFV) was approximately the same compared to the total numbers of virus-infected cells in larvae infected singly with FV or SFV. CPV and SFV multiplied in the same columnar cell showing CPV in cytoplasm and SFV in the nucleus. The larvae infected with two kinds of viruses showed an increased mortality and a low E_{50} value as compared to those larvae infected with single virus. The studies revealed the independent multiplication of virus in a larva infected with two kinds of viruses.

Inoue (1981) conducted studies on the double infection of midgut epithelial cells with nuclear polyhedrosis virus (NPV) and cytoplasmic polyhedrosis virus (CPV). Double infection occurred in majority of columnar cells. Both viruses were observed to be multiplying independently and fusion of two kinds of proteinaceous crystals was not observed. However, a lot of nucleocapsids of NPV attached to the surface of CPV inclusion body, followed with incorporation of some of them into the inclusion body. It was inconsistent to NPV polyhedron which incorporated NPV and its empty envelopes.

The associative effect of bacteria S. marcescens and Staphylococcus spp. in silkworm was studied by Vasantharajan and Munirathnamma (1978). Two isolates VK 42 and VK 45 belonging to Staphylococcus were potential synergists of S. marcescens. S. marcescens VK1 together with VK42 or VK45 was highly lethal, whereas, singly none of these strains caused any damage. When VK1 and VK42 were fed together, out of 50 silkworms 24 died amongst which 16 exhibited Serratia symptoms, while infection with VK1 two days prior to VK42 resulted in mortality of 19 worms from which no worm expressed Serratia symptoms and further infection with VK1 two days after VK42 resulted in mortality of 24 out of 50 worms, and 20 worms exhibited Serratia symptoms. Similar trend was observed even in association with VK45. They inferred that Serratia was invasive only when the associative organism was already present in the gut. If, instead, Serratia was fed first to be followed later by Staphylococcus, no damage was seen.

The bacteria, Aerobacter aereogenes and Proteus mirabilis affected digestive tract of geometrid, Borania selenaria and the former was not considered highly pathogenic since it lacked invasive power to penetrate through midgut wall. The possible proteolytic activity of P. mirabilis made the gut wall more vulnerable to entrance of secondary

bacterium A. aereogenes indicating the synergistic effect of the two species (Wysoki and Raccach, 1980).

Matsumoto et al. (1985) conducted studies on mixed infection with infectious flacherie virus (IFV) and endogeneous bacterium - Streptococcus faecalis that forms the main component of intestinal flora of healthy silkworm larvae and exogeneous bacterium - Serratia marcescens. Higher virulence of IFV was associated with these bacteria. S. faecalis at 10^2 , 10^4 and 10^6 cells/ml with IFV at 10^{-3} dilution resulted in one hundred per cent mortality of fifth instar worms when inoculated during initial fourth instar, while the single larval weight at the end of fourth instar was 0.86, 0.84 and 0.81 g., respectively, compared to 1.14 g in uninfected control batch. Inoculation of fourth instar worms with IFV (10^{-3}) alone, S. faecalis alone (10^6) and S. marcescens alone (10^6) resulted in fifth instar larval weight of 2.95, 4.63 and 4.31 g with larval mortality of 20.0, 0.0 and 0.0 per cent while simultaneous infections with IFV + S. faecalis, IFV + S. marcescens caused larval weight of 2.06 and 2.40 g respectively, with mortality of 100 per cent in each case. Infection with IFV followed by S. faecalis and S. marcescens gave larval weight of 3.12 and 3.55 g respectively of fifth instar with mortality of 100 per cent

in both. On the other hand, infection with S. faecalis followed by IFV yielded fifth instar larval weight of 3.13 g while that of S. marcescens followed by IFV resulted in 3.95 g of larval weight with mortality of 100 per cent. Uninfected control had a larval weight of 4.58 g with no mortality. Ordinary larvae fed on artificial diet or on mulberry leaves were 10 to 30 times more sensitive to IFV infection than gnotobiotic larvae in each instar.

Matsumoto et al. (1986) studied the effects of antijuvenile hormone (AJH) on mixed infection of infectious flasherie virus (IFV) and bacteria viz., Streptococcus faecalis and Serratia marcescens in the silkworm larvae. Trimoultar larvae were induced by administration of AJH in the third instar. The ^Suceptibility of trimoulters to mixed infection of IFV and bacteria was determined. When a mixture of IFV at 10^{-2} , 10^{-3} , 10^{-4} and 10^{-5} dilutions with S. faecalis (10^6) was fed to fourth instar larvae that received AJH, the per cent mortality was 17.4, 34.8, 21.7 and 4.3 respectively when compared to 100, 100, 30 and 10 per cent in respective controls that did not receive AJH. When dilutions of 10^{-2} , 10^{-3} , 10^{-4} and 10^{-5} of IFV were fed with S. faecalis (10^6) to the fourth instar worms that had received AJH treatment, the mortality was 21.7, 21.7, 17.4 and 0 in comparison to 100, 100 and 25 per cent in case of the first three

controls, respectively. The administration of AJH to the infected larvae prevented death from occurring and the presence of either of the bacteria in the intestine of the larvae could not be detected. When larvae, that were administered AJH during third instar, were infected with IFV in the fourth instar in the presence of S. faecalis, the bacteria accumulated in the larval intestine. However, in case of mixed infection of IFV and S. marcescens to fourth instar silkworms that had received AJH during third instar, the bacteria were not noticed in the larval intestine.

MATERIAL AND METHODS

III. MATERIAL AND METHODS

During the periods of 1981-1986, studies were conducted on Kenchu virus disease of silkworm, Bombyx mori L. at Department of Agricultural Entomology, Agricultural College, Bangalore-560 024 and Department of Sericulture, College of Agriculture, Dharwad-580 005, University of Agricultural Sciences, Bangalore-560 065. The techniques employed in the studies are presented in the following pages under different headings.

3.1 Silkworm rearing techniques

3.1.1 Disinfection of rearing room

Two separate silkworm rearing venues, one for rearing healthy silkworms and the other for rearing experimentally infected worms having similar conditions were used. Six days prior to the hatching of eggs, the rearing rooms and equipments were cleaned, washed and properly disinfected with four per cent formalin at the rate of 800 ml per 10 m² (Krishnaswami et al., 1973) using Gator Rocker sprayer. For effective disinfection, the rooms were kept closed for 24 hours after sealing cracks in doors, windows and ventilators with papers. Afterwards, the rearing venues were used for silkworm rearing.

3.1.2 Silkworm rearing

Pure Mysore breed of silkworm, B. mori was employed in all the experiments since it was proved to be the most susceptible breed to Kenohu virus (Bhaskar, 1984; Narayanaswamy et al., 1985b). Eggs were surface sterilized with two per cent formalin, washed with sterile water and dried in shade. They were kept at 25 to 27°C and 85 to 90 per cent relative humidity, created using moist foam rubber strip and covering with paraffin paper. The eggs were black-boxed for 24 hours in pin head stage followed by exposure in diffused light to obtain uniform hatching (Krishnaswami, 1978b). At 10 a.m. fresh tender mulberry leaf grown as per recommended practices (Krishnaswami, 1978a), chopped to bits of 0.5 cm² were spread over the emerged larvae on egg card and they were brushed on to a paraffin paper spread in a wooden rearing tray. Wet foam rubber strip was kept all around the silkworm bed in the tray and another paraffin sheet was used to cover the bed. The paraffin paper and wet foam rubber strip were used only upto the end of third larval instar (except during moulting periods). The trays were arranged in rearing racks with legs resting on ant wells. The worms were fed five times a day (Marayanan and Chowla, 1965; Krishnaswami, 1978b; 1979) at 6 a.m., 10 a.m., 2 p.m., 6 p.m. and 9.30 p.m.

with suitable quality mulberry leaf of variety Kanva-2 in all experiments unless specified under specific experiments. Chopped leaf and unchopped leaf were fed to young and late age worms, respectively. The spacing for worms and cleaning of beds and other rearing procedures were used as recommended by Krishnaswami (1978b). The actual rearing site was covered with nylon net to prevent uji-fly infestation as recommended by Jolly (1981). The ripe worms were mounted on bamboo mountages at the rate of 50 worms/ft² of mounting space and the cocoons were harvested five days after spinning.

3.2 Preparation of cell free Kenchu virus extract

Surface sterilized mulberry leaves treated with Kenchu virus suspension were fed to second instar Pure Mysore silkworms and the worms showing typical "Kenchu" symptoms were collected and the virus was extracted by following the method of Inoue and Ayuzawa (1972a), employed for infectious flacherie virus of silkworm, as modified by Hadimani (1980). For isolation of the virus, the experimentally diseased worms were homogenized in sterile pestle and mortar containing Sorensen's phosphate buffer (0.72 M, pH 7.2). The ratio of larvae to buffer was 1:4(W/V). The homogenized material was kept in refrigerator for 24 hours (Poinar and Thomas, 1978).

Then it was filtered first through several layers of sterile muslin cloth twice and later with sterile Whatman No.1 filter paper. This filtrate was centrifuged at 3000 rpm for 10 minutes and repeated thrice. The supernatant was centrifuged twice at 18,000 rpm for 20 minutes each time in a Remi C-24 refrigerated centrifuge. The resultant supernatant was collected and the presence of the Kenchu virus (Plate 3) was confirmed by electron microscopy (Hitachi HU 11F). This formed the virus stock suspension. The stock suspension was stored at 5°C.

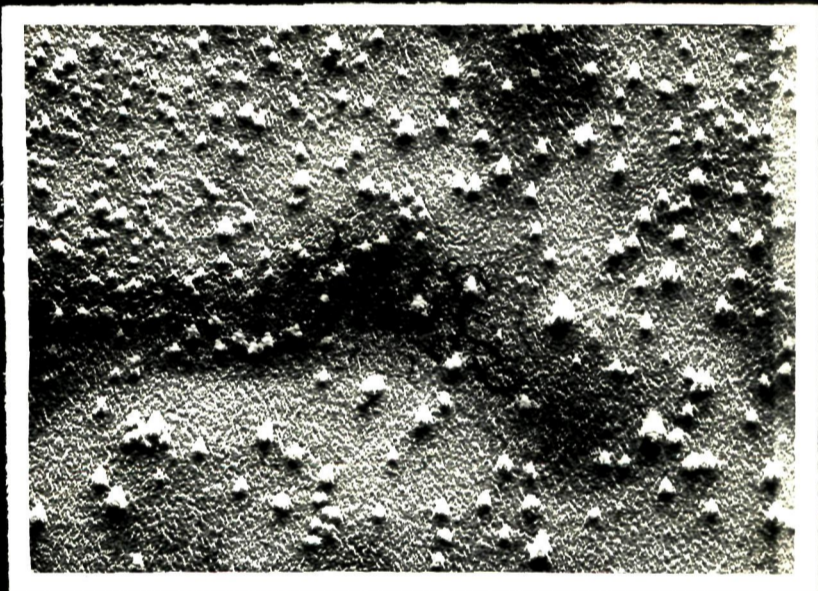
3.3 Testing of isolates of Kenchu virus against antisera of infectious flacherie virus and denonucleosis virus of silkworm

The antisera for infectious flacherie virus (IFV) and denonucleosis virus (DNV) of silkworm were obtained from Sericultural Experiment Station, Ibaraki, Japan. To know the antigenic relationship of Kenchu virus with the antisera of IFV and Ina DNV, double gel diffusion test was carried out in this laboratory. Fluorescent Antibody test on the Kenchu virus infected midgut cells was carried out by Dr. Hajime Inoue.

3.3.1 Collection of diseased material

Silkworms showing typical Kenchu symptoms were collected from different sericultural areas of Karnataka.

Plate 3. Platinum shadowcasted Kenchu virus (22,000X).



All the samples collected were from silkworm crops which had suffered from an epizootic of Kenchu disease. The samples were collected from III and IV instars. The areas covered were Bangalore, Kanakapura, Chamarajanagar and Chitradurga.

3.3.2 Double gel diffusion test

Saline gel (1.5 per cent) with sodium azide (1:1000) was uniformly poured on a clean glass microslide to a thickness of 2 mm. Wells were dug at equidistance from central well. One ml of distilled water was added to the original stock antisera and then it was diluted 16-fold with physiological saline and added to central well. Antigens-Kenchu isolates collected from Bangalore, Kanakapura, Chamarajanagar and Chitradurga were added separately to the peripheral wells. It was incubated at room temperature for 48 to 72 hours. Then the gel was examined for precipitin bands. Separate tests were run with IFV and DNV antisera.

3.3.3 Fluorescent antibody technique

Epithelial cells from both anterior and posterior part of midgut from Kenchu virus infected silkworm, were smeared on to clean glass slides. The preparation was dried at room temperature for 15 minutes. Then the cells

were fixed with acetone for 10 minutes at room temperature. These microslides were sent to Dr. Hajime Inoue for the fluorescent antibody test (Inoue and Ayuzawa, 1971).

3.4 Infectivity technique

Infection (per os) with Kenchu virus was done by feeding virus contaminated mulberry leaf. Mulberry leaves, washed with tap water, were surface sterilized with 70 per cent ethyl alcohol using sterile cotton. Sufficient time was allowed for evaporation of moisture from leaf surface. Then the required dilution of Kenchu virus suspension was uniformly smeared at the rate of 0.2 ml for about 50 cm² leaf area and sufficient quantity of such contaminated leaf was provided for first feed and further feeds were of uncontaminated mulberry leaf.

In the studies on interaction between Kenchu virus and bacterial isolates, 0.2 ml of the appropriate virus extract was mixed with 0.2 ml of appropriate bacterial extract and the mixture was used on 50 cm² of leaf.

3.5 Effect of refrigeration of silkworm eggs and Kenchu virus infection in silkworm

Pure Mysore silkworm eggs of one, three, five and seven days' age were refrigerated for a duration of two, four, six and eight days each at 5°C in a refrigerator.

After the required duration of refrigeration, the eggs were released and kept at room temperature. Hatching percentage was recorded. The resultant worms were infected with Kenchu virus stock suspension and observations were recorded on weight of worms on seventh and 15th day after infection, number of worms entering subsequent instars and time taken for 50 per cent mortality. In addition, the other parameters like maximum larval weight, cocooning, cocoon weight, pupal weight, shell weight, number of cocoons formed and moth emergence were also recorded.

3.6 Effect of releasing Kenchu virus infected silkworms into healthy populations in various proportions

Second, third and fourth instar silkworms were separately fed with Kenchu virus stock suspension and they were released at a density of 4, 8, 16, 20 and 24 per cent in healthy populations of respective instars. Observations were made on larval weight, number of worms entering subsequent instars, instar duration, number of larvae competing^l cocooning, cocoon weight, shell weight, pupal weight and moth emergence.

3.7 Influence of nutrition on intensity of Kenchu virus infection

3.7.1 Effect of underfeeding in early instar on subsequent infection by Kenchu virus

Silkworms from first to third instar were reared separately by providing two, three, four and five feeds

per day. When they entered fourth and fifth instars, immediately after third and fourth moult, the worms were separately administered 10^{-3} dilution of stock Kenchu virus extract for the first feed. In both fourth and fifth instars five feedings were provided per day with uncontaminated mulberry leaf. Observations were recorded on larval weight, instar duration, cocooning, cocoon weight, pupal weight, cocoon shell weight and moth emergence.

3.7.2 Effect of starvation on Kenchu virus infection in silkworm

Third, fourth and fifth instar silkworms immediately after moulting were separately starved for 12, 24, 36 and 48 hours and after starving period, they were infected with 10^{-3} dilution of Kenchu virus for first feed only. Suitable controls were also maintained. Following were the treatments.

1. No starvation + infection
2. No starvation and no infection
3. 12 hours starvation + infection
4. 12 hours starvation only
5. 24 hours starvation + infection
6. 24 hours starvation only

7. 36 hours starvation + infection
8. 36 hours starvation only
9. 48 hours starvation + infection
10. 48 hours starvation only

Observations were made on larval weight, duration of moulting and instars, mortality, cocooning and weights of cocoon, pupa and cocoon shell and moth emergence.

3.7.3 Effect of feeding silkworms with leaves of different maturity on Kenchu virus infection

From the beginning of third, fourth and fifth instar, separate batches of worms were fed with (i) only tender leaf (ii) only middle leaves (medium mature leaf) and (iii) only lower most leaves (coarse leaf) in addition to two batches fed with leaf suitable to the instar as recommended to that instar, the other batch being fed with leaf recommended to that instar but pretreated with water. In the beginning of every instar, the worms were separately infected with Kenchu virus of 10^{-3} dilution. Following were the treatments:

1. Feeding with tender leaf + Kenchu virus infection
2. Feeding with tender leaf only
3. Feeding with medium mature leaf (middle leaf order) + Kenchu virus infection

4. Feeding with medium mature leaf only
5. Feeding with coarse leaf + Kenchu virus infection
6. Feeding with coarse leaf only
7. Feeding with leaf recommended to that instar pretreated with water + Kenchu virus infection
8. Feeding with leaf recommended to that instar pretreated with water only
9. Feeding with leaf recommended to that instar + Kenchu virus infection
10. Feeding with leaf recommended for that instar only.

Observations on larval weight, mortality, durations of moulting and instars, cocooning, cocoon weight, pupal weight, shell weight and moth emergence were made.

3.10 Interaction effect of simultaneous infection with Kenchu virus and bacterium - *Staphylococcus aureus* Rosenbach in silkworm

Bacterial culture: The *Staphylococcus aureus* culture was originally isolated from flacherie infected silkworms in the Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore. The culture obtained from the Department was subcultured and maintained in the University Sericulture Laboratory at Dharwad.

The bacterium, S. aureus was maintained on nutrient agar (Beef Extract - 3 g; Peptone - 5 g; Agar - 15 g and distilled water - 1 litre) slants. Different dilutions of S. aureus were arrived at by employing dilution plate technique and bacterial suspensions containing 10^7 , 10^6 , 10^5 , 10^4 and 10^3 cells/ml were prepared in sterile distilled water.

Dilutions of 10^{-2} , 10^{-3} and 10^{-4} of stock Kenchu virus extract were also prepared.

Simultaneous infection with different concentrations of Kenchu virus and the bacterial suspensions individually as well as with all their possible combinations was effected in the beginning of third, fourth and fifth instars as well as one day and two days' old fifth instar silkworm. The following treatments were given .

V_1	= Kenchu virus at 10^{-2} dilution
V_2	= " " 10^{-3} "
V_3	= " " 10^{-4} "
B_1	= <u>S. aureus</u> at 10^7 cells per ml
B_2	= " " 10^6 " "
B_3	= " " 10^5 " "
B_4	= " " 10^4 " "
B_5	= " " 10^3 " "

V ₁ B ₁	=	Kenchu virus (10 ⁻²)	+	<u>S. aureus</u>	at 10 ⁷ cells/ml
V ₁ B ₂	=	..	+	..	10 ⁶ ..
V ₁ B ₃	=	..	+	..	10 ⁵ ..
V ₁ B ₄	=	..	+	..	10 ⁴ ..
V ₁ B ₅	=	..	+	..	10 ³ ..
V ₂ B ₁	=	Kenchu virus (10 ⁻³)	+	..	10 ⁷ ..
V ₂ B ₂	=	..	+	..	10 ⁶ ..
V ₂ B ₃	=	..	+	..	10 ⁵ ..
V ₂ B ₄	=	..	+	..	10 ⁴ ..
V ₂ B ₅	=	..	+	..	10 ³ ..
V ₃ B ₁	=	Kenchu virus (10 ⁻⁴)	+	..	10 ⁷ ..
V ₃ B ₂	=	..	+	..	10 ⁶ ..
V ₃ B ₃	=	..	+	..	10 ⁵ ..
V ₃ B ₄	=	..	+	..	10 ⁴ ..
V ₃ B ₅	=	..	+	..	10 ³ ..

C₁ = Buffer control

C₂ = Distilled water control

C₃ = Normal untreated control

Observations were made on instar duration, moulting duration, number of worms entering subsequent instars, larval weight, larval mortality, cocooning, cocoon weight, shell weight, pupal weight and moth emergence.

3.11 Observations recorded and statistical analysis

The regular parameters like larval weight, moulting duration, instar duration, progression of instar, mortality,

extent of cocooning, weights of cocoon, cocoon shell and pupa, cocoon shell ratio and moth emergence were recorded. The duration of instar did not include the moulting duration. Though symptom exhibition is not an accurate parameter for measurement of any disease, the Kenohu symptoms were clear enough for approximate estimate of the intensity of the disease and hence it was considered useful as a parameter. Per cent cocooning and moth emergence were based on initial number of larvae used.

In all the experiments, three replications of 50 worms each were used. For recording larval weight, cocoon weight, cocoon shell weight and pupal weight, ten individuals from each of three replications were employed. In cases where individual weights of these were provided, usually the mean of five individuals for each of the three replications was arrived at. The data were analysed statistically by one way analysis of variance after transformation to $\sqrt{x + 0.5}$ wherever necessary (Sundararaj *et al.*, 1972). The data with percentages were analysed after angular transformation of original figures (Snedecor and Cochran, 1972). The level of significance used in "F" and "t" tests was $P = 0.05$.

EXPERIMENTAL RESULTS

IV. EXPERIMENTAL RESULTS

The results of experiments conducted under different objectives are presented in the following pages under concerned headings.

4.1 Testing of isolates of Kenchu virus against antisera of infectious flacherie virus and Ina densonucleosis virus of silkworm

In double gel diffusion test, precipitation bands were not formed by Kenchu virus isolates, collected from Bangalore, Kanakapura, ChamaraJanagar and Chitradurga, with the antisera of either infectious flacherie virus or Ina densonucleosis virus of silkworm.

Even in the fluorescent antibody examination of Kenchu virus infected midgut cells, specific fluorescence was not produced with the fluorescen-isothiocyanate conjugated antibodies of infectious flacherie virus as well as densonucleosis virus, when examined under fluorescent microscope.

4.2 Effect of refrigeration of silkworm eggs on the development and intensity of Kenchu disease

Eggs of the multivoltine race Pure Mysore, were refrigerated at different stages of growth (one, three, five and seven days) for periods varying from 2 to 8 days. Infection experiment was conducted by per oral

infection immediately after hatching. Appropriate uninfected controls were maintained throughout.

Hatching of eggs was not affected much as it ranged from 83.65 to 96.09 per cent in different treatments compared to 93.49 per cent in unrefrigerated eggs(Appendix I).

The larval weights of ten worms each recorded on the 7th and 15th day are presented in Table 1. The adverse effect of refrigeration of eggs for more than two days was evident at all stages of embryonic growth. Statistically significant reduction in larval weight ranging from 0.0243 g to 0.0349 g was seen on 7th day as compared to unrefrigerated or 2 day refrigerated treatments (0.0673 to 0.0768 g) in larvae hatching from one, three, five and seven day-old eggs refrigerated for periods above four days. More marked, statistically significant differences in larval weight and size were also observed on the 15th day (Plates 4,5,6 & 7) which further substantiated the growth retarding effect of refrigeration of eggs for more than two days at any stage of development in the egg. Unrefrigerated control larvae and larvae from seven day-old eggs refrigerated for two days manifested body weights ranging from 0.5105 to 0.5992 g as compared to the range of body weights 0.1243 to 0.3751 g observed in the other treatments on 15th day.

Table 1. Effect of refrigeration of silkworm eggs and Kenohu virus infection on larval weight (g)

Sl. No.	Treatments		Weight of 10 worms on			
	Age of egg (days)	Duration of refrigeration (days)	7th day		15th day	
			Infected	Healthy	Infected	Healthy
1.	One day	Two days	0.0381	0.0746	0.0863	0.5220
2.	One day	Four days	0.0275	0.0309	0.0840	0.2159
3.	One day	Six days	0.0235	0.0311	0.0630	0.2101
4.	One day	Eight days	0.0232	0.0248	0.0629	0.1713
5.	Three days	Two days	0.0357	0.0682	0.0638	0.5177
6.	Three days	Four days	0.0316	0.0331	0.0637	0.2747
7.	Three days	Six days	0.0256	0.0315	0.0557	0.1918
8.	Three days	Eight days	0.0193	0.0283	0.0338	0.1393
9.	Five days	Two days	0.0449	0.0673	0.1054	0.5657
10.	Five days	Four days	0.0236	0.0299	0.0669	0.2767
11.	Five days	Six days	0.0219	0.0296	0.0658	0.1835
12.	Five days	Eight days	0.0197	0.0275	0.0316	0.1709
13.	Seven days	Two days	0.0360	0.0768	0.0712	0.5992
14.	Seven days	Four days	0.0287	0.0349	0.0631	0.3751
15.	Seven days	Six days	0.0189	0.0248	0.0401	0.2005
16.	Seven days	Eight days	0.0185	0.0243	0.0298	0.1243
17.	Unrefrigerated control		0.0211	0.0669	0.0324	0.5105
S.E.M. \pm			0.0018		0.0119	
C.D. at 5%			0.00509		0.0336	

**Plate 4. Silkworms on 15th day, that resulted from
seven days old eggs refrigerated for two days.**

A: Uninfected

a: Infected

**Plate 5. Silkworms on 15th day, that resulted from
seven days old eggs refrigerated ^{eight} days.**

M: Uninfected

m: Infected

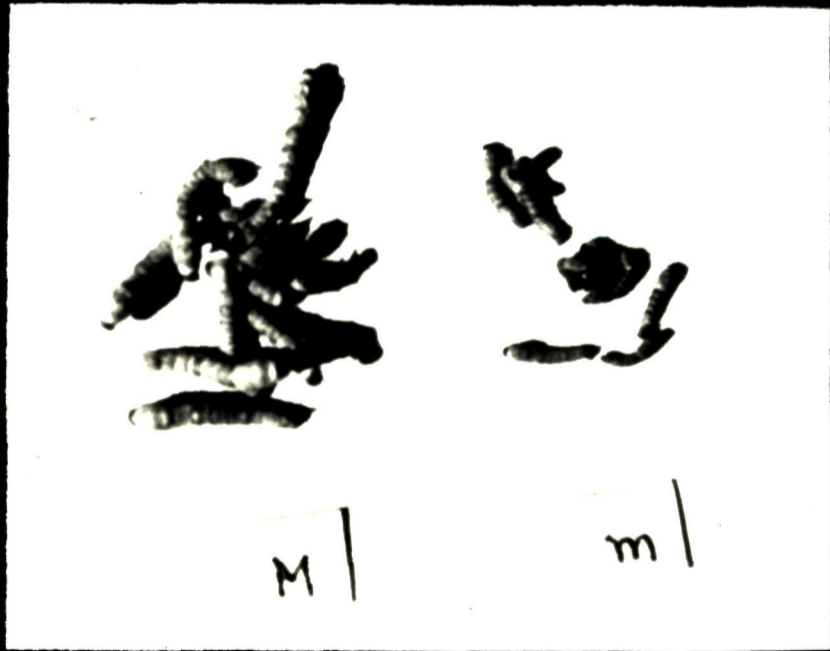


Plate 6. Silkworms on 15th day, that resulted from five days old eggs refrigerated for eight days.

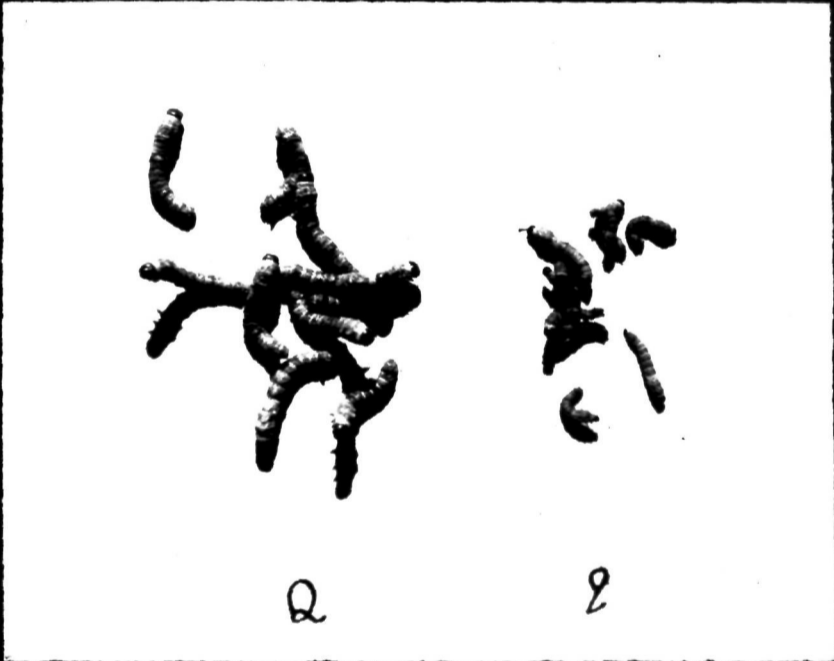
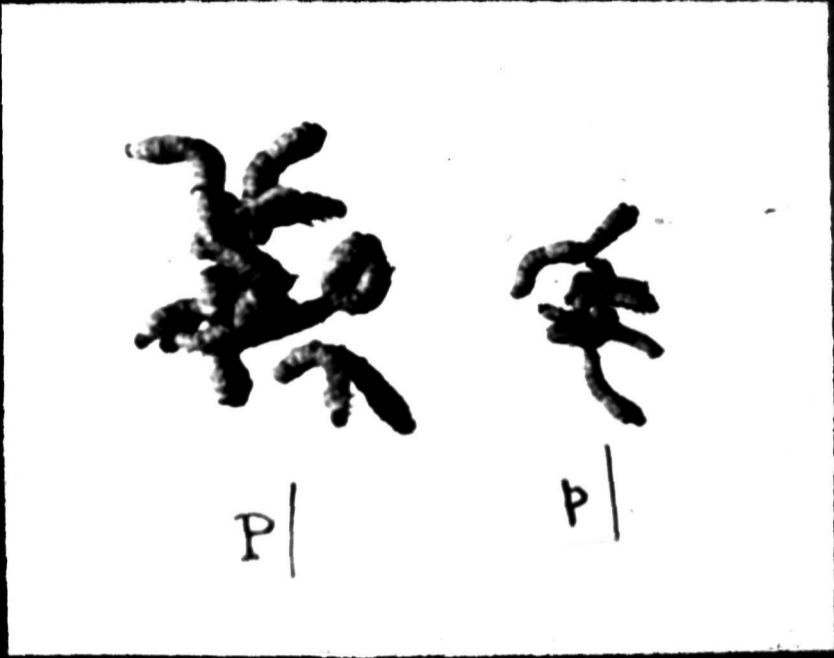
P: Uninfected

p: Infected

Plate 7. Silkworms on 15th day, that resulted from unrefrigerated eggs.

Q: Uninfected

q: Infected



A superimposition of Kenchu virus infection on larvae resulting from refrigerated eggs caused a drastic reduction in larval weight as observed on the 15th day, a trend which was already evident even on the 7th day (Fig.1).

Inhibition of larval growth was manifested in unrefrigerated treatment as also in the two day refrigeration treatment. On the 7th day, larvae from infected, unrefrigerated treatment registered 0.0211 g as against 0.0669 g seen in the corresponding uninfected control. The larval weights in the different infected batches on the 7th day ranged from 0.0185 g to 0.0449 g. Higher body weights were recorded in the corresponding uninfected controls - 0.0243 g to 0.0768 g (Table 1). Statistically distinct reduction in body weight as a consequence of Kenchu virus infection was seen after 15 days when the infected batches corresponding to the five to seven days old eggs showed weights ranging from 0.0298 g to 0.1054 g as compared to 0.1243 g to 0.5992 g in uninfected, control larvae. The results also indicated a lack of significant interaction between refrigeration and Kenchu virus infection.

**Fig.1. Effect of refrigeration of silkworm eggs and
Keshu virus infection on larval weight on
seventh day**

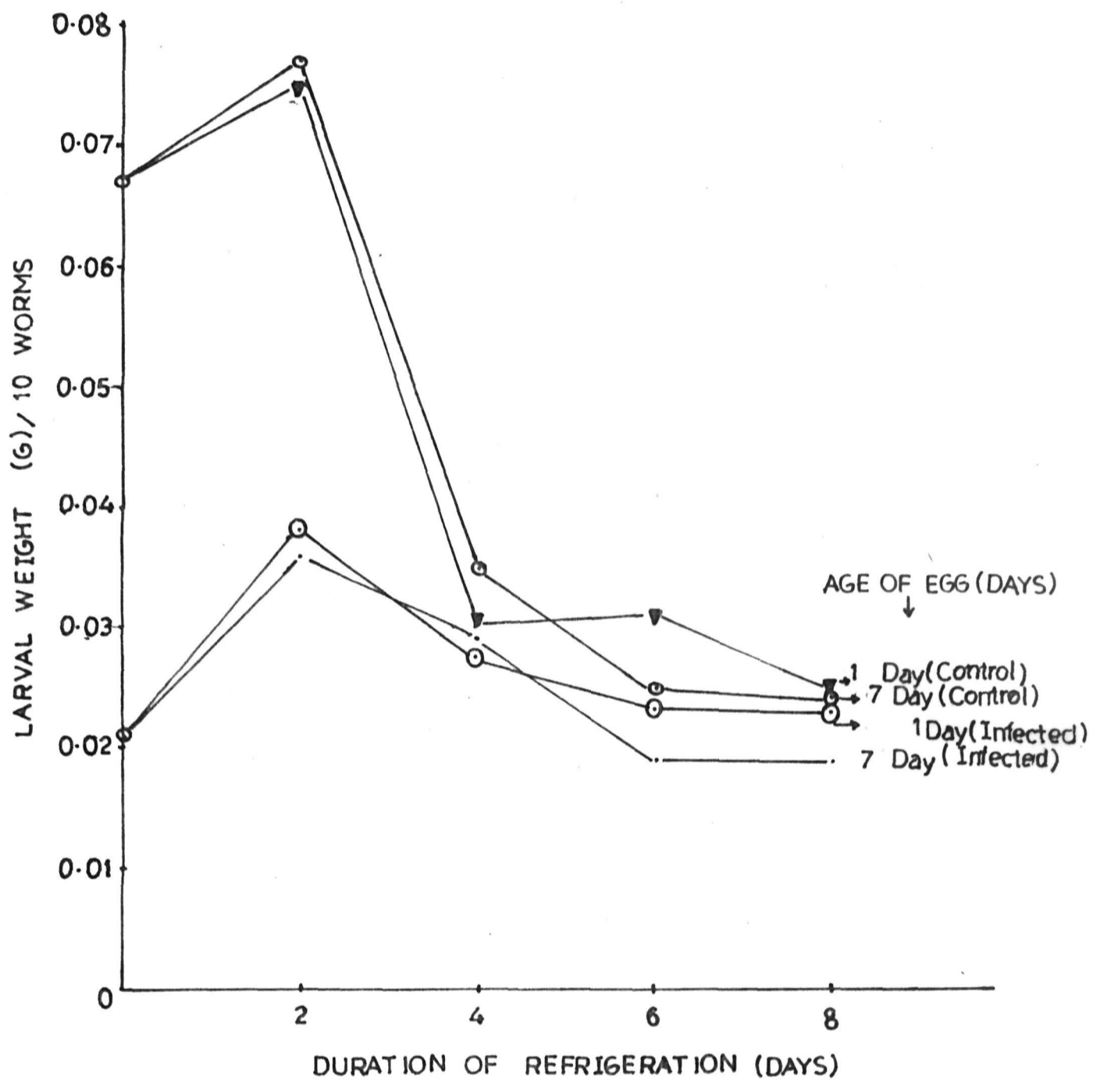


FIGURE - 1

4.2.2 Instar progression

Successful completion of instars is a fair pointer to the vigour and health of the silkworm. Instar progression, especially into the III instar, has furnished useful information on this aspect (Table 2 and Fig.2). In the healthy, uninfected batches subjected to a mild refrigeration of 2 days in the case of one to five days old eggs, the larval progression into the III instar ranged high from 92 to 95 per cent. However, longer periods of refrigeration of eggs tended to decrease the percentage of worms progressing into the III instar (56.66 to 76.00 per cent). As revealed from the data, the cold tolerance capacity of Pure Mysore silkworm eggs tended to decrease with age. To cite the extreme examples of one and seven day old egg treatments, the percentages of worms entering III instar were 96.33 and 75.34 per cent for a 2-day period of refrigeration and 76.00 and 56.66 per cent for 8-day period.

Infection with Kenchu virus led to a drastic reduction in the percentage of worms crossing the moult barriers from 93.66 to 71.34 and from 97.34 to 18.66 per cent for II and III instar entry respectively in larvae from unrefrigerated eggs (Fig.2). Refrigeration

Table 2. Effect of refrigeration of silkworm eggs and Kenchu virus infection on extent of worms entering subsequent instars and ET₅₀ for mortality

Sl. No.	Treatments	Per cent worms entering II instar		Per cent worms entering III instar		ET ₅₀ for mortality (days)		
		Infected	Uninfected	Infected	Uninfected	Infected	Uninfected	
	Age of egg (days)	3	4	5	6	7	8	9
1.	One day		83.34 (72.82)*	98.66 (84.58)*	60.66 (51.18)*	96.33 (79.60)*	18.67 (4.537)**	0.00 (0.707)**
2.	"		80.66 (64.09)	96.00 (80.68)	40.66 (39.59)	92.33 (74.53)	17.67 (4.262)	0.00 (0.707)
3.	"		80.66 (63.96)	82.66 (65.88)	17.34 (24.45)	80.66 (64.31)	17.67 (4.262)	0.00 (0.707)
4.	"		79.34 (63.07)	82.00 (64.97)	18.66 (25.55)	76.00 (60.67)	16.67 (4.143)	0.00 (0.707)
5.	Three days		85.34 (68.16)	98.66 (86.15)	44.00 (41.54)	95.34 (79.80)	20.67 (4.600)	0.00 (0.707)
6.	"		84.66 (67.02)	98.00 (83.44)	26.66 (31.07)	92.66 (74.32)	17.67 (4.262)	0.00 (0.707)
7.	"		76.00 (60.83)	84.66 (79.13)	17.34 (24.59)	87.34 (69.40)	16.67 (4.143)	0.00 (0.707)
8.	"		64.00 (53.13)	87.34 (69.40)	4.66 (12.03)	74.00 (59.50)	15.67 (4.020)	0.00 (0.707)

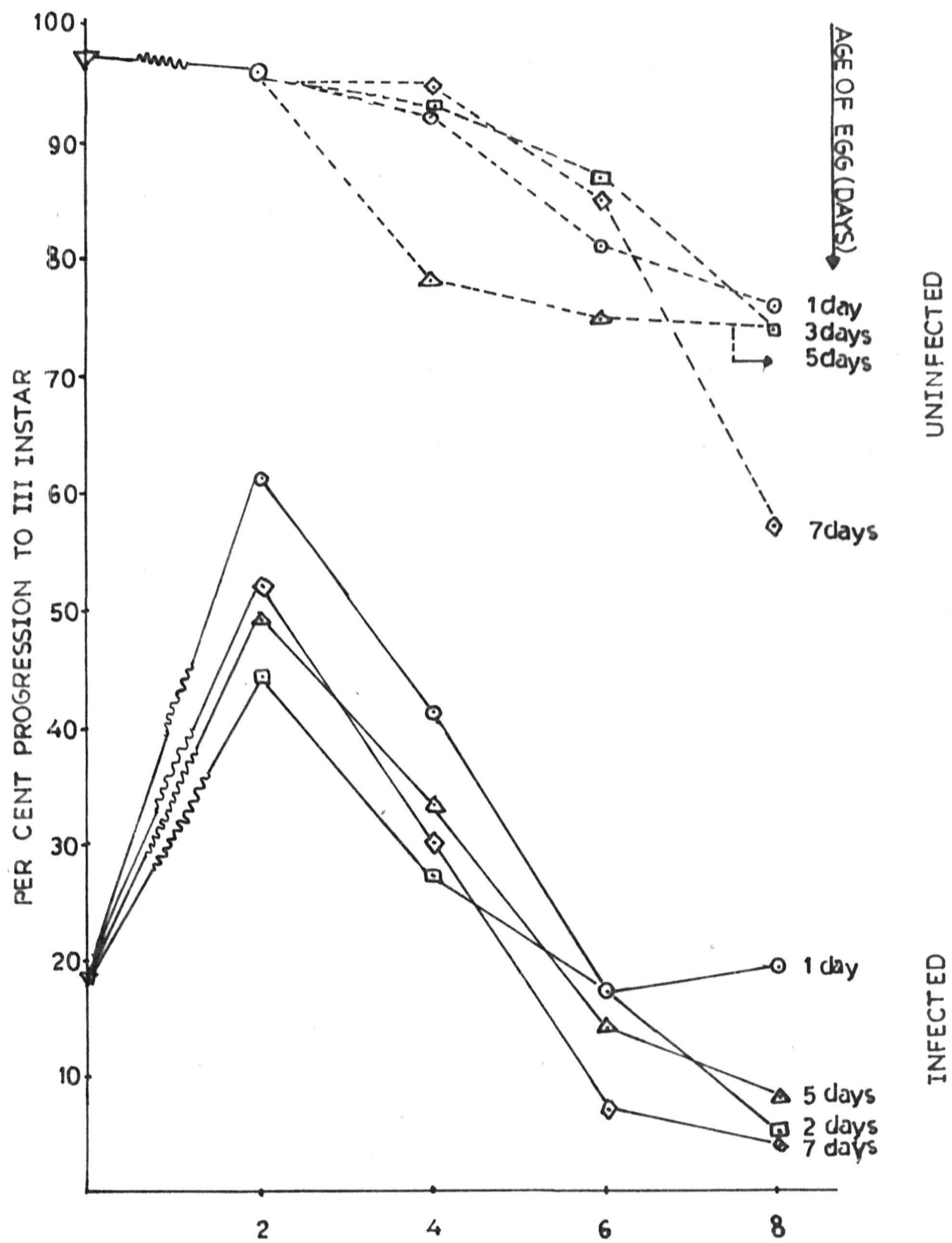
Continued

Table 2. (Continued)

	1	2	3	4	5	6	7	8	9
9. Five days			Two days	87.34 (66.45)	98.00 (83.44)	48.66 (44.24)	95.34 (77.97)	18.00 (4.300)	0.00 (0.707)
10. "			Four days	78.00 (62.05)	88.00 (69.91)	33.34 (35.23)	78.00 (62.09)	17.33 (4.222)	0.00 (0.707)
11. "			Six days	78.00 (62.53)	87.34 (69.98)	14.00 (21.88)	75.34 (60.25)	16.67 (4.143)	0.00 (0.707)
12. "			Eight days	69.34 (56.39)	75.34 (60.25)	8.00 (16.35)	74.66 (59.89)	15.00 (3.935)	0.00 (0.707)
13. Seven days			Two days	76.00 (60.72)	97.34 (82.56)	56.66 (48.84)	95.34 (80.01)	18.33 (4.339)	0.00 (0.707)
14. "			Four days	71.34 (57.68)	96.66 (79.60)	30.00 (34.16)	94.66 (76.70)	17.67 (4.262)	0.00 (0.707)
15. "			Six days	66.66 (54.92)	86.00 (68.12)	7.34 (15.47)	84.66 (67.07)	16.33 (4.102)	0.00 (0.707)
16. "			Eight days	62.66 (52.42)	62.00 (52.68)	4.00 (11.54)	56.66 (49.08)	15.67 (4.020)	0.00 (0.707)
17. Unrefrigerated control				71.34 (57.68)	98.66 (84.58)	18.66 (25.55)	97.34 (88.73)	18.00 (4.300)	0.00 (0.707)
			S.E.M. $\frac{t}{\sqrt{n}}$	3.36		2.26		0.031	
			C.D. at 5%	9.503		6.392		0.0876	

* Figures in parentheses are angular transformed values
 ** Figures in parentheses are $\sqrt{x+0.5}$ transformed values

**Fig.2. Effect of refrigeration of silkworm eggs
and Kenchu virus infection on progression
to third instar.**



DURATION OF REFRIGERATION (DAYS)

FIGURE - 2

in combination with Kenchu virus infection also led to a marked adverse influence on instar progression. However, refrigeration, per se, did not have appreciable influence on the severity of Kenchu infection as evidenced by instar progression.

Irrespective of whether the eggs were refrigerated or not, ET_{50} for mortality ranged from 15.00 to 20.67 days in virus infection treatments.

4.3 Effect of releasing Kenchu virus inoculated silkworms into healthy populations in various proportions

4.3.1 Second instar

4.3.1.1 Larval weight

The larval weights corresponding to 10 worms on 7th day after infection were 0.3850, 0.3953 and 0.3997 g in respect of 24, 20 and 16 per cent density of infected individuals, respectively which were similar with each other and significantly lower compared to others. Larval weight corresponding to control (0.4620 g) and 4 per cent density (0.4650 g) was maximum and significantly more than in all the others (Table 3).

The weight of ten worms in control was the highest (2.0980 g) on 15th day after infection. There was a

Table 3. Impact of releasing Kenchu virus inoculated silkworms into healthy populations in various proportions in second instar on larval weight

Sl. No.	Density of Kenchu virus infected individuals released	Weight of 10 worms on 7th day (g)	Weight of 10 worms on 15th day (g)	Maximum larval weight of 10 worms (g)
1.	4 per cent	0.4650	1.5952	11.1635
2.	8 per cent	0.4310	1.5069	10.5740
3.	12 per cent	0.4190	1.2694	10.1857
4.	16 per cent	0.3997	1.0423	9.4750
5.	20 per cent	0.3953	0.9958	8.5340
6.	24 per cent	0.3850	0.8545	8.0897
7.	Untreated control	0.4620	2.0980	14.5270
S.E.M. \pm		0.0081	0.0478	0.5931
C.D. at 5%		0.0249	0.1472	1.827

gradual yet statistically significant decrease in larval weight attendant on releasing infected individuals (1.5952 to 0.8545 g). A similar trend was also observed in the maximum larval weights recorded.

4.3.1.2 Moulting duration

Duration of second moult in different treatments ranged from 22.67 to 27.33 hr. The delay in moulting time in 20 and 24 per cent treatments was found to be statistically significant and higher than in the other treatments (Table 4). Duration of third moult in different treatments ranged from 24.33 to 34.00 hr. At the higher releases of 12 to 24 per cent (30 to 34 hr) the delay was statistically highly significant. A more pronounced prolongation of IV moult duration as compared to II and III moults was seen in all concentrations above 8 per cent (32.33 to 38.00 hr) as against 28.00 to 30.33 hr in control and 4 per cent treatment respectively.

4.3.1.3 Instar duration

There was a statistically significant prolongation of all the instars from II to V. The second instar duration was 3.08 days in healthy control as against 4.00 to 4.67 days in the different releases of infected worms. The III

Table 4. Impact of releasing Kenchu virus inoculated silkworms into healthy populations in various proportions in second instar on moulting duration

Sl. No.	Density of Kenchu virus infected individuals released	Second moulting duration (Hr)	Third moulting duration (Hr)	Fourth moulting duration (Hr)
1.	4 per cent	22.67	25.00	30.33
2.	8 per cent	23.33	26.33	32.33
3.	12 per cent	23.33	30.00	33.33
4.	16 per cent	24.67	31.67	34.67
5.	20 per cent	27.33	33.33	36.33
6.	24 per cent	26.67	34.00	38.00
7.	Untreated control	23.33	24.33	28.00
	S.E.M. \pm	0.94	0.81	0.77
	C.D. at 5%	2.896	2.496	2.372

instar was 4.75 days long in control which was increased to 4.92 to 6.75 in the treated ones and the IV instar duration was again minimum in control (5.08 days) as compared to 6.08 to 12.08 days in different releases. The magnitude of difference in duration of V instar was comparable to that recorded for IV instar (9.08 days in control and 10.50 to 15.58 days in batches containing the released infected individuals) (Table 5).

4.3.1.4 Instar progression

There was no significant difference in the percentage of worms entering the III instar. Release of eight to 24 per cent density of infected individuals into healthy batches in the II instar resulted in a statistically significant decrease in the percentage of worms progressing to the IV instar (94.00 to 73.33 per cent) as compared to 98.00 per cent in control larvae. Differences in worms entering V instar in 8 to 24 per cent treatments were statistically borne out as compared to 90.67 per cent in control larvae (Table 6).

4.3.1.5 ET₅₀ for symptom exhibition and mortality

There was a gradual decrease in the ET₅₀ for symptom expression as the percentage of released individuals increased (22.33 to 13.33 days). Similar effect was also

Table 5. Impact of releasing Kenchu virus inoculated silkworms into healthy populations in various proportions in second instar on duration of instars (days)

Sl. No.	Density of Kenchu virus inoculated individuals released	Second instar	Third instar	Fourth instar	Fifth instar
1.	4 per cent	4.08	4.92	6.08	10.50
2.	8 per cent	4.08	5.25	6.50	11.42
3.	12 per cent	4.00	5.50	6.58	12.67
4.	16 per cent	4.25	5.75	7.42	13.33
5.	20 per cent	4.33	6.25	9.08	14.25
6.	24 per cent	4.67	6.75	12.08	15.58
7.	Untreated control	3.08	4.75	5.08	9.08
	S.E.M. \pm	0.10	0.15	0.23	0.23
	C.D. at 5%	0.308	0.462	0.708	0.708

Table 6. Impact of releasing Kenchu virus inoculated silkworms into healthy populations in various proportions in second instar on extent of worms entering to subsequent instars(Per cent)

Sl. No.	Density of Kenchu virus inoculated individuals released	Worms entering third instar	Worms entering fourth instar	Worms entering fifth instar
1.	4 per cent	96.00 (81.21)*	94.00 (76.59)*	81.33 (65.73)*
2.	8 per cent	95.00 (79.80)	90.00 (71.80)	82.67 (65.49)
3.	12 per cent	96.67 (81.81)	82.00 (65.04)	79.33 (63.00)
4.	16 per cent	94.00 (76.34)	79.33 (66.03)	63.33 (52.76)
5.	20 per cent	93.33 (78.29)	76.67 (62.68)	59.33 (50.42)
6.	24 per cent	91.33 (75.26)	73.33 (59.12)	55.33 (48.07)
7.	Untreated control	100.00 (90.00)	98.00 (83.44)	90.67 (72.82)
	S.E.M. \pm	4.92	2.70	2.92
	C.D. at 5%	15.161	8.320	8.998
		NS		

* Figures in parentheses are angular transformed values

NS = Non-significant

recorded for ET_{50} for mortality which ranged between 30.00 to 17.67 days in release percentages ranging from 4 to 24 per cent (Table 7).

4.3.1.6 Cocooning and cocoon, pupal and cocoon shell weights

The drastic effect of releasing Kenchu virus infected silkworms into healthy populations at as low a level as 4 per cent (the least examined in the present investigations) was clearly felt in the number of cocoons spun which happens to be one of the important economic traits in practical sericulture. It was 30 per cent as compared to 90.67 per cent in healthy, control worms. The severity of effect was also evident in the 24 per cent treatment where 100 per cent mortality was noticed (Table 7 and Fig.3). The percentage of worms spinning reduced gradually with the increase in the percentage of release.

There was a gradual decrease in cocoon weight as the percentage of infected-individuals released increased (Table 8). The cocoon weight reduction from 8 to 20 per cent treatments was found to be statistically significant (7.010 g in control and 6.471 to 6.102 g in treatments). A similar trend was also noticed in the pupal weight and cocoon shell weight. However, it was interesting to note that differences in cocoon shell ratio were of small

Table 7. Impact of releasing Kenchu virus inoculated silkworms into healthy populations in various proportions in second instar on ET_{50} for Kenchu symptom exhibition and mortality and extent of cocooning

S1. No.	Density of Kenchu virus inoculated individuals released	ET_{50} for Kenchu symptom exhibition(days)	ET_{50} for mortality (days)	Per cent cocooning
1.	4 per cent	22.33 (4.773)*	30.00 (5.504)*	30.00 (33.20)**
2.	8 per cent	22.00 (4.740)	28.00 (5.290)	24.33 (29.55)
3.	12 per cent	19.00 (4.414)	26.67 (5.163)	22.00 (27.95)
4.	16 per cent	17.33 (4.220)	22.00 (4.742)	10.00 (18.42)
5.	20 per cent	16.33 (4.100)	20.33 (4.509)	7.00 (15.24)
6.	24 per cent	13.33 (3.718)	17.67 (4.222)	0.00 (0.00)
7.	Untreated control	0.00 (0.707)	0.00 (0.707)	90.67 (72.15)
	S.Em. \pm	0.068	0.068	3.747
	G.D. at 5%	0.209	0.209	11.544

* Figures in parentheses are $\sqrt{x + 0.5}$ transformed values

** Figures in parentheses are angular transformed values

Fig.3. Extent of cocooning as influenced by release of Keshu virus infected individuals into healthy populations in different proportions in second, third and fourth instars.

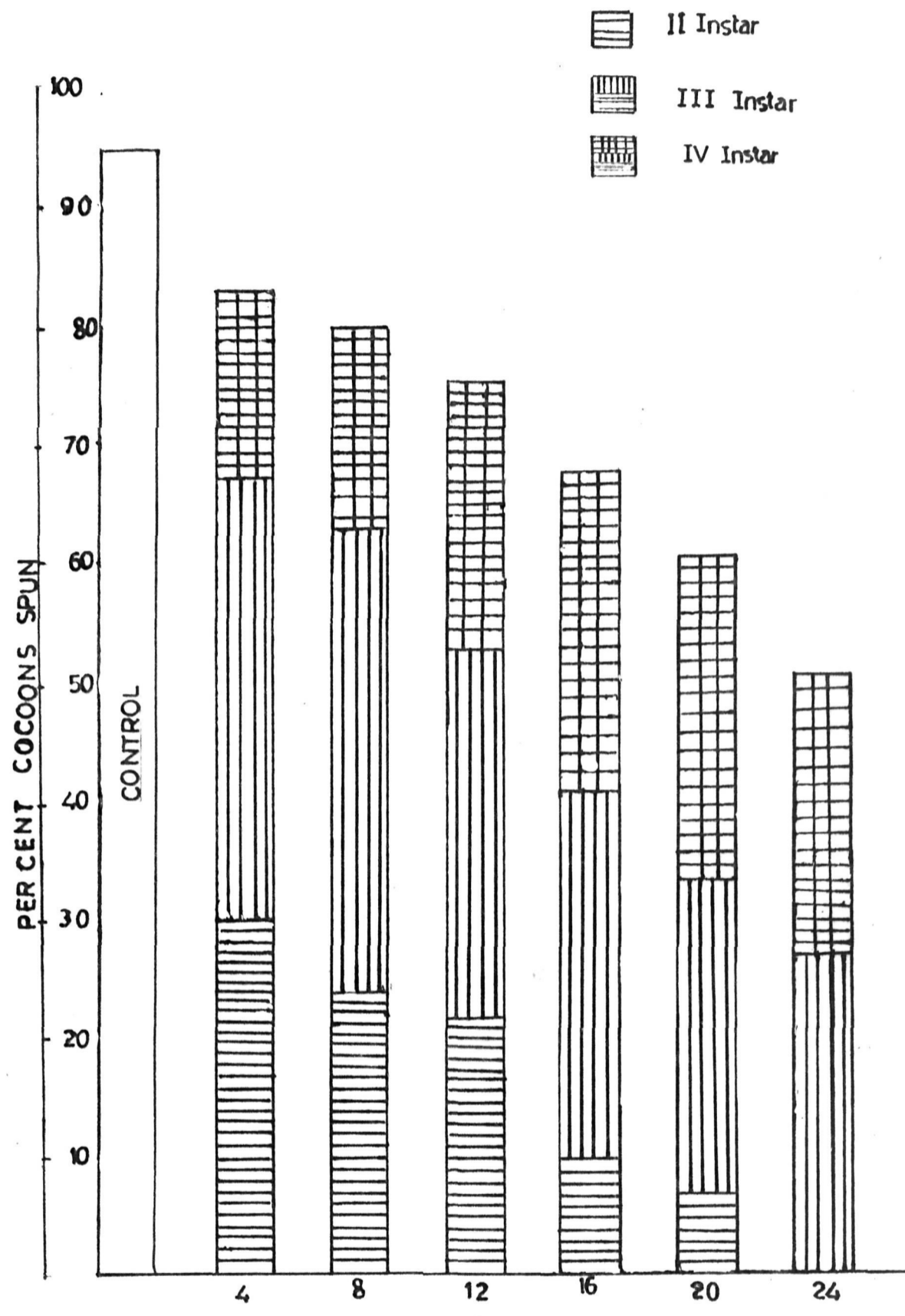


Figure-3 PER CENT DENSITY OF INFECTED SILK WORMS

Table 8. Impact of releasing Kenchu virus inoculated silkworms into healthy populations in various proportions in second instar on cocoon weight, pupal weight, cocoon shell weight and cocoon shell ratio

Sl. No.	Density of Kenchu virus inoculated individuals released	Weight of 10 cocoons (g)	Weight of 10 pupae (g)	Weight of 10 cocoon shells (g)	Cocoon shell ratio (%)
1.	4 per cent	6.925 (2.742)*	5.9813 (2.5456)*	0.8707 (1.1707)*	12.26 (20.49)**
2.	8 per cent	6.471 (2.639)	5.5750 (2.4643)	0.8602 (1.1662)	13.29 (21.36)
3.	12 per cent	6.261 (2.587)	5.3890 (2.4266)	0.8110 (1.1449)	12.95 (21.07)
4.	16 per cent	6.158 (2.576)	5.3154 (2.4108)	0.8069 (1.1430)	13.10 (21.19)
5.	20 per cent	6.102 (2.568)	5.2840 (2.4033)	0.7770 (1.1299)	12.74 (20.88)
6.	24 per cent	0.00 (0.707)	0.00 (0.7071)	0.00 (0.7071)	0.00 (0.00)
7.	Untreated control	7.010 (2.740)	6.0247 (2.5537)	0.9372 (1.1987)	13.39 (21.44)
S.E.m. \pm		0.032	0.0326	0.0087	0.26
C.D. at 5%		0.0986	0.1004	0.0268	0.801

* Figures in parentheses are $\sqrt{x + 0.5}$ transformed values

** Figures in parentheses are angular transformed values.

magnitude and a definite trend was not seen (range 12.26 to 13.29 per cent) in the treated batches.

4.3.2 Third instar

4.3.2.1 Larval weight

There was no significant decrease in larval weight on the 7th day, the weight ranging from 0.6638 to 0.7642 g/10 larvae (Table 9). The differences became more pronounced on the 15th day. There was a gradual decrease accompanying the increase in the percentage release (1.9435 to 1.4797 g). The corresponding control larvae registered 2.7897 g/10 larvae.

The adverse effect of high releases was clearly evident in the pronounced reduction in maximum larval weight (7.7437 g to 6.3480 g/10 larvae) as compared to 14.3207 g in healthy larvae (Table 9).

4.3.2.2 Moulting duration

The duration of III moult was not significantly affected by the release of infected individuals in all the levels tried. Fourth moult duration, however, was significantly increased in 12 to 24 per cent treatments (31.67 to 36.33 hr) as compared to 28.00, 26.33 and 30.00 hr for control, 4 per cent and 8 per cent, respectively (Table 10).

Table 9. Effect of releasing Kenchu virus inoculated silkworms into healthy populations in various proportions in third instar on larval weight (g)

Sl. No.	Density of Kenchu virus inoculated individuals released	Weight of 10 worms on 7th day (g)	Weight of 10 worms on 15th day (g)	Maximum larval weight of 10 worms (g)
1.	4 per cent	0.7573	1.9455	11.0863
2.	8 per cent	0.7474	1.9598	10.5923
3.	12 per cent	0.7444	1.7475	9.3943
4.	16 per cent	0.7128	1.7312	7.7437
5.	20 per cent	0.7114	1.6797	7.1253
6.	24 per cent	0.6638	1.4797	6.3480
7.	Untreated control	0.7642	2.7897	14.3207
	S.E.M. \pm	0.0229	0.0827	0.3914
	C.D. at 5%	0.0705	0.2548	1.206
		NS		

NS = Non-significant

Table 10. Effect of releasing Kenchu virus inoculated silkworms into healthy populations in various proportions in third instar on moulting duration and ET_{50} for Kenchu symptom exhibition

Sl. No.	Density of Kenchu virus inoculated individuals released	Third moulting duration (Hr)	Fourth moulting duration (Hr)	ET_{50} for Kenchu symptom exhibition (Days)
1.	4 per cent	24.00	26.35	25.33 (5.074)*
2.	8 per cent	23.67	30.00	23.35 (4.881)
3.	12 per cent	24.00	31.67	20.33 (4.562)
4.	16 per cent	24.67	33.33	18.00 (4.297)
5.	20 per cent	27.33	32.67	16.67 (4.142)
6.	24 per cent	28.67	36.33	14.67 (3.894)
7.	Untreated control	24.67	28.00	0.00 (0.707)
S.E.m. \pm		1.29	1.08	0.040
C.D. at 5%		3.975	3.328	0.123
		NS		

* Figures in parentheses are $\sqrt{x + 0.5}$ transformed values

NS = Non-significant

4.3.2.3 Instar duration

There was a significant increase in III instar duration in 8 to 24 per cent treatments (5.17 to 5.58 days as against 4.75 days in healthy worms). Fourth instar duration also manifested similar behaviour. The data were significant statistically. The drag in the V instar was more pronounced. For example, with 24 per cent release, the delay in III instar was 0.83 day, IV instar - 3.41 days and V instar - 5.17 days (Table 11).

4.3.2.4 Instar progression

There was very little mortality in any of the treatments and most of them progressed to the IV instar. However, the deleterious effect began to be felt in the entry to V instar. In control batches, 96.33 per cent of worms progressed into the V instar as compared to 89.33 to 69.33 per cent in release treatments of 4 to 24 per cent (Table 12).

4.3.2.5 ET₅₀ for Kenchu symptom manifestation and mortality

None of the healthy larvae exhibited Kenchu symptoms. ET₅₀ for symptom expression decreased gradually with increasing percentage release -25.33 to 14.67 days in 4 to 24 per cent treatments, thereby indicating the high significance of the result (Table 10).

Fifty per cent mortality was not reached in control and 4 to 12 per cent releases. In 16 to 24 per cent

Table 11. Effect of releasing Kenohu virus inoculated silkworms into healthy populations in various proportions in third instar on instar duration

Sl. No.	Density of Kenohu virus inoculated individuals released	Third instar duration (Days)	Fourth instar duration (Days)	Fifth instar duration (Days)
1.	4 per cent	5.08	5.75	10.25
2.	8 per cent	5.17	6.08	10.50
3.	12 per cent	5.17	6.50	11.00
4.	16 per cent	5.25	6.42	12.08
5.	20 per cent	5.33	7.08	13.50
6.	24 per cent	5.58	8.58	14.00
7.	Untreated control	4.75	5.17	8.93
	S.E.m. \pm	0.13	0.23	0.17
	C.D. at 5%	0.40	0.708	0.523

Table 12. Effect of releasing Kenohu virus inoculated silkworms into healthy populations in various proportions in third instar on extent of worms entering subsequent instars, ET_{50} for mortality and extent of cocooning

Sl. No.	Density of Kenohu virus inoculated individuals released	Per cent worms entering IV instar	Per cent worms entering V instar	ET_{50} for mortality (Days)	Per cent cocooning
1.	4 per cent	98.00 (83.44)*	89.33 (71.01)*	0.00 (0.707)**	67.33 (55.15)*
2.	8 per cent	100.00 (90.00)	81.33 (64.61)	0.00 (0.707)	62.67 (52.34)
3.	12 per cent	100.00 (90.00)	76.00 (60.85)	0.00 (0.707)	53.00 (46.72)
4.	16 per cent	100.00 (90.00)	74.67 (59.83)	18.67 (4.297)	41.00 (39.82)
5.	20 per cent	100.00 (90.00)	70.00 (56.81)	18.33 (4.339)	31.33 (34.02)
6.	24 per cent	96.67 (81.43)	69.33 (56.39)	16.33 (4.102)	27.00 (31.30)
7.	Untreated control	99.33 (87.29)	96.33 (79.14)	0.00 (0.707)	92.33 (73.99)
	S.E.M. \pm	2.39	1.19	0.045	0.74
	C.D. at 5%	7.364	5.885	0.1386	2.280
		NS			

* Figures in parentheses are angular transformed values

** Figures in parentheses are $\sqrt{x + 0.5}$ transformed values

NS = Non-significant

releases, the ET_{50} values ranged from 18.67 to 16.33 days (Table 12).

4.3.2.6 Cocooning and cocoon and shell weights

Highest percentage of cocooning was observed in healthy controls (92.33). There was a gradual, drastic reduction in cocooning from 67.33 to 27.00 per cent in the treatments ranging from 4 to 24 per cent release (Table 12 and Fig. 3).

Cocoon weight was significantly reduced in releases of 12 and higher percentage (6.3497 to 5.4514 g/10 cocoons) as compared to 7.2228 g in control. Similar trend was observed even with regard to pupal weight. Statistical differences did not exist among the different experimental treatments in respect of cocoon shell weight and cocoon shell ratio (Table 13).

4.3.3 Fourth instar

4.3.3.1 Larval weight

Significant reduction in larval weight was observed on the 7th day in all treatments, the weights ranging from 2.5031 to 2.6563 g/10 larvae. The corresponding weight in control was 2.8312 g (Table 14).

There was a gradual, significant reduction in larval weight on the 15th day from 8.0270 g in 4 per cent release

Table 13. Effect of releasing Kenchu virus inoculated silkworms into healthy populations in various proportions in third instar on cocoon weight, pupal weight, cocoon shell weight and cocoon shell ratio

Sl. No.	Density of Kenchu virus inoculated individuals released	Weight of 10 cocoons (g)	Weight of 10 pupae (g)	Weight of 10 cocoon shells (g)	Cocoon shell ratio (%)
1.	4 per cent	6.7997	5.9250	0.8565	12.56 (20.68)*
2.	8 per cent	6.6845	5.8164	0.8333	12.47 (20.70)
3.	12 per cent	6.3497	5.4700	0.8461	13.33 (21.39)
4.	16 per cent	6.2965	5.4295	0.8057	12.81 (20.95)
5.	20 per cent	5.8356	5.0360	0.7784	13.42 (21.45)
6.	24 per cent	5.4514	4.5206	0.7441	13.65 (21.66)
7.	Untreated control	7.2228	6.2794	0.8900	12.20 (20.47)
	S.E.M. †	0.2127	0.1984	0.0684	0.39
	G.D. at 5%	0.655	0.611	0.2100	2.742
				NS	NS

* Figures in parentheses are angular transformed values

NS = Non-significant

Table 14. Effect of releasing Kenchu virus inoculated silkworms into healthy populations in various proportions in fourth instar on larval weight

Sl. No.	Density of Kenchu virus inoculated individuals released	Weight of 10 worms on 7th day (g)	Weight of 10 worms on 15th day (g)	Maximum larval weight of 10 worms (g)
1.	4 per cent	2.5912	8.0270	12.3427
2.	8 per cent	2.6563	8.0373	10.7097
3.	12 per cent	2.5651	7.5866	10.3247
4.	16 per cent	2.5290	7.4048	10.3200
5.	20 per cent	2.5280	6.8673	9.4390
6.	24 per cent	2.5031	6.6007	8.7997
7.	Untreated control	2.8512	10.5343	14.1817
	S.E.m. \pm	0.038	0.1829	0.4987
	C.D. at 5%	0.117	0.5636	1.536

to 6.6007 g in 24 per cent release as compared to 10.5343 g in uninfected worms. The variation was similar in respect of maximum larval weight also (12.3427 to 8.7987 g in 4 to 24 per cent release). Ten larvae weighed 14.1817 g without infection.

4.3.3.2 Moultling and instar duration and instar progression

Duration of IV instar was extended only at the higher percentage releases of 20 and 24 per cent, the values for instar duration being 6.50 and 7.00 days, respectively. Control worms showed IV instar duration of 5.80 days (Table 15). Duration of IV moult was not significantly affected.

Percentage of worms entering V instar registered significantly lower values of 92.00, 91.00, 89.00 and 86.67 per cent for 12, 16, 20 and 24 per cent releases, respectively compared to 98.33 per cent in uninfected control. Duration of V instar was affected to a larger extent, a gradual increase from 10.08 to 13.00 days being manifested in 4 to 24 per cent releases. The control worms required 9.25 days for completing the instar.

4.3.3.3 ET₅₀ for Kenohu symptom manifestation

Fifty per cent of the worms showed Kenohu symptoms only in 20 and 24 per cent releases (19.33 and 10.33 days respectively) (Table 16).

Table 15. Effect of releasing Kenchu virus inoculated silkworms into healthy populations in various proportions in fourth instar on instar duration, moulting duration and extent of worms entering fifth instar

Sl. No.	Density of Kenchu virus inoculated individuals released	Fourth instar duration (Days)	Fourth moulting duration (Hr)	Per cent worms entering V instar	Fifth instar duration
1.	4 per cent	5.50	26.67	97.67 (81.28)*	10.08
2.	8 per cent	5.33	26.67	96.00 (78.46)	10.25
3.	12 per cent	6.00	28.00	92.00 (73.57)	10.33
4.	16 per cent	6.42	28.67	91.00 (72.54)	11.00
5.	20 per cent	6.50	30.67	89.00 (70.63)	12.08
6.	24 per cent	7.00	31.33	86.67 (68.61)	13.00
7.	Untreated control	5.80	27.67	98.33 (82.51)	9.25
	S.E.m. \pm	0.22	1.20	1.044	0.16
	C.D. at 5%	0.677	3.697	3.217	0.493
			N.S.		

* Figures in parentheses are angular transformed values

N.S. = Non-significant

Table 16. Effect of releasing Kenchu virus inoculated silkworms into healthy populations in various proportions in fourth instar on EF_{50} for Kenchu symptom exhibition, extent of cocooning and cocoon weight

Sl. No.	Density of Kenchu virus inoculated individuals released	EF_{50} for Kenchu symptom exhibition (Days)	Per cent cocooning	Weight of 10 cocoons (g)
1.	4 per cent	0.00 (0.707)*	82.67 (65.43)**	7.1011
2.	8 per cent	0.00 (0.707)	80.00 (63.53)	7.0727
3.	12 per cent	0.00 (0.707)	76.00 (60.69)	6.9596
4.	16 per cent	0.00 (0.707)	68.00 (55.56)	6.9361
5.	20 per cent	19.33 (4.452)	61.33 (51.55)	6.9042
6.	24 per cent	10.33 (2.902)	51.33 (45.77)	6.4211
7.	Untreated control	0.00 (0.707)	97.00 (80.02)	7.2957
<hr/>				
	S.E.m. \pm	0.418	0.87	0.222
	C.D. at 5%	1.288	2.68	0.684
				NS

* Figures in parentheses are $\sqrt{x + 0.5}$ transformed values

** Figures in parantheses are angular transformed values

NS = Non-significant

4.3.3.4 Cocooning and cocoon and shell weights

A significant reduction in the percentage of larvae spinning cocoons was evident. There was a gradual fall from 82.67 to 51.33 per cent in 4 to 24 per cent treatments. With healthy batches, 97.00 per cent worms spun cocoons (Fig.3).

No significant differences were observed in cocoon weights (Table 16), weight of pupae and cocoon shell ratio (Table 17). Small but significant differences in shell weight were seen at all the levels of release (0.9162 to 0.6777 g) as compared to 0.9307 g/10 shells in control.

A perusal of the data so far collected on the effect of releasing different proportions of Kenchu virus infected individuals to healthy populations revealed the following information.

The larval weight at the end of the fifth instar was significantly reduced irrespective of stage of infection and per cent release of infected larvae. In general, the extension in moulting duration was dependent on stage of infection. The moult immediately after infection was not significantly affected whereas the effect could be prominently seen in the subsequent moults. Instar durations were found to be significantly increased when releases were made in early instars. If releases of infected worms were

Table 17. Effect of releasing Kenohu virus inoculated silkworms into healthy populations in various proportions in fourth instar on pupal weight, cocoon shell weight and cocoon shell ratio

Sl. No.	Density of Kenohu virus inoculated individuals released	Weight of 10 pupae (g)	Weight of 10 cocoon shells (g)	Cocoon shell ratio (%)
1.	4 per cent	6.1202	0.9162	12.97 (21.11)*
2.	8 per cent	6.1783	0.8930	12.57 (20.73)
3.	12 per cent	5.9464	0.8843	12.70 (20.85)
4.	16 per cent	6.0023	0.8467	12.27 (20.45)
5.	20 per cent	5.9890	0.8173	11.86 (20.07)
6.	24 per cent	5.5638	0.6777	10.58 (18.94)
7.	Untreated control	6.3252	0.9307	12.76 (20.88)
	S.E.m. \pm	0.2043	0.0211	0.49
	C.D. at 5%	0.629	0.065	1.509
		N.S.		N.S.

* Figures in parentheses are angular transformed values
N.S. = Non-significant

made in later instars, the instar prolongation was not of high magnitude but still remained statistically significant. The instar progression was dependent significantly on the stage at which the infected individuals were released into healthy populations. The effect was not marked in the instar in which they were released, but the subsequent instars were prolonged. In case of releases in IV instar, appreciable effect was not seen in progression into V instar although duration of V instar was slightly increased. The EF_{50} values for symptom exhibition reduced with the increase in the percentage release of infected individuals. Mortality could be observed, irrespective of percentage of infected worms released, when the stage of infection was early instars. However, release of infected individuals in IV instar did not result in the appreciable larval mortality in any of the releases. Cocooning and cocoon parameters were also observed to come down with increase in the percentage of released infected individuals.

4.4 Influence of nutrition on intensity of Kenohu virus infection

4.4.1 Underfeeding

The early instar silkworms were started on a feeding regime of 2, 3, 4 and 5 feeds/day, the last one being the standard practice. Infection treatment was imposed in the

IV instar and V instar: separately. Five feeds per day were given in the IV and V instars.

4.4.1.1 Fourth instar

4.4.1.1.1 Symptom manifestation and larval weight

Healthy worms did not exhibit any symptoms of disease irrespective of the number of feeds/day. Disease infected treatments indicated significant differences in ET_{50} for symptom expression (12.66, 14.00, 15.00 and 15.53 days in batches corresponding to 2, 3, 4 and 5 feeds/day respectively) (Table 18).

The maximum body weight attained by underfeeding in early instars followed by normal feeding in IV and V instars was significantly different, but the differences in magnitude among the treatments was low. In infected batches, there was a lowering in body weight irrespective of the number of feeds/day (13.438 to 14.533 g/10 larvae) as against 15.675 g in standard control. However, statistically significant differences were seen in 3 and 5 feed - treatments (Table 18).

4.4.1.1.2 Cocooning, cocoon and shell weights and moth emergence

The percentage cocooning in normal, uninfected larvae was 54.66 and higher than that in all other treatments. The low percentage cocooning in normal batch could be attributed to non-spinning of silk in larvae which showed

Table 18. Influence of underfeeding early instar silkworms followed by Kenchu virus infection in fourth instar on ET50 for Kenchu symptom exhibition, maximum larval weight, extent of cocooning and cocoon weight

Sl. No.	Treatments	ET50 for Kenchu symptom exhibition (days)	Maximum larval weight of 10 worms (g)	Per cent cocooning	Weight of 10 cocoons (g)
1.	Two feeds/day + Virus infection	12.66 (3.6280)*	14.533	19.33 (25.83)**	8.589
2.	Two feeds/day	0.00 (0.7071)	15.977	35.00 (36.24)	9.219
3.	Three feeds/day + Virus infection	14.00 (3.8060)	14.078	30.66 (33.61)	9.322
4.	Three feeds/day	0.00 (0.7071)	15.775	40.66 (39.62)	9.286
5.	Four feeds/day + Virus infection	15.00 (3.9356)	13.438	21.33 (27.44)	8.163
6.	Four feeds/day	0.00 (0.7071)	14.728	42.66 (40.77)	9.117
7.	Five feeds/day + Virus infection	15.33 (3.9775)	14.001	20.66 (26.48)	8.483
8.	Five feeds/day	0.00 (0.7071)	15.675	54.66 (47.69)	10.285
	S.E.M. \pm	0.0451	0.532	2.390	0.352
	C.D. at 5%	0.136	1.613	7.248	1.067

* Figures in parentheses are $\sqrt{x + 0.5}$ transformed values

** Figures in parentheses are angular transformed values

normal body weight. There was significant, high magnitude difference in percentage cocooning in all treatments of underfeeding and underfeeding accompanied by Kenchu virus infection. In uninfected batches the percentage ranged from 35.00 to 54.66.

In infection with Kenchu virus, the percentage cocooning was 19.33, 30.66, 21.33 and 20.66 in treatments receiving 2, 3, 4 and 5 feeds/day.

There was marginal difference in cocoon weight in uninfected normal and underfed batches. Infection, however, resulted in a significant reduction in cocoon weights ranging from 8.163 to 9.322 g for 10 cocoons as compared to 10.285 g in standard control (Table 18).

There was no significant change in pupal weight and cocoon shell ratio as a result of underfeeding and/or infection. It is interesting to note that statistically significant differences occurred in cocoon shell weight. The weights were less as a result of underfeeding either alone or with infection (0.987 to 1.188 g as compared to 1.282 g in controls). Moth emergence was considerably lower in all treatments (healthy as well as infected). In general, moth emergence was higher with increasing number of feeds in both infected and uninfected batches (Table 19).

Table 19. Influence of underfeeding early instar silkworms followed by Kenchu virus infection in fourth instar on pupal weight, cocoon shell weight, cocoon shell ratio and moth emergence.

Sl. No.	Treatments	Weight of 10 pupae (g)	Weight of 10 cocoon shells (g)	Cocoon shell ratio (%)	Per cent moth emergence
1.	Two feeds/day + Virus infection	7.536	1.009	12.35 (20.51)*	11.00 (18.95)*
2.	Two feeds/day	8.186	1.030	13.71 (21.74)	25.33 (30.11)
3.	Three feeds/day + Virus infection	8.225	1.017	12.48 (20.67)	18.00 (24.84)
4.	Three feeds/day	8.276	0.987	11.92 (20.21)	28.00 (31.91)
5.	Four feeds/day + Virus infection	6.985	1.007	14.54 (22.39)	14.00 (21.62)
6.	Four feeds/day	7.906	1.188	15.03 (22.82)	28.66 (32.55)
7.	Five feeds/day + Virus infection	7.901	1.060	13.52 (21.53)	22.66 (28.05)
8.	Five feeds/day	8.962	1.282	14.33 (22.25)	50.66 (45.38)
	S.E.M. +	0.395	0.045	0.700	2.680
	C.D. at 5%	1.198	0.136	0.212	8.128
		NS		NS	

*Figures in parentheses are angular transformed values

NS = Non-significant

4.4.1.2 Fifth instar

Maximum larval weight was not significantly affected by either underfeeding in early instars followed by normal feeding in IV and V instars and/or infection (Table 20).

Percentage of larvae spinning cocoons was high in uninfected, fully fed larvae (89.33). Underfeeding alone caused a gradual but significant reduction in cocooning with reduction in the number of feeds/day (72.00 to 39.33 per cent). Infection in normally fed larvae resulted in a low percentage of cocooning (45.33). Infection in underfed larvae resulted in lower percentage cocooning ranging from 28.66 to 34.66 (Table 20).

The differences in cocoon weight, pupal weight and also shell weight were marginal yet statistically significant especially in the infected lots. A similar trend was seen for cocoon shell ratio also.

Moth emergence, however, was significantly lowered as a result of either underfeeding or infection or both (59.33 in control and 38.00 to 22.00 in all other treatments). A high degree of difference in moth emergence was not shown as a result of infection alone. Underfeeding appeared to be the more decisive factor affecting moth emergence (Table 21).

The above results indicated a slight reduction in larval and cocoon weights, but the per cent cocooning was

Table 20. Influence of underfeeding early instar silkworms followed by Kenchu virus infection in fifth instar on maximum larval weight, extent of cocooning and weight of cocoon and pupa

Sl. No.	Treatments	Maximum larval weight of 10 worms (g)	Per cent cocooning	Weight of 10 cocoons (g)	Weight of 10 pupae (g)
1.	Two feeds/day + Virus infection	13.888	34.66 (36.05)*	8.472	7.414
2.	Two feeds/day	13.083	39.33 (38.80)	9.289	8.282
3.	Three feeds/day + Virus infection	14.062	31.33 (33.99)	8.432	7.317
4.	Three feeds/day	13.816	64.00 (53.45)	8.994	8.486
5.	Four feeds/day + Virus infection	13.457	28.66 (32.37)	8.372	7.547
6.	Four feeds/day	15.464	72.00 (58.21)	8.747	7.685
7.	Five feeds/day + Virus infection	14.124	45.33 (42.29)	8.938	7.842
8.	Five feeds/day	14.379	89.33 (71.54)	9.596	8.366
S.E.M. \pm		0.419	2.990	0.171	0.185
C.D. at 5%		1.270	9.068	0.518	0.561
NS					

* Figures in parentheses are angular transformed values

NS = Non-significant

Table 21. Influence of underfeeding early instar silkworms followed by Kanshu virus infection in fifth instar on cocoon shell weight, cocoon shell ratio and moth emergence

Sl. No.	Treatments	Weight of 10 cocoon shells (g)	Cocoon shell ratio (%)	Per cent moth emergence
1.	Two feeds/day + Virus infection	1.049	12.38 (20.59)*	22.00 (27.72)*
2.	Two feeds/day	0.988	10.65 (19.05)	22.66 (28.30)
3.	Three feeds/day + Virus infection	1.101	13.06 (21.19)	23.33 (28.46)
4.	Three feeds/day	0.970	10.82 (19.19)	32.00 (34.42)
5.	Four feeds/day + Virus infection	0.924	11.04 (19.42)	27.33 (31.50)
6.	Four feeds/day	1.049	11.99 (20.26)	35.33 (36.41)
7.	Five feeds/day + Virus infection	0.988	11.07 (19.46)	38.00 (38.04)
8.	Five feeds/day	1.207	12.57 (20.78)	59.33 (50.42)
S.E.m. \pm		0.043	0.390	2.310
C.D. at 5%		0.130	0.118	7.006

* Figures in parentheses are angular transformed values

significantly reduced as a result of underfeeding. Similarly, moth emergence was also drastically reduced. Superimposition of underfeeding and virus infection further brought down the extent of moth emergence.

4.4.2 Post-moult starvation

In these investigations silkworms were given the recommended number of feeds throughout the larval period. The starvation was imposed after the II, III and IV moults separately, the periods of starvation being 12, 24, 36 and 48 hours. Infection was carried out immediately after the starvation period, separately for each instar.

4.4.2.1 Third instar

4.4.2.1.1 Larval weight

On 7th as well as 15th day after the commencement of the experiment, a gradual but significant lowering in growth was seen as evidenced by reduced body weights in uninfected larvae starved for different periods after moults (1.519 to 0.638 g as against 1.432 g in normal larvae on 7th day and 4.326 g to 5.849 g as against 6.821 g in normal larvae on 15th day). There was marginal difference in maximum body weight as a result of post-moult starvation for different periods.

Table 22. Effect of starvation and Kenchu virus infection in third instar silkworm on larval weight

S1. No.	Treatments	Weight of 10 worms on 7th day (g)	Weight of 10 worms on 15th day (g)	Single maximum larval weight (g)
1.	No starvation + virus infection	0.528	3.683	1.615
2.	No starvation	1.432	6.821	1.730
3.	12 hr starvation + Virus infection	1.021	3.316	1.078
4.	12 hr starvation	1.319	4.717	1.460
5.	24 hr starvation + Virus infection	0.670	3.291	1.277
6.	24 hr starvation	1.118	5.849	1.553
7.	36 hr starvation + Virus infection	0.595	2.250	0.955
8.	36 hr starvation	0.884	5.231	1.661
9.	48 hr starvation + Virus infection	0.583	2.712	0.838
10.	48 hr starvation	0.638	4.326	1.628
	S.E.m. \pm	0.056	0.307	0.0419
	C.D. at 5%	0.166	0.912	0.1244

A decrease in larval weight on the 7th day as a result of infection was quite marked especially at the higher levels of starvation (Table 22). On the 15th day a marked, highly significant reduction in body weight was recorded with infected larvae (6.821, 4.717, 5.849, 5.231 and 4.326 g for 0, 12, 24, 36 and 48 hr starvations respectively without infection; 3.683, 3.316, 3.291, 2.250 and 2.712 g/10 larvae for 0, 12, 24, 36 and 48 hr starvation respectively with infection).

Maximum larval weight was also similarly affected though to a lesser extent.

4.4.2.1.2 Instar and moulting duration

Post-moult starvation led to a significant prolongation of III instar, III moult and IV moult and V instar duration. Fourth instar duration was also significantly affected but the degree of prolongation was minimal (Tables 23 and 24). Infection superimposed on starvation did not produce any noticeable additional effect of prolonging III instar, III moult and IV instar. However, the retarding influence of infection was evident in the duration of IV moult especially at the higher levels of starvation.

Table 23. Effect of starvation and Kenchu virus infection in third instar silkworm on moulting duration and instar duration

Sl. No.	Treatments	Third instar duration (Days)	Third moulting duration (Hr)	Fourth instar duration (Days)	Fourth moulting duration (Hr)
1.	No starvation + Virus infection	4.83	30.67	5.50	32.00
2.	No starvation	4.08	25.33	5.00	24.00
3.	12 hr starvation + Virus infection	4.33	32.00	5.33	32.00
4.	12 hr starvation	5.08	23.00	5.67	26.00
5.	24 hr starvation + Virus infection	5.17	36.00	5.42	36.00
6.	24 hr starvation	5.17	30.00	5.25	32.00
7.	36 hr starvation + Virus infection	5.50	38.00	6.08	38.00
8.	36 hr starvation	5.42	34.00	5.83	32.00
9.	48 hr starvation + Virus infection	5.33	40.00	5.58	42.00
10.	48 hr starvation	5.33	37.33	5.58	36.00
	S.E.M. \pm	0.100	1.760	0.180	1.750
	C.D. at 5%	0.297	5.228	0.534	5.199

Table 24. Effect of starvation and Kenchu virus infection in third instar silkworm on fifth instar duration, extent of cocooning, cocoon weight and pupal weight

Sl. No.	Treatments	Fifth instar duration (Days)	Per cent cocooning	Single cocoon weight (g)	Single pupal weight (g)
1.	No starvation + Virus infection	8.50	29.33 (32.77)*	1.0302	0.8953
2.	No starvation	7.67	64.00 (53.13)	1.0417	0.8824
3.	12 hr starvation + Virus infection	8.00	14.00 (21.97)	0.9536	0.8250
4.	12 hr starvation	8.00	25.33 (30.20)	0.9493	0.7934
5.	24 hr starvation + Virus infection	8.50	10.00 (18.44)	0.9525	0.8770
6.	24 hr starvation	8.75	26.00 (30.66)	0.9690	0.8337
7.	36 hr starvation + Virus infection	8.58	10.67 (19.09)	0.8707	0.7520
8.	36 hr starvation	9.00	30.00 (33.21)	0.9866	0.8335
9.	48 hr starvation + Virus infection	9.17	9.33 (17.76)	0.8706	0.7543
10.	48 hr starvation	8.67	28.67 (32.39)	1.0074	0.8668
	S.E.M. +	0.190	1.955	0.0222	0.0206
	C.D. at 5%	0.564	5.810	0.0659	0.0612

*Figures in parentheses are angular transformed values

4.4.2.1.3 Cocooning, cocoon and shell weights and moth emergence

The per cent cocoons spun showed less value with starvation (25.33 to 30.00) compared against 64.00 per cent with no starvation in uninfected worms. Almost 50 per cent reduction in cocoon spinning was observed in case of all periods of starvation clubbed with virus infection (9.33 to 14.00 per cent) (Table 24) thus revealing their combined effect.

The single cocoon weight did not show much difference in batches starved and uninfected (0.9493 to 1.0074 g). But the starved infected batches showed differences in cocoon weight in that it was same with 12 and 24 hr (0.9525 to 0.9536 g) which was higher compared to 0.8706 to 0.8707 g observed with more duration of starvation. The cocoon shell weight, pupal weight and cocoon shell ratio changes were more or less in the same pattern (Tables 24 and 25).

Further, the per cent moth emergence was almost similar in starved uninfected worms (23.33 to 28.00) (Table 25) and starved infected worms (6.00 to 11.33 per cent) separately compared to 62.00 and 26.67 per cent respectively in their unstarved control and unstarved infected batches, thus indicating that there was combined

Table 25. Effect of starvation and Kenchu virus infection in third instar silkworm on cocoon shell weight, cocoon shell ratio and moth emergence

Sl. No.	Treatments	Single cocoon shell weight (g)	Shell ratio (%)	Per cent moth emergence
1.	No starvation + Virus infection	0.1313	12.75 (20.93)*	26.67 (30.97)*
2.	No starvation	0.1401	13.45 (21.53)	62.00 (52.00)
3.	12 hr starvation + Virus infection	0.1240	13.08 (21.22)	11.33 (19.66)
4.	12 hr starvation	0.1357	14.24 (22.15)	23.33 (28.85)
5.	24 hr starvation + Virus infection	0.1305	13.70 (21.72)	9.33 (17.36)
6.	24 hr starvation	0.1285	13.28 (21.36)	24.00 (29.21)
7.	36 hr starvation + Virus infection	0.1115	12.80 (20.96)	8.00 (16.35)
8.	36 hr starvation	0.1354	13.73 (21.69)	27.33 (31.50)
9.	48 hr starvation + Virus infection	0.1113	12.79 (20.96)	6.00 (14.05)
10.	48 hr starvation	0.1321	13.13 (21.53)	28.00 (31.77)
	S.E.M. \pm	0.0054	0.340	1.570
	C.D. at 5%	0.0160	1.010	4.664

* Figures in parentheses are angular transformed values

effect of both starvation and infection on moth emergence too.

4.4.2.2 Fourth instar

4.4.2.2.1 Larval weight

The larval weight on seventh day after beginning the experiment did not vary much in uninfected starved batches (2.582 to 2.809 g/10 worms) but difference of higher degree could be noticed amongst worms that were starved and infected (2.062 to 2.756 g). Even the maximum larval weight showed the same trend (Table 26).

4.4.2.2.2 Cocooning, cocoon and cocoon shell weight and moth emergence

The extent of cocooning was drastically affected by starvation itself (12.66 to 20.00 per cent) compared to 70.66 per cent observed for uninfected, unstarved control worms. Though infection clubbed with starvation did bring down cocooning significantly, the differences were marginal (4.66 to 10.66 per cent) (Table 26).

Cocoon weight and cocoon shell weight exhibited only marginal differences in starved uninfected lots, but appreciable differences could be noticed in case of starved and infected worms (Tables 26 and 27). Pupal weight also showed the same trend. However, the difference

Table 26. Effect of starvation and Kenchu virus infection in fourth instar silkworm on larval weight, extent of cocooning and cocoon weight

Sl. No.	Treatments	Weight of 10 worms on 7th day (g)	Maximum larval weight of 10 worms(g)	Per cent cocooning	Single cocoon weight (g)
1.	No starvation + Virus infection	2.144	13.74	13.33 (18.38)*	0.850
2.	No starvation	2.723	16.97	70.66 (57.60)	0.987
3.	12 hr starvation + Virus infection	2.530	14.24	10.66 (18.95)	0.859
4.	12 hr starvation	2.582	16.37	20.00 (26.12)	0.943
5.	24 hr starvation + Virus infection	2.756	15.18	8.66 (16.78)	1.031
6.	24 hr starvation	2.727	15.14	16.66 (23.98)	0.969
7.	36 hr starvation + Virus infection	2.174	16.23	10.00 (18.20)	0.845
8.	36 hr starvation	2.809	18.39	20.00 (26.45)	1.063
9.	48 hr starvation + Virus infection	2.062	6.93	4.66 (11.57)	0.622
10.	48 hr starvation	2.741	14.97	12.66 (20.73)	1.031
	S.E.M. \pm	0.088	1.140	6.85	0.039
	C.D. at 5%	0.261	3.386	20.351	0.115

*Figures in parentheses are angular transformed values

Table 27. Effect of starvation and Kenchu virus infection in fourth instar silkworm on pupal weight, cocoon shell weight, cocoon shell ratio and moth emergence

Sl. No.	Treatments	Single pupal weight (g)	Single cocoon shell weight (g)	Cocoon shell ratio (%)	Moth emergence (%)
1.	No starvation + Virus infection	0.740	0.105	12.32 (20.53)*	9.33 (17.71)*
2.	No starvation	0.854	0.128	12.99 (21.13)	68.66 (56.20)
3.	12 hr starvation + Virus infection	0.760	0.095	11.03 (19.32)	10.00 (18.28)
4.	12 hr starvation	0.823	0.116	12.26 (20.47)	18.00 (23.37)
5.	24 hr starvation + Virus infection	0.901	0.125	12.14 (20.38)	8.00 (15.90)
6.	24 hr starvation	0.828	0.137	14.02 (21.95)	16.00 (23.37)
7.	36 hr starvation + Virus infection	0.736	0.086	10.39 (18.70)	9.33 (17.63)
8.	36 hr starvation	0.921	0.138	12.99 (21.08)	19.33 (26.00)
9.	48 hr starvation + Virus infection	0.553	0.065	10.45 (18.81)	4.00 (10.90)
10.	48 hr starvation	0.896	0.130	12.81 (20.75)	12.00 (20.25)
S.E.M. \pm		0.034	0.0097	0.830	2.280
C.D. at 5%		0.101	0.0288	2.465	6.773
				NS	

* Figures in parentheses are angular transformed values

NS = Non-significant

in starved and uninfected worms was not appreciable. The cocoon shell ratio did not result in significant difference. Moth emergence was 12.00 to 19.33 per cent in starved, uninfected lots showing difference only with 36 hr starvation, while the same with starvation and infection was reduced to a considerable extent (10.00 to 4.00 per cent). In general the moth emergence came down with increase in the duration of starvation.

4.4.2.3 Fifth instar

4.4.2.3.1 Larval weight

The differences in larval weights on 7th day were more marked in starved and uninfected (7.656 to 10.891 g/100 worms) as well as in starved and infected batches (7.096 to 9.236 g) compared to 12.153 and 11.476 g in their respective controls (Table 28). The maximum larval weight also exhibited marginal difference (1.400 to 1.543 g/larva) in starved and uninfected worms when compared to 0.977 to 1.113 g in case of starved and infected worms.

4.4.2.3.2 Cocooning, cocoon and cocoon shell weights and moth emergence

The percentage of cocoons spun was 20.00 to 30.00 per cent in starved, uninfected worms compared to the control (60.00 per cent), the higher value being for

Table 28. Effect of starvation and Kanchu virus infection in fifth instar silkworm on larval weight, extent of cocooning and cocoon weight

Sl. No.	Treatments	Weight of 10 worms on 7th day (g)	Single maximum larval weight (g)	Per cent cocooning	Single cocoon weight (g)
1.	No starvation + Virus infection	11.476	1.230	22.66 (28.25)*	0.848
2.	No starvation	12.153	1.593	60.00 (48.84)	0.958
3.	12 hr starvation + viral infection	9.236	1.047	26.00 (30.51)	0.738
4.	12 hr starvation	10.891	1.400	30.00 (35.18)	0.878
5.	24 hr starvation + Viral infection	8.405	0.977	14.67 (21.23)	0.588
6.	24 hr starvation	10.007	1.487	20.00 (26.52)	0.949
7.	36 hr starvation + Virus infection	7.434	1.113	18.00 (23.50)	0.762
8.	36 hr starvation	8.051	1.400	23.33 (28.73)	0.965
9.	48 hr starvation + virus infection	7.096	1.000	14.00 (21.09)	0.764
10.	48 hr starvation	7.656	1.543	26.00 (30.50)	0.916
	S.E.M. \pm	0.508	0.083	2.85	0.056
	C.D. at 5%	1.509	0.246	8.468	0.166

* Figures in parentheses are angular transformed values

12 hr starvation period only, but very much less than that of control thus revealing the detrimental effect of starvation alone. Similar trend of less cocooning was observed in starved and infected batches (14.00 to 26.00 per cent). The cocoons spun by unstarved, infected worms was 22.66 per cent (Table 28).

The cocoon weight has not shown difference due to the effect of starvation (0.878 to 0.965 g/cocoon) but it was affected to a considerable extent (0.588 to 0.7648 g) by the combined effect of starvation and Kenchu virus infection (Table 28). The unstarved, uninfected worms and unstarved-infected worms gave 0.958 and 0.848 g cocoon weights, respectively. Similar trend was noticed even with regard to pupal weight, cocoon shell weight and shell ratio (Table 29). The difference in moth emergence was marginal (18.00 to 22.66 per cent) in starved-uninfected worms except with 12 hr starvation (28.66 per cent) and the pattern was similar even with starved and infected worms resulting in lesser moth emergence (11.33 to 24.00 per cent) compared to 18.66 per cent with unstarved and infected worms (Table 29).

The above data revealed the general reduction in growth and drastic reduction in cocooning and moth emergence due to starvation. Starvation combined with

Table 29. Effect of starvation and Kenchu virus infection in fifth instar silkworm on pupal weight, cocoon shell weight, cocoon shell ratio and moth emergence

Sl. No.	Treatments	Single pupal weight (g)	Single cocoon shell weight (g)	Cocoon shell ratio (%)	Per cent moth emergence
1.	No starvation + Virus infection	0.750	0.095	11.40 (19.37)*	18.66 (25.91)*
2.	No starvation	0.824	0.131	13.71 (21.72)	62.00 (51.94)
3.	12 hr starvation + Virus infection	0.651	0.079	10.74 (19.12)	24.00 (29.16)
4.	12 hr starvation	0.771	0.102	11.69 (19.99)	28.66 (32.38)
5.	24 hr starvation + Virus infection	0.512	0.071	12.09 (20.37)	13.33 (20.90)
6.	24 hr starvation	0.810	0.135	14.20 (22.13)	18.00 (25.04)
7.	36 hr starvation + Virus infection	0.668	0.090	11.95 (20.19)	14.66 (22.23)
8.	36 hr starvation	0.837	0.123	12.72 (20.93)	22.66 (28.35)
9.	48 hr starvation + virus infection	0.671	0.089	11.60 (19.91)	11.33 (19.06)
10.	48 hr starvation	0.795	0.117	12.69 (20.89)	22.66 (28.24)
	S.E.M. \pm	0.049	0.0091	0.560	2.870
	C.D. at 5%	0.145	0.0270	1.663	8.526

* Figures in parentheses are angular transformed values

Kenchu virus infection caused a slight reduction in larval and cocoon weights, but a marked falling down of cocooning and moth emergence. Additive effect of starvation and infection was more apparent in III and IV instar infections compared to V instar.

4.4.3 Effect of feeding tender, medium, coarse or water-supplemented leaves to silkworms on the intensity of Kenchu virus infection

The feeding of tender, medium, coarse and water supplemented leaves was effected from III, IV and V instars separately, where artificial infection of Kenchu virus was resorted to. The infection was carried out at the beginning of the respective instars.

4.4.3.1 Third instar

4.4.3.1.1 Larval weight

Small magnitude but significant differences were observed in the 7th day larval weights (Table 30). Leaf suitable for the instar enabled the larvae to attain a weight of 0.908 g. Larvae fed on medium and coarse leaves registered a small but significant reduction in larval weight. Infection with Kenchu virus led to a lowering of body weight but this effect was not distinguishable from the lowering resulting from differences in leaf quality.

Table 30. Effect of mulberry leaf maturity and Kenchu virus infection in third instar on larval weight, undersized worms, ET_{50} for Kenchu symptom exhibition and mortality.

Sl. No.	Treatments	Weight of 10 worms on 7th day	Per cent undersized worms on 15th day	ET_{50} for Kenchu symptoms (days)	Per cent mortality upto 15th day
1.	Tender leaf + Virus	0.805	30.66 (33.56)*	13.33 (3.715)**	13.33 (21.40)*
2.	Tender leaf	0.984	14.66 (22.19)	0.00 (0.707)	7.33 (14.42)
3.	Middle order leaf + Virus	0.791	29.33 (32.74)	14.00 (3.806)	16.00 (23.55)
4.	Middle order leaf	0.894	9.33 (17.63)	0.00 (0.707)	7.33 (14.94)
5.	Coarse leaf + Virus	0.715	19.33 (25.97)	12.33 (3.581)	10.00 (18.38)
6.	Coarse leaf	0.786	10.00 (18.38)	0.00 (0.707)	3.33 (10.40)
7.	Water soaked leaf suitable to the instar + Virus	0.709	22.66 (28.41)	14.33 (3.850)	25.33 (30.22)
8.	Water soaked leaf suitable to the instar	0.764	19.33 (25.65)	0.00 (0.707)	10.00 (18.36)
9.	Leaf suitable to the instar+Virus	0.765	30.00 (33.20)	15.33 (3.979)	26.66 (30.09)
10.	Leaf suitable to the instar	0.908	19.33 (25.33)	0.00 (0.707)	17.33 (24.51)
	S.E.M. \pm	0.0348	2.35	0.052	3.02
	C.D. at 5%	0.1030	6.981	0.154	8.972

* Figures in parentheses are angular transformed values

** Figures in parentheses are $\sqrt{x + 0.5}$ transformed values

Body weight differences in maximum weight were insignificant between the different leaf treatments. Infection with Kenchu virus, however, led to a significant lowering of maximum body weight (Table 31).

4.4.3.1.2 Symptom manifestation and mortality

Appearance of undersized worms as on the 15th day indicated a low value of 9.33 and 10.00 per cent in the medium and coarse leaf treatments, respectively. Differences in the other leaf types were not significant. Infection with Kenchu virus resulted in a statistically significant marked increase in the appearance of undersized worms with tender and medium leaf feeding (Table 30).

Kenchu symptoms were not seen in uninfected treatments receiving leaves of different maturity. All the infection treatments led to the manifestation of Kenchu symptoms (minimum ET_{50} of 12.33 days in coarse leaf treatment and maximum ET_{50} of 15.33 days in the control with recommended leaf feeding).

Mortality upto the 15th day was minimum (3.33 per cent) in coarse leaf feeding followed by medium leaf treatment. It was 10.0 and 17.33 per cent in water supplemented and recommended leaf treatments, respectively. Significant mortality due to infection was seen with (a) leaf suitable for the instar (b) water fortified leaf

Table 31. Effect of mulberry leaf maturity and Kenchu virus infection in third instar on maximum larval weight, extent of cocooning, cocoon weight and pupal weight

Sl. No.	Treatments	Single maximum larval weight (g)	Per cent cocooning	Single cocoon weight (g)	Single pupal weight (g)
1.	Tender leaf + Virus	1.033	32.66 (35.15)*	0.927	0.785
2.	Tender leaf	1.561	47.33 (43.47)	0.986	0.880
3.	Middle order leaf + Virus	1.055	31.33 (33.83)	0.811	0.696
4.	Middle order leaf	1.624	56.66 (48.88)	0.989	0.852
5.	Coarse leaf + Virus	1.255	36.66 (37.26)	0.935	0.811
6.	Coarse leaf	1.536	68.00 (55.57)	0.999	0.859
7.	Water soaked leaf suitable to the instar + Virus	1.005	18.66 (25.55)	0.842	0.695
8.	Water soaked leaf suitable to the instar	1.553	73.33 (58.69)	1.009	0.955
9.	Leaf suitable to instar + Virus	1.250	36.66 (37.13)	0.902	0.782
10.	Leaf suitable to instar	1.561	71.33 (58.10)	1.068	0.928
	S.E.M. \pm	0.0739	2.77	0.0485	0.0371
	C.D. at 5%	0.2200	8.229	0.1440	0.1100

*Figures in parentheses are angular transformed values

and (c) medium leaf (Table 30).

4.4.3.1.3 Percentage cocooning, cocoon and shell weights and moth emergence

The percentage of worms spinning cocoons was found to be high with recommended leaf feeding (71.33). Addition of water to the leaf gave a cocooning value of 73.33 per cent. A significantly lesser cocooning was observed with tender, and medium leaf treatments (47.33 and 56.66 per cent) whereas coarse leaf treatment was not found to significantly affect cocooning (68.00 per cent) as compared to standard leaf feeding. Disease incidence substantially reduced cocooning in all the treatments, maximum effect being seen with water treated leaves (Table 31).

Cocoon weights were similar in all uninoculated treatments. Significant reduction in cocoon weight occurred with infected worms fed on either water soaked leaves or medium maturity leaf. Pupal weights exhibited a similar trend (Table 31). In all healthy lots, differing leaf feedings did not exert any influence on shell weight. Decreased shell weight was registered with (a) recommended leaf (b) watersoaked leaf and (c) medium leaf treatments in the case of infection with Kenchu virus. Cocoon shell ratios were not influenced by the different leaf feedings either with or without infection (Table 32).

Table 32. Effect of mulberry leaf maturity and Kenohu virus infection in third instar on cocoon shell weight, cocoon shell percentage and moth emergence

Sl. No.	Treatments	Single cocoon shell weight (g)	Cocoon shell percentage	Per cent moth emergence
1.	Tender leaf + Virus	0.1262	13.59 (21.63)*	28.66 (32.35)*
2.	Tender leaf	0.1289	13.13 (21.23)	30.00 (33.19)
3.	Middle order leaf + Virus	0.1098	13.48 (21.55)	28.66 (32.33)
4.	Middle order leaf	0.1358	13.76 (21.78)	62.66 (46.14)
5.	Coarse leaf + Virus	0.1226	13.11 (21.19)	27.33 (31.50)
6.	Coarse leaf	0.1359	13.60 (21.64)	51.33 (45.77)
7.	Water soaked leaf suitable to the instar + Virus	0.0699	8.50 (16.18)	16.66 (24.04)
8.	Water soaked leaf suitable to the instar	0.1293	12.00 (20.27)	38.66 (38.43)
9.	Leaf suitable to the instar + Virus	0.1147	12.70 (20.85)	25.33 (30.07)
10.	Leaf suitable to the instar	0.1347	12.61 (20.79)	50.66 (45.38)
S.E.M. \pm		0.0066	0.730	2.170
C.D. at 5%		0.0196	2.168	6.447

* Figures in parentheses are angular transformed values

Moth emergence was high with recommended leaf and coarse leaf feedings (50.66 and 51.33 per cent respectively). Moth emergence was only 30 per cent with tender leaf. Infection resulted in a substantial decrease in moth emergence with recommended leaf, water supplemented leaf, coarse leaf and medium leaf but not with tender leaf feeding (Table 32).

4.4.3.2 Fourth instar

4.4.3.2.1 Larval weight

On the 7th day all the leaf treatments indicated similar body weights with the exception of water treated leaves which showed a slight reduction. Superimposing infection invariably resulted in a reduction in body weight in comparison with the respective controls, the only exception being tender leaf treatment (Table 33).

No significant maximum body weight variation was noticed in the uninfected larvae receiving different types of leaf. Infection also did not have appreciable effect except in one instance - water soaked leaf accompanied by infection.

4.4.3.2.2 Symptom manifestation and mortality

There were no significant differences in larval growth resulting in undersized worms in all the treatments on the 10th day (Table 33).

Table 33. Effect of mulberry leaf maturity and Kenchu virus infection in fourth instar on larval weight, undersized worms, mortality and ET₅₀ for Kenchu symptom exhibition

Sl. No.	Treatments	Weight of 10 worms on 7th days (g)	Per cent undersized worms on 10th day	Per cent mortality upto 10th day	ET ₅₀ for Kenchu symptom exhibition (Days)
1.	Tender leaf + Virus	3.053	8.66 (19.99)*	22.00 (27.87)*	14.57 (3.894)**
2.	Tender leaf	3.211	6.00 (14.18)	6.00 (13.31)	0.00 (0.707)
3.	Middle order leaf + Virus	2.836	6.66 (14.93)	22.00 (27.91)	16.00 (4.046)
4.	Middle order leaf	3.376	5.33 (12.91)	4.66 (12.03)	0.00 (0.707)
5.	Coarse leaf + Virus	2.518	12.66 (20.48)	19.33 (23.55)	12.67 (3.628)
6.	Coarse leaf	3.267	10.66 (19.05)	4.00 (11.28)	0.00 (0.707)
7.	Water soaked leaf suitable to the instar + Virus	2.723	18.00 (24.96)	13.33 (21.09)	14.67 (3.894)
8.	Water soaked leaf suitable to the instar	3.057	9.33 (16.55)	4.00 (10.90)	0.00 (0.707)
9.	Leaf suitable to the instar + Virus	3.009	11.33 (19.32)	5.33 (13.30)	15.67 (4.020)
10.	Leaf suitable to the instar	3.524	8.00 (16.35)	3.33 (10.40)	0.00 (0.707)
	S.E.M. \pm	0.122	2.540	2.100	0.033
	G.D. at 5%	0.366	7.546	6.239	0.098

NS

* Figures in parentheses are angular transformed values

** Figures in parentheses are $\sqrt{x + 0.5}$ transformed values

NS = Nonsignificant

Mortality upto the 10th day was low and statistically insignificant between leaf treatments without infection. Tender, medium, coarse and water treated leaf feeding coupled with infection resulted in a significant increase in mortality on the 10th day. Kenchu virus symptoms were not exhibited in larvae from different leaf treatments. ET_{50} for Kenchu symptom expression ranged from 12.67 to 16.00 days in the different treatments where infection was carried out (Table 33).

4.4.3.2.3 Cocooning, cocoon and shell weights and moth emergence

Coarse leaf feeding gave a maximum percentage cocooning of 79.33. With recommended leaf feeding it was 71.33 per cent. There was a tendency for a reduction in percentage cocooning as the coarseness of the fed leaf decreased. There was a statistically significant decrease in the percentage cocooning as a result of Kenchu infection in all leaf treatments. An interesting point was noted that the response to infection was more with water soaked and coarse leaves as compared to medium and tender leaves.

There were no significant differences in cocoon, pupa and cocoon shell weights and also cocoon shell ratio in the different treatments (Tables 34 and 35) under uninfected treatments.

Table 34. Effect of mulberry leaf maturity and Kenchu virus infection in fourth instar silkworm on larval weight, extent of cocooning, cocoon weight and pupal weight

Sl. No.	Treatments	Single maximum larval weight (g)	Per cent cocooning	Single cocoon weight (g)	Single pupal weight (g)
1.	Tender leaf + Virus	1.368	33.33 (35.19)*	0.9283	0.7959
2.	Tender leaf	1.495	58.67 (50.04)	0.9986	0.8732
3.	Middle order leaf + Virus	1.443	47.33 (43.46)	0.9429	0.8123
4.	Middle order leaf	1.526	59.33 (50.68)	1.0145	0.8813
5.	Coarse leaf + Virus	1.383	48.67 (44.24)	0.8298	0.7217
6.	Coarse leaf	1.410	79.33 (65.92)	0.8754	0.7516
7.	Water soaked leaf suitable to the instar + Virus	1.244	21.33 (27.48)	0.8554	0.7038
8.	Water soaked leaf suitable to the instar	1.488	65.33 (54.07)	0.9774	0.8323
9.	Leaf suitable to instar + Virus	1.379	36.00 (38.70)	1.0457	0.9038
10.	Leaf suitable to instar	1.506	71.33 (57.68)	0.9883	0.8580
	S.Em. \pm	0.0463	4.39	0.0459	0.0421
	C.D. at 5%	0.1390	13.042	0.1363	0.1250

* Figures in parentheses are angular transformed values
NS = Non-significant

Table 35. Effect of mulberry leaf maturity and Kenchu virus infection in fourth instar on cocoon shell weight, cocoon shell ratio and moth emergence

Sl. No.	Treatments	Single cocoon shell weight (g)	Cocoon shell ratio (%)	Per cent moth emergence
1.	Tender leaf + Virus	0.1291	13.90 (21.86) *	24.00 (29.32) *
2.	Tender leaf	0.1337	13.39 (21.40)	43.33 (41.13)
3.	Middle order leaf + Virus	0.1256	13.33 (21.39)	27.33 (31.50)
4.	Middle order leaf	0.1349	13.29 (21.39)	44.00 (41.42)
5.	Coarse leaf + Virus	0.0997	12.02 (20.26)	32.66 (34.82)
6.	Coarse leaf	0.1197	13.64 (21.65)	72.00 (59.02)
7.	Water soaked leaf suitable to the instar + Virus	0.1133	13.20 (21.27)	17.33 (21.70)
8.	Water soaked leaf suitable to the instar	0.1396	14.29 (22.21)	38.66 (38.42)
9.	Leaf suitable to instar + Virus	0.1362	13.11 (21.17)	28.66 (32.12)
10.	Leaf suitable to instar	0.1318	13.36 (21.41)	46.00 (42.70)
	S.E.M. \pm	0.0071	0.490	3.540
	C.D. at 5%	0.0210	1.455	10.517
		NS	NS	

* Figures in parentheses are angular transformed values

NS = Non-significant

Moth emergence was highest with coarse leaf feeding (72.00 per cent) which was significantly higher than that for other leaf treatments. Infection resulted in a significant decrease in moth emergence in all cases. As in the case of percentage cocooning, moth emergence was maximally affected in the case of superimposition of infection on water soaked leaf and coarse leaf treatments (Table 35).

4.4.3.3 Fifth instar

Maximum larval weight, cocoon and pupal weights in larvae from different treatments with or without infection were not significantly affected (Tables 36 and 37). Cocoon shell weights were of the same order in all the treatments except in the virus infected batch fed on water treated leaves. No appreciable differences were noticed in shell ratios in different treatments even though the data were statistically significant.

Percentage of cocooning ranged from 57.33 to 78.00 in healthy treatments. Significant difference was seen only with coarse leaf where maximum cocooning occurred. Infection with virus substantially reduced cocooning in water soaked leaf, coarse leaf and tender leaf fed batches (Table 36). A similar trend was observed even in moth emergence (Table 37).

Table 36. Effect of mulberry leaf maturity and Kenchu virus infection in fifth instar on maximum larval weight, extent of cocooning, cocoon weight and pupal weight

Sl. No.	Treatments	Single maximum larval weight (g)	Per cent cocooning	Single cocoon weight (g)	Single pupal weight (g)
1.	Tender leaf + Virus	1.284	41.33 (39.68)*	0.9416	0.8181
2.	Tender leaf	1.297	69.33 (57.20)	0.9890	0.8513
3.	Middle order leaf + Virus	1.281	54.66 (47.69)	0.8893	0.7737
4.	Middle order leaf	1.283	57.33 (49.22)	0.9817	0.8479
5.	Coarse leaf + Virus	1.326	51.33 (45.76)	0.8873	0.7716
6.	Coarse leaf	1.368	78.00 (62.34)	0.9312	0.8045
7.	Water soaked leaf suitable to the instar + Virus	1.335	52.66 (48.0)	0.9019	0.7892
8.	Water soaked leaf suitable to the instar	1.258	73.33 (58.93)	0.8963	0.8076
9.	Leaf suitable to the instar + Virus	1.109	58.66 (50.02)	0.9219	0.7898
10.	Leaf suitable to the instar	1.283	59.33 (50.45)	0.9339	0.8191
	S.E.M. \pm	0.0710	3.820	0.0297	0.0256
	C.D. at 5%	0.2109	11.349	0.0882	0.0760
		NS		NS	NS

* Figures in parentheses are angular transformed values

NS = Non-significant

Table 37. Effect of mulberry leaf maturity and Kenchu virus infection in fifth instar on cocoon shell weight, cocoon shell ratio and moth emergence

Sl. No.	Treatments	Single cocoon shell weight (g)	Shell ratio (%)	Per cent moth emergence
1.	Tender leaf + Virus	0.1175	12.46 (20.67)*	28.66 (31.90)*
2.	Tender leaf	0.1316	13.31 (21.34)	64.66 (53.65)
3.	Middle order leaf + Virus	0.1106	12.41 (20.64)	40.00 (39.18)
4.	Middle order leaf	0.1304	13.30 (21.41)	46.86 (43.08)
5.	Coarse leaf + Virus	0.1107	12.44 (20.64)	36.66 (37.26)
6.	Coarse leaf	0.1217	13.08 (21.19)	74.00 (59.44)
7.	Water soaked leaf suitable to the instar + Virus	0.1077	11.94 (20.20)	39.33 (38.83)
8.	Water soaked leaf suitable to the instar	0.1247	13.96 (21.94)	66.66 (54.74)
9.	Leaf suitable to the instar + Virus	0.1271	13.78 (21.80)	45.33 (42.31)
10.	Leaf suitable to the instar	0.1265	13.54 (21.58)	52.66 (46.53)
	S.E.M. \pm	0.0041	0.290	2.510
	C.D. at 5%	0.0121	0.861	7.457

* Figures in parentheses are angular transformed values

A survey of the above data revealed that larval and cocoon weights were not appreciably affected while extent of cocooning and moth emergence were much significantly influenced. Coarse leaf feeding caused more cocooning and moth emergence. The effect of leaf maturity and Kenchu virus infection was one of causing body weight reduction, instar and moult prolongation and reduction in survival. No appreciable influence of leaf quality on the development and intensity of Kenchu infection was evident from the present experiments.

4.5 Interaction effect of simultaneous infection with Kenchu virus and *Staphylococcus aureus* in the silkworm

Simultaneous infection with Kenchu virus and *S. aureus* was investigated. Virus extract was used at three concentrations and the bacterium was used at five concentrations. The combination effects were studied by infecting the early III, IV and V instar silkworms. Silkworms infected on the 2nd and 3rd day of V instar were also investigated separately and the results are presented below.

4.5.1 Third instar

4.5.1.1 Larval weight

A statistically significant reduction in growth as evidenced by larval weight was clear in all infected

Table 38. Interaction effect of simultaneous infection with Kenchu virus and *Staphylococcus aureus* in third instar silkworm on larval weight and duration of fourth moult

Sl. No.	Treatments		Weight of 10 worms on 7th day (g)	Weight of single worm on 15th day (g)	Duration of fourth moult (hr)
	Kenchu virus	<i>S. aureus</i>			
V ₁	10 ⁻²	-	0.698	0.219	34.00
V ₂	10 ⁻³	-	0.759	0.317	36.00
V ₃	10 ⁻⁴	-	0.863	0.327	34.00
B ₁	-	10 ⁷ cells/ml	0.939	0.567	34.00
B ₂	-	10 ⁶ "	0.946	0.570	32.00
B ₃	-	10 ⁵ "	0.901	0.603	32.00
B ₄	-	10 ⁴ "	0.814	0.470	34.00
B ₅	-	10 ³ "	0.875	0.681	30.00
V ₁ B ₁	10 ⁻² +	10 ⁷ "	0.664	0.125	40.00
V ₁ B ₂	10 ⁻² +	10 ⁶ "	0.806	0.165	34.00
V ₁ B ₃	10 ⁻² +	10 ⁵ "	0.737	0.133	36.00
V ₁ B ₄	10 ⁻² +	10 ⁴ "	0.961	0.241	34.00
V ₁ B ₅	10 ⁻² +	10 ³ "	0.644	0.424	34.00
V ₂ B ₁	10 ⁻³ +	10 ⁷ "	0.827	0.166	32.00
V ₂ B ₂	10 ⁻³ +	10 ⁶ "	0.577	0.240	30.00
V ₂ B ₃	10 ⁻³ +	10 ⁵ "	0.767	0.239	32.00
V ₂ B ₄	10 ⁻³ +	10 ⁴ "	0.561	0.245	30.00
V ₂ B ₅	10 ⁻³ +	10 ³ "	0.736	0.217	32.00
V ₃ B ₁	10 ⁻⁴ +	10 ⁷ "	0.904	0.301	32.00
V ₃ B ₂	10 ⁻⁴ +	10 ⁶ "	0.827	0.252	30.00
V ₃ B ₃	10 ⁻⁴ +	10 ⁵ "	0.533	0.251	32.00
V ₃ B ₄	10 ⁻⁴ +	10 ⁴ "	0.761	0.285	32.00
V ₃ B ₅	10 ⁻⁴ +	10 ³ "	0.754	0.293	30.00
C ₁	Buffer control		1.092	0.751	24.00
C ₂	Distilled water control		1.073	0.724	26.00
C ₃	Untreated control		1.021	0.710	24.00
S.E.m. +			0.037	0.023	1.65
C.D. at 5%			0.105	0.0654	4.694

Plate 8. Silkworms on 15th day of infection with different dilutions of Kenchu virus in third instar

Worms infected with: 10^{-2} , 10^{-3} and 10^{-4} Kenchu virus
Control worms : Buffer, Distilled water and Untreated

Plate 9. Silkworms on 15th day of infection with different concentrations of Staphylococcus aureus in third instar.

Worms infected with: 10^7 , 10^6 , 10^5 , 10^4 and 10^3 cells/ml
Control worms : Buffer, Distilled water and Untreated



Plate 10. Silkworms on 15th day of simultaneous infection with Kenchu virus and Staphylococcus aureus in third instar.

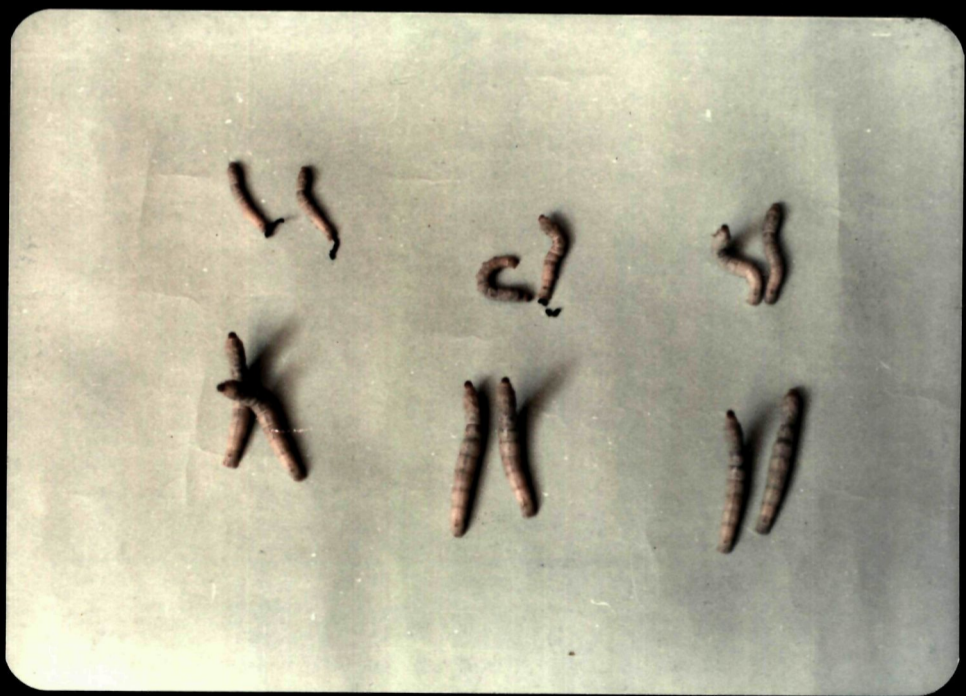
Worms

infected with: V₁B₁ V₁B₂ V₁B₃ V₁B₄ V₁B₅ V₂B₁ V₂B₂ V₂B₃ V₂B₄
V₂B₅ V₃B₁ V₃B₂ V₃B₃ V₃B₄ V₃B₅

Control worms: Buffer, Distilled water and untreated

Plate 11: Silkworms on 11th day of infection with different dilutions of Kenchu virus in fourth instar.

Worms infected with: 10⁻², 10⁻³ and 10⁻⁴ Kenchu virus
Control worms : Buffer, Distilled water and Untreated



treatments on 7th day (0.533 to 0.961 g/10 worms) and 15th day (0.125 to 0.681/worm) (Fig.4 and Plates 8, 9 and 10) as compared to their respective controls of 1.092 g and 0.751 g, respectively (Table 38).

4.5.1.2 Moulting duration, instar duration and instar progression

Fourth moulting duration was significantly delayed in all infected batches. The per cent worms entering into IV instar was non-significant. But the same entering to V instar exhibited significant differences in that in all infected batches there was less per cent of progression (2.66 to 54.67) compared to uninfected buffer control (86.67 per cent) (Table 38 and Fig.5). Differences in third instar duration were also non-significant while there was considerable extension of IV instar (4.58 to 6.50 days) compared to uninfected control (4.17 days) (Table 39).

4.5.1.3 Cocooning, cocoon and shell weights and moth emergence

Cocooning was absent with 10^{-2} virus as well as with all combinations of virus and bacteria, thus indicating the pronounced interaction effect in III instar (Tables 40 and 41). However, among the surviving infected batches, cocooning was significantly reduced (4.66 to 25.33 per cent) as against 81.33 per cent in control. Reduction in cocoon weight, pupal weight, shell weight, cocoon

Fig.4. Interaction effect of simultaneous infection with Kenchu virus and Staphylococcus aureus in third instar silkworm on larval weight on 15th day.

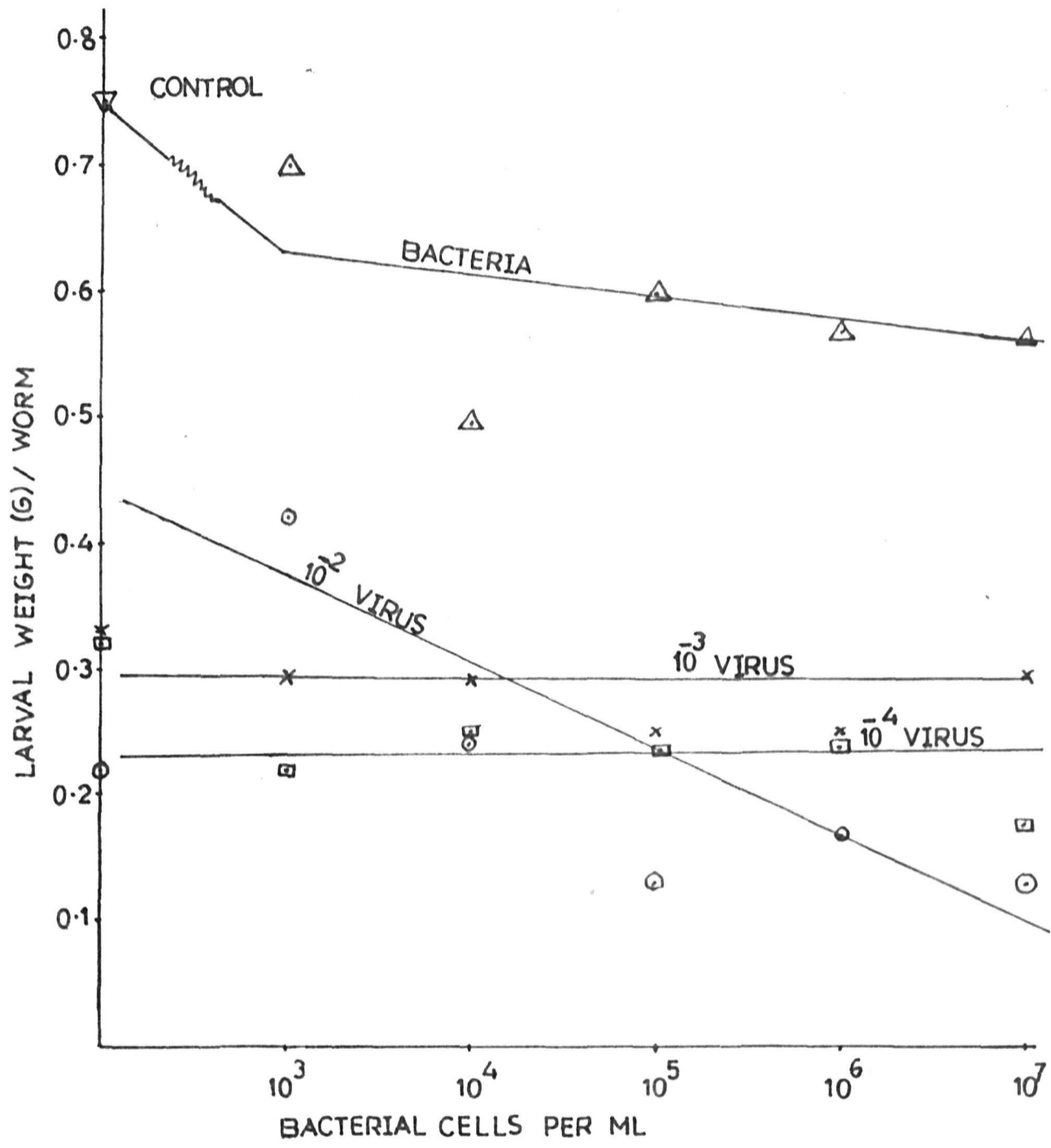


FIGURE - 4

Table 39. Interaction effect of simultaneous infection with Kenchu virus and *Staphylococcus aureus* in third instar silkworm on extent of worms entering subsequent instars and instar duration

Sl. No.	Treatments		Per cent worms entering IV instar	Per cent worms entering V instar	III instar duration (Hr)	IV instar duration (Days)
	Kenchu virus	<i>S. aureus</i>				
V ₁	10 ⁻²	-	98.67(86.15)*	13.33(21.37)*	92.00	5.58
V ₂	10 ⁻³	-	97.67(74.58)	28.67(32.33)	88.00	5.67
V ₃	10 ⁻⁴	-	100.00(90.00)	32.00(34.42)	89.33	5.17
B ₁	-	10 ⁷ cells/ml	99.33(87.29)	42.67(40.75)	88.00	5.58
B ₂	-	10 ⁶	100.00(90.00)	43.33(41.16)	88.00	5.58
B ₃	-	10 ⁵	98.67(86.15)	44.67(44.24)	84.00	5.25
B ₄	-	10 ⁴	99.33(87.29)	54.67(47.68)	86.00	5.00
B ₅	-	10 ³	100.00(90.00)	52.67(46.53)	88.00	5.00
V ₁ B ₁	10 ⁻²	+ 10 ⁷	98.00(83.44)	2.66(9.27)	92.00	5.67
V ₁ B ₂	10 ⁻²	+ 10 ⁶	99.33(87.29)	6.00(14.05)	90.00	5.58
V ₁ B ₃	10 ⁻²	+ 10 ⁵	98.00(83.44)	7.33(15.47)	86.00	5.83
V ₁ B ₄	10 ⁻²	+ 10 ⁴	98.67(86.15)	18.00(25.01)	86.00	6.50
V ₁ B ₅	10 ⁻²	+ 10 ³	100.00(90.00)	12.66(20.76)	88.00	6.00
V ₂ B ₁	10 ⁻³	+ 10 ⁷	98.67(86.15)	20.67(27.00)	86.00	5.42
V ₂ B ₂	10 ⁻³	+ 10 ⁶	99.33(87.29)	20.00(26.45)	84.00	5.33
V ₂ B ₃	10 ⁻³	+ 10 ⁵	100.00(90.00)	20.00(26.49)	86.00	5.42
V ₂ B ₄	10 ⁻³	+ 10 ⁴	100.00(90.00)	16.67(23.56)	86.00	5.08
V ₂ B ₅	10 ⁻³	+ 10 ³	100.00(90.00)	16.67(23.78)	84.00	5.17
V ₃ B ₁	10 ⁻⁴	+ 10 ⁷	99.33(84.35)	32.67(34.84)	86.00	4.83
V ₃ B ₂	10 ⁻⁴	+ 10 ⁶	98.67(83.45)	26.67(30.96)	84.67	4.67
V ₃ B ₃	10 ⁻⁴	+ 10 ⁵	100.00(90.00)	26.00(30.32)	84.00	4.83
V ₃ B ₄	10 ⁻⁴	+ 10 ⁴	100.00(90.00)	34.00(35.40)	86.00	4.67
V ₃ B ₅	10 ⁻⁴	+ 10 ³	100.00(90.00)	36.00(36.70)	88.00	4.58
C ₁	Buffer control		99.33(87.29)	86.67(68.83)	86.00	4.17
C ₂	Distilled water control		100.00(90.00)	84.67(67.14)	86.00	4.17
C ₃	Untreated control		100.00(90.00)	87.33(69.34)	88.00	4.17

S.E.m. +

C.D. at 5%

3.17

9.019

2.34

6.658

2.03

5.776

0.21

0.597

* Figures in parentheses are angular transformed values
NS = Non-significant

Fig.5. Interaction effect of simultaneous infection with Kenchu virus and Staphylococcus aureus in third instar silkworms on extent of worms entering fifth instar.

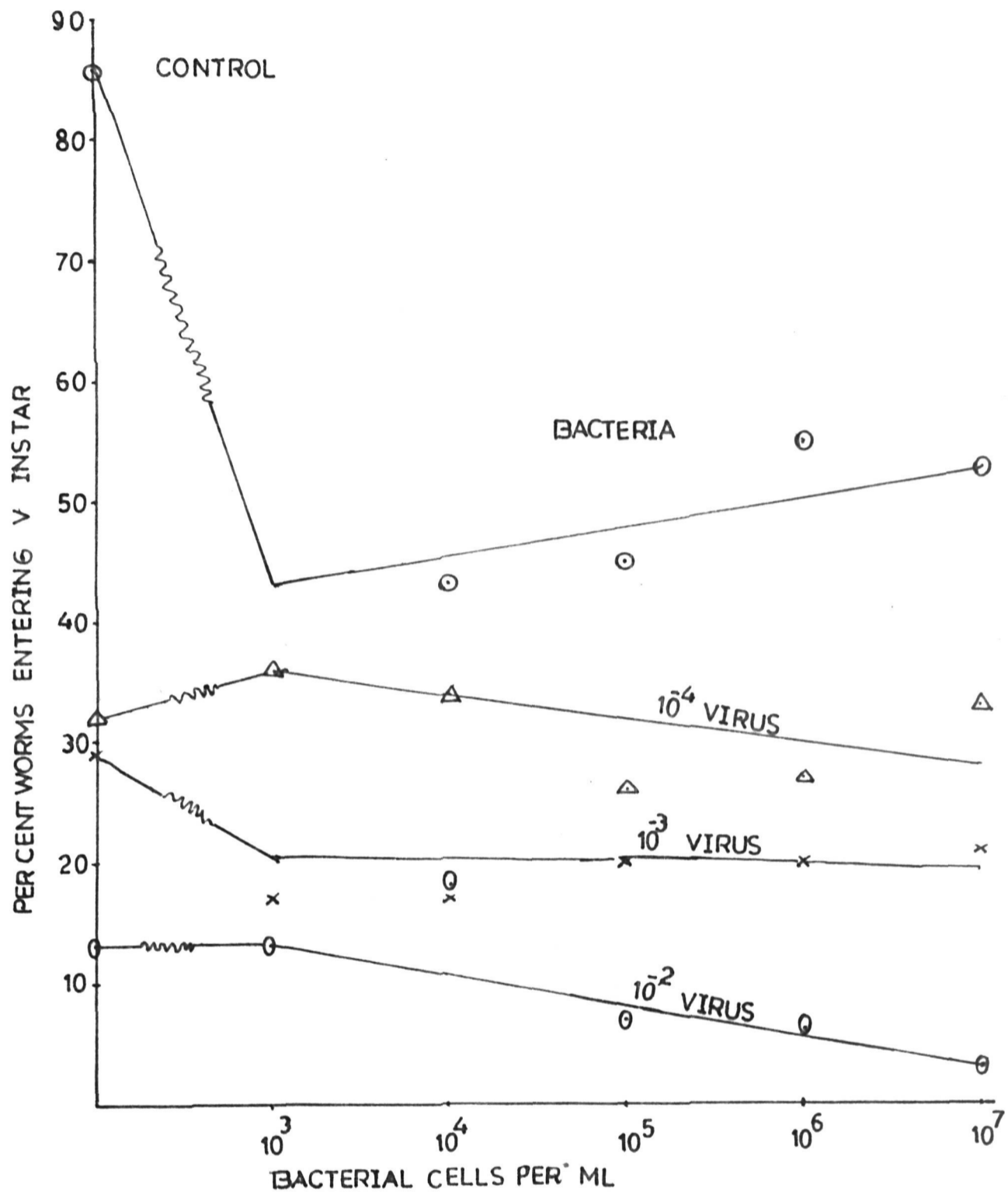


FIGURE - 5

Table 40. Interaction effect of simultaneous infection with Kenchu virus and *Staphylococcus aureus* in third instar silkworm on extent of cocooning and cocoon and pupal weights

No.	Treatments		Per cent cocooning	Single cocoon weight (g)	Single pupal weight (g)
	Kenchu virus	<i>S. aureus</i>			
V ₁	10 ⁻²	-	0.00(0.00)*	0.000(0.707)**	0.000(0.707)**
V ₂	10 ⁻³	-	8.00(16.08)	0.678(1.084)	0.580(1.039)
V ₃	10 ⁻⁴	-	4.66(12.42)	0.649(1.071)	0.559(1.028)
B ₁	-	10 ⁷ cells/ml	14.66(22.48)	0.799(1.139)	0.692(1.091)
B ₂	-	10 ⁶	16.00(23.37)	0.670(1.083)	0.584(1.041)
B ₃	-	10 ⁵	18.00(24.96)	0.616(1.054)	0.528(1.012)
B ₄	-	10 ⁴	22.66(28.36)	0.644(1.069)	0.553(1.025)
B ₅	-	10 ³	25.33(30.17)	0.637(1.065)	0.552(1.024)
V ₁ B ₁	10 ⁻²	+ 10 ⁷	0.00(0.00)	0.000(0.707)	0.000(0.707)
V ₁ B ₂	10 ⁻²	+ 10 ⁶	0.00(0.00)	0.000(0.707)	0.000(0.707)
V ₁ B ₃	10 ⁻²	+ 10 ⁵	0.00(0.00)	0.000(0.707)	0.000(0.707)
V ₁ B ₄	10 ⁻²	+ 10 ⁴	0.00(0.00)	0.000(0.707)	0.000(0.707)
V ₁ B ₅	10 ⁻²	+ 10 ³	0.00(0.00)	0.000(0.707)	0.000(0.707)
V ₂ B ₁	10 ⁻³	+ 10 ⁷	0.00(0.00)	0.000(0.707)	0.000(0.707)
V ₂ B ₂	10 ⁻³	+ 10 ⁶	0.00(0.00)	0.000(0.707)	0.000(0.707)
V ₂ B ₃	10 ⁻³	+ 10 ⁵	0.00(0.00)	0.000(0.707)	0.000(0.707)
V ₂ B ₄	10 ⁻³	+ 10 ⁴	0.00(0.00)	0.000(0.707)	0.000(0.707)
V ₂ B ₅	10 ⁻³	+ 10 ³	0.00(0.00)	0.000(0.707)	0.000(0.707)
V ₃ B ₁	10 ⁻⁴	+ 10 ⁷	0.00(0.00)	0.000(0.707)	0.000(0.707)
V ₃ B ₂	10 ⁻⁴	+ 10 ⁶	0.00(0.00)	0.000(0.707)	0.000(0.707)
V ₃ B ₃	10 ⁻⁴	+ 10 ⁵	0.00(0.00)	0.000(0.707)	0.000(0.707)
V ₃ B ₄	10 ⁻⁴	+ 10 ⁴	0.00(0.00)	0.000(0.707)	0.000(0.707)
V ₃ B ₅	10 ⁻⁴	+ 10 ³	0.00(0.00)	0.000(0.707)	0.000(0.707)
C ₁	Buffer control		81.33(64.50)	0.906(1.185)	0.784(1.133)
C ₂	Distilled water control		78.67(62.56)	0.892(1.179)	0.756(1.120)
C ₃	Untreated control		76.67(61.17)	0.910(1.296)	0.783(1.133)

S.E.m. +

C.D. at 5%

1.16

3.300

0.025

0.071

0.014

0.0398

* Figures in parentheses are angular transformed values

** Figures in parentheses are $\sqrt{x + 0.5}$ transformed values

Table 41. Interaction effect of simultaneous infection with Kenchu virus and Staphylococcus aureus in third instar silkworm on cocoon shell weight, shell ratio and moth emergence.

No.	Treatments		Single cocoon shell weight (g)	Shell ratio (%)	Moth emergence (%)
	Kenchu virus	<u>S. aureus</u>			
V ₁	10 ⁻²	-	0.000(0.707)*	0.00(0.00)**	0.00(0.00)**
V ₂	10 ⁻³	-	0.091(0.768)	13.10(21.17)	4.66(12.42)
V ₃	10 ⁻⁴	-	0.083(0.763)	12.94(21.05)	4.66(12.42)
B ₁	-	10 ⁷ cells/ml	0.101(0.775)	12.68(20.83)	13.33(21.40)
B ₂	-	10 ⁶ ..	0.079(0.760)	11.85(20.11)	12.00(20.09)
B ₃	-	10 ⁵ ..	0.081(0.762)	13.15(21.28)	13.33(21.27)
B ₄	-	10 ⁴ ..	0.083(0.763)	12.96(21.09)	17.33(24.54)
B ₅	-	10 ³ ..	0.077(0.759)	12.07(20.25)	18.00(24.93)
V ₁ B ₁	10 ⁻²	+ 10 ⁷ ..	0.000(0.707)	0.00(0.00)	0.00(0.00)
V ₁ B ₂	10 ⁻²	+ 10 ⁶ ..	0.000(0.707)	0.00(0.00)	0.00(0.00)
V ₁ B ₃	10 ⁻²	+ 10 ⁵ ..	0.000(0.707)	0.00(0.00)	0.00(0.00)
V ₁ B ₄	10 ⁻²	+ 10 ⁴ ..	0.000(0.707)	0.00(0.00)	0.00(0.00)
V ₁ B ₅	10 ⁻²	+ 10 ³ ..	0.000(0.707)	0.00(0.00)	0.00(0.00)
V ₂ B ₁	10 ⁻³	+ 10 ⁷ ..	0.000(0.707)	0.00(0.00)	0.00(0.00)
V ₂ B ₂	10 ⁻³	+ 10 ⁶ ..	0.000(0.707)	0.00(0.00)	0.00(0.00)
V ₂ B ₃	10 ⁻³	+ 10 ⁵ ..	0.000(0.707)	0.00(0.00)	0.00(0.00)
V ₂ B ₄	10 ⁻³	+ 10 ⁴ ..	0.000(0.707)	0.00(0.00)	0.00(0.00)
V ₂ B ₅	10 ⁻³	+ 10 ³ ..	0.000(0.707)	0.00(0.00)	0.00(0.00)
V ₃ B ₁	10 ⁻⁴	+ 10 ⁷ ..	0.000(0.707)	0.00(0.00)	0.00(0.00)
V ₃ B ₂	10 ⁻⁴	+ 10 ⁶ ..	0.000(0.707)	0.00(0.00)	0.00(0.00)
V ₃ B ₃	10 ⁻⁴	+ 10 ⁵ ..	0.000(0.707)	0.00(0.00)	0.00(0.00)
V ₃ B ₄	10 ⁻⁴	+ 10 ⁴ ..	0.000(0.707)	0.00(0.00)	0.00(0.00)
V ₃ B ₅	10 ⁻⁴	+ 10 ³ ..	0.000(0.707)	0.00(0.00)	0.00(0.00)
C ₁	Buffer control		0.130(0.793)	14.39(22.27)	79.33(63.07)
C ₂	Distilled water control		0.129(0.793)	14.54(22.40)	78.00(62.09)
C ₃	Untreated control		0.124(0.789)	13.60(21.63)	75.33(60.30)
S.E.m. +			0.003	0.48	1.05
C.D. at 5%			0.0085	1.365	2.987

* Figures in parentheses are $\sqrt{x + 0.5}$ transformed values

** Figures in parentheses are angular transformed values

shell ratio and moth emergence was evident (Tables 40 and 41).

4.5.2 Fourth instar

4.5.2.1 Larval weight

The weight of 10 worms on 7th day was considerably reduced amongst infected batches (2.372 to 3.672 g/10 worms) compared to that of control (4.771 g) (Fig.6 and Plates 11, 12 and 13). It was interesting to note that the weight reduction was more with simultaneous infection of higher doses of both bacteria and virus (Table 42). The maximum larval weight also exhibited the same tendency. Higher dose of virus as well as its combination with all bacterial doses had detrimental effect.

4.5.2.2 Instar progression, moulting duration and instar duration

Though the progression of worms to V instar was statistically significant, the degree of differences was minimal compared to healthy control (Table 42). Fourth instar duration, fourth moulting duration as well as fifth instar duration showed statistically significant differences in the infected batches compared to the control (Table 43).

Table 42. Interaction effect of simultaneous infection with Kenchu virus and *Staphylococcus aureus* in fourth instar silkworm on extent of worms entering fifth instar and larval weight

No.	Treatments		Per cent worms entering fifth instar	Weight of 10 worms on 7th day (g)	Single maximum larval weight (g)
	Kenchu virus	<i>S. aureus</i>			
V ₁	10 ⁻²	-	88.67(70.11)*	2.643	0.000(0.707)**
V ₂	10 ⁻³	-	94.67(76.83)	2.982	1.094(1.262)
V ₃	10 ⁻⁴	-	90.00(71.80)	2.747	1.147(1.283)
B ₁	-	10 ⁷ cells/ml	94.00(76.69)	2.372	0.975(1.214)
B ₂	-	10 ⁶	92.67(74.53)	2.761	0.904(1.185)
B ₃	-	10 ⁵	92.00(73.92)	2.739	0.939(1.199)
B ₄	-	10 ⁴	94.00(76.42)	2.920	1.189(1.299)
B ₅	-	10 ³	94.00(76.69)	2.829	1.158(1.286)
V ₁ B ₁	10 ⁻²	+ 10 ⁷	89.33(71.05)	2.841	0.000(0.707)
V ₁ B ₂	10 ⁻²	+ 10 ⁶	89.33(71.01)	2.589	0.000(0.707)
V ₁ B ₃	10 ⁻²	+ 10 ⁵	88.00(70.09)	2.673	0.000(0.707)
V ₁ B ₄	10 ⁻²	+ 10 ⁴	84.00(66.67)	2.729	0.000(0.707)
V ₁ B ₅	10 ⁻²	+ 10 ³	92.67(74.67)	2.928	0.000(0.707)
V ₂ B ₁	10 ⁻³	+ 10 ⁷	88.67(70.52)	3.265	1.219(1.310)
V ₂ B ₂	10 ⁻³	+ 10 ⁶	86.00(68.56)	3.276	1.054(1.246)
V ₂ B ₃	10 ⁻³	+ 10 ⁵	81.33(66.67)	2.865	1.033(1.238)
V ₂ B ₄	10 ⁻³	+ 10 ⁴	84.67(67.14)	3.175	1.075(1.254)
V ₂ B ₅	10 ⁻³	+ 10 ³	82.00(65.07)	3.429	1.247(1.321)
V ₃ B ₁	10 ⁻⁴	+ 10 ⁷	80.67(64.00)	3.327	1.477(1.401)
V ₃ B ₂	10 ⁻⁴	+ 10 ⁶	84.00(66.88)	3.671	1.461(1.418)
V ₃ B ₃	10 ⁻⁴	+ 10 ⁵	81.33(64.53)	3.448	1.285(1.298)
V ₃ B ₄	10 ⁻⁴	+ 10 ⁴	88.00(70.01)	3.199	1.246(1.321)
V ₃ B ₅	10 ⁻⁴	+ 10 ³	90.67(72.37)	3.287	1.197(1.302)
C ₁	Buffer control		91.33(72.98)	4.771	1.771(1.480)
C ₂	Distilled water control		94.67(76.70)	4.873	1.730(1.494)
C ₃	Untreated control		93.33(75.20)	5.162	1.696(1.481)
S.E.m. ±			2.47	0.145	0.030
C.D. at 5%			7.853	0.412	0.085

* Figures in parentheses are angular transformed values

** Figures in parentheses are $\sqrt{x + 0.5}$ transformed values

Fig.6. Interaction effect of simultaneous infection with Kenchu virus and Staphylococcus aureus in fourth instar silkworm on larval weight on seventh day.

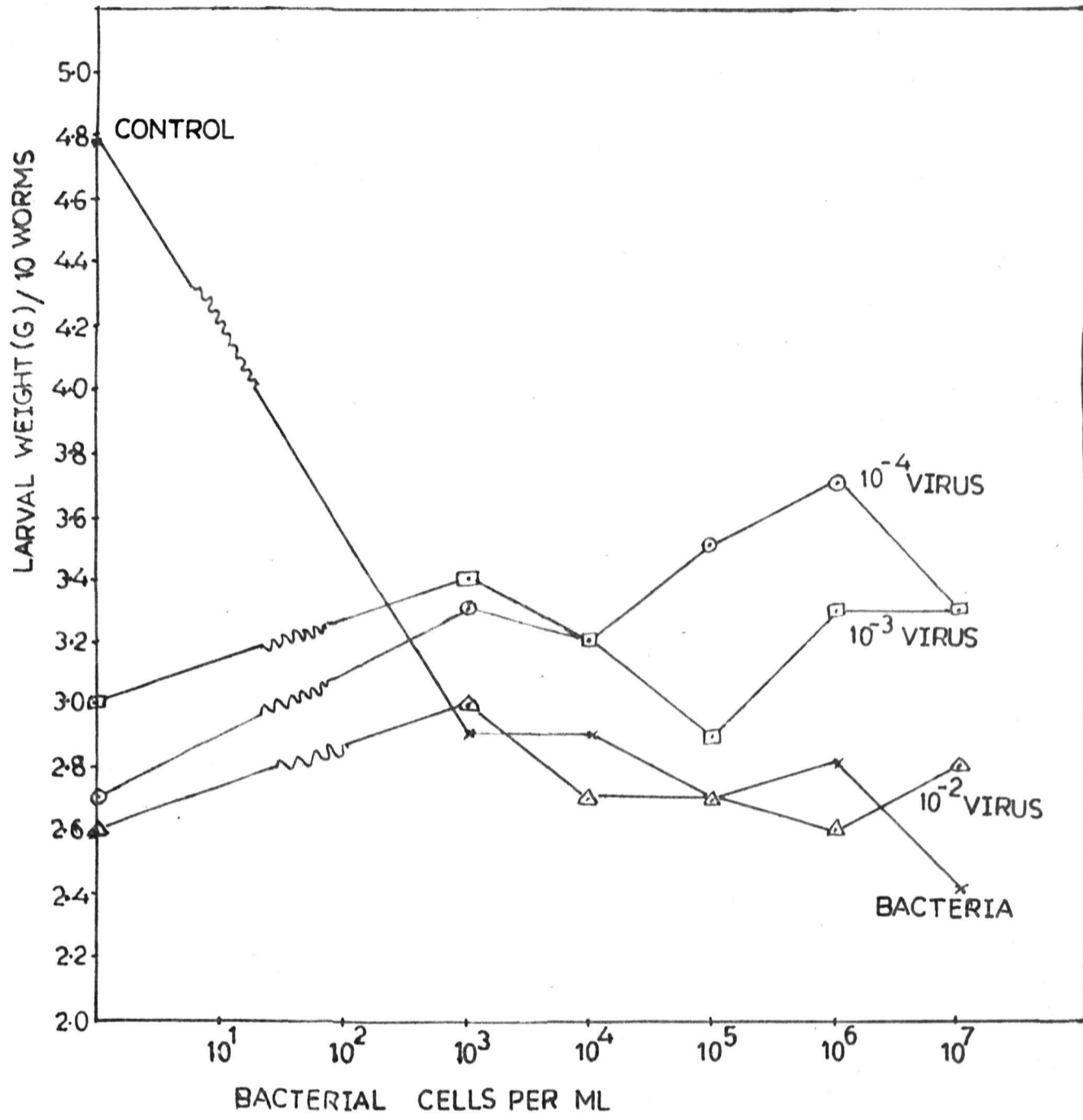


FIGURE-6

**Plate 12. Silkworms on 11th day of infection with
different concentrations of Staphylococcus
aureus in fourth instar.**

**Worms infected with: 10^7 , 10^6 , 10^5 , 10^4 and 10^3 cells/ml.
Control worms: Buffer, Distilled Water and Untreated**

**Plate 13: Silkworms on 11th day of simultaneous infection
with Kenchu virus and Staphylococcus aureus
in fourth instar.**

**Worms infected with: V_1B_1 , V_1B_2 , V_1B_3 , V_1B_4 , V_1B_5
 V_2B_1 , V_2B_2 , V_2B_3 , V_2B_4 , V_2B_5
 V_3B_1 , V_3B_2 , V_3B_3 , V_3B_4 , V_3B_5**

Control worms : Buffer, Distilled Water and Untreated

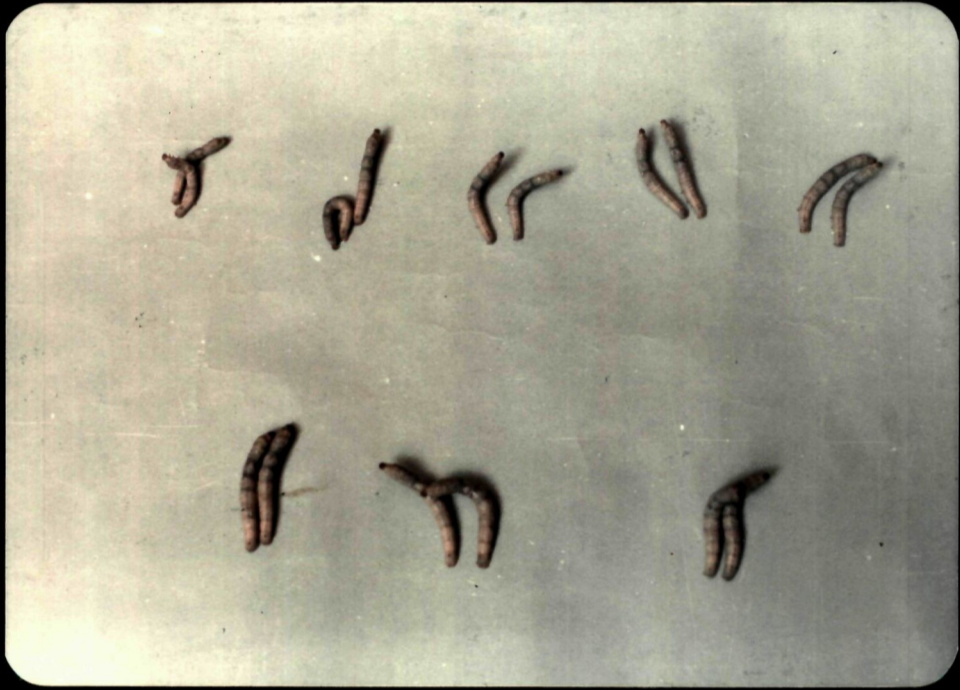


Table 43. Interaction effect of simultaneous infection with Kenchu virus and *Staphylococcus aureus* in fourth instar on fourth and fifth instars duration and fourth moulting duration

No.	Treatments		Fourth instar duration (Days)	Fourth moulting duration (Hours)	Fifth instar duration (Days)
	Kenchu virus	<i>S. aureus</i>			
V ₁	10 ⁻²	-	5.17	32.00	10.75
V ₂	10 ⁻³	-	4.83	32.00	10.08
V ₃	10 ⁻⁴	-	4.75	30.00	10.08
B ₁	-	10 ⁷ cells/ml	4.83	32.00	8.75
B ₂	-	10 ⁶ ..	4.50	30.00	8.50
B ₃	-	10 ⁵ ..	4.50	30.00	8.33
B ₄	-	10 ⁴ ..	4.67	28.00	8.42
B ₅	-	10 ³ ..	4.50	24.00	8.42
V ₁ B ₁	10 ⁻²	+ 10 ⁷ ..	5.17	36.00	9.83
V ₁ B ₂	10 ⁻²	+ 10 ⁶ ..	5.17	36.00	9.75
V ₁ B ₃	10 ⁻²	+ 10 ⁵ ..	5.08	34.00	9.50
V ₁ B ₄	10 ⁻²	+ 10 ⁴ ..	5.08	32.00	9.75
V ₁ B ₅	10 ⁻²	+ 10 ³ ..	5.00	30.00	9.08
V ₂ B ₁	10 ⁻³	+ 10 ⁷ ..	4.58	34.00	9.17
V ₂ B ₂	10 ⁻³	+ 10 ⁶ ..	4.67	34.00	9.08
V ₂ B ₃	10 ⁻³	+ 10 ⁵ ..	4.58	30.00	9.08
V ₂ B ₄	10 ⁻³	+ 10 ⁴ ..	4.58	34.00	9.08
V ₂ B ₅	10 ⁻³	+ 10 ³ ..	4.67	30.00	8.67
V ₃ B ₁	10 ⁻⁴	+ 10 ⁷ ..	4.58	30.00	8.67
V ₃ B ₂	10 ⁻⁴	+ 10 ⁶ ..	4.58	30.00	8.33
V ₃ B ₃	10 ⁻⁴	+ 10 ⁵ ..	4.42	30.00	8.33
V ₃ B ₄	10 ⁻⁴	+ 10 ⁴ ..	4.33	30.00	8.25
V ₃ B ₅	10 ⁻⁴	+ 10 ³ ..	4.25	28.00	8.00
C ₁	Buffer control		4.17	24.00	7.08
C ₂	Distilled water control		4.08	24.00	7.00
C ₃	Untreated control		4.17	24.00	7.00
S.E.M. ±			0.09	1.28	0.17
C.D. at 5%			0.256	3.642	0.483

4.5.2.3 Cocooning, cocoon and shell weights and moth emergence

Even in case of IV instar infection, cocooning was not encountered with 10^{-2} virus infection as well as in its combinations with all bacterial dilutions. The percentage of cocooning increased with increase in the dilution of virus and bacteria (6.00 to 24.00 per cent with 10^{-3} virus and bacteria and 28.00 to 40.67 per cent with 10^{-4} virus and bacteria) compared to control (84.00 per cent) (Table 44 and Figs.7 and 8). Cocoon weight, pupal weight, shell weight and moth emergence (Fig.9) behaved in a similar manner. However, cocoon shell ratio exhibited statistically significant difference, eventhough the magnitude of difference was less (Table 45).

4.5.3 Fifth instar

4.5.3.1 Initial fifth instar

The maximum larval weight was significantly different in the different treatments. It was considerably reduced with higher titre of virus and bacteria (12.691 to 14.389 g/10 worms) compared to control (16.030 g) (Table 46).

The per cent cocoons spun was considerably low with infected batches, particularly so in higher concentration of virus and bacteria (48.00) compared to that with lower concentration of virus and bacteria (84.66 per cent). In

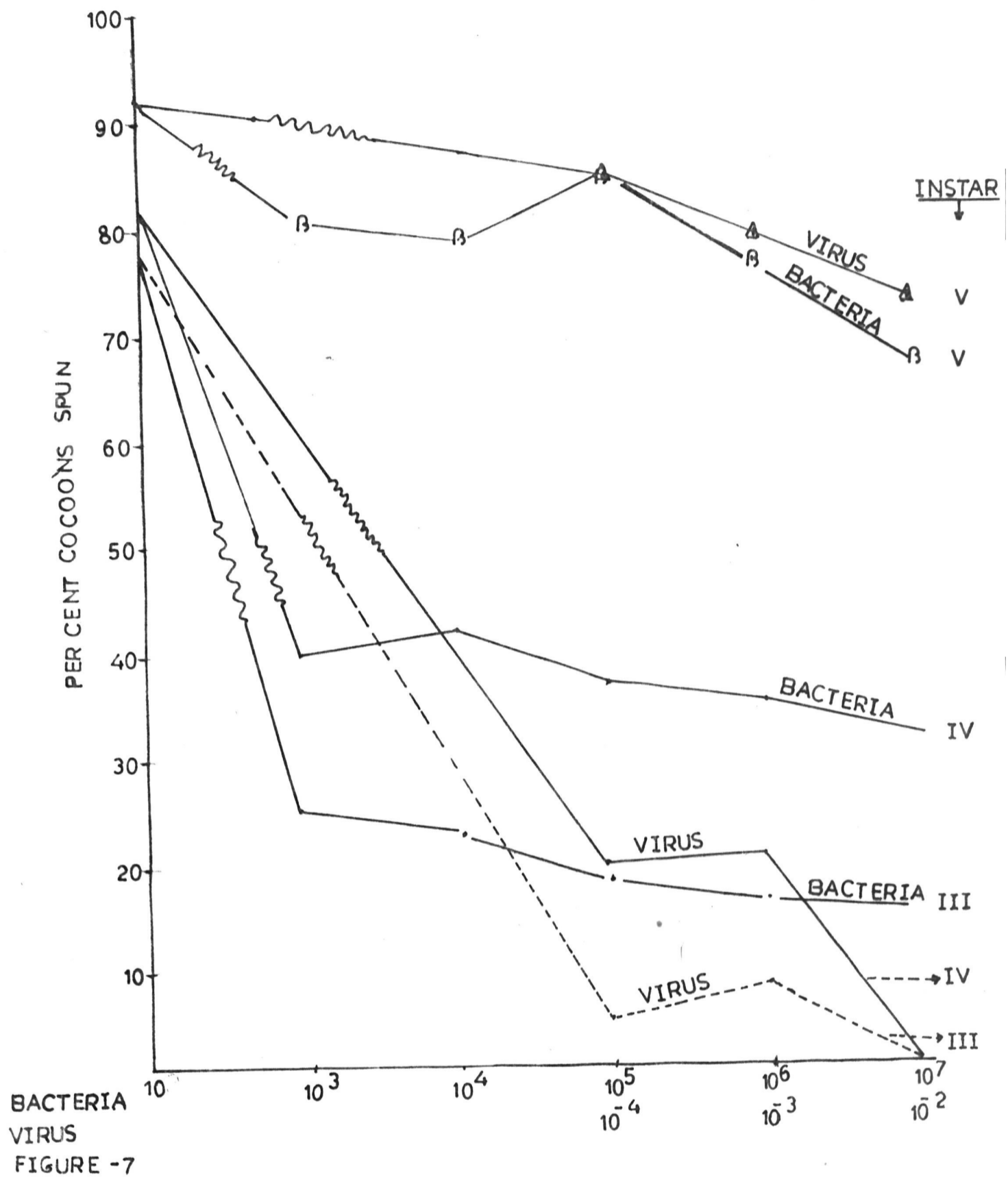
Table 44. Interaction effect of simultaneous infection with Kenchu virus and *Staphylococcus aureus* in fourth instar on extent of cocooning, cocoon weight and pupal weight

No.	Treatments		Per cent cocooning	Single cocoon weight (g)	Single pupal weight (g)
	Kenchu virus	<i>S. aureus</i>			
V ₁	10 ⁻²	-	0.00 (0.00)*	0.00(0.707)**	0.000(0.707)**
V ₂	10 ⁻³	-	20.00(26.49)	0.757(1.118)	0.653(1.071)
V ₃	10 ⁻⁴	-	19.33(25.95)	0.654(1.075)	0.560(1.067)
B ₁	-	10 ⁷ cells/ml	32.00(34.42)	0.663(1.077)	0.572(1.035)
B ₂	-	10 ⁶ ..	34.67(36.02)	0.758(1.121)	0.657(1.075)
B ₃	-	10 ⁵ ..	37.33(37.65)	0.634(1.065)	0.540(1.019)
B ₄	-	10 ⁴ ..	42.00(40.39)	0.611(1.054)	0.508(1.004)
B ₅	-	10 ³ ..	40.00(39.22)	0.678(1.084)	0.583(1.040)
V ₁ B ₁	10 ⁻²	+ 10 ⁷ ..	0.00(0.00)	0.000(0.707)	0.000(0.707)
V ₁ B ₂	10 ⁻²	+ 10 ⁶ ..	0.00(0.00)	0.000(0.707)	0.000(0.707)
V ₁ B ₃	10 ⁻²	+ 10 ⁵ ..	0.00(0.00)	0.000(0.707)	0.000(0.707)
V ₁ B ₄	10 ⁻²	+ 10 ⁴ ..	0.00(0.00)	0.000(0.707)	0.000(0.707)
V ₁ B ₅	10 ⁻²	+ 10 ³ ..	0.00(0.00)	0.000(0.707)	0.000(0.707)
V ₂ B ₁	10 ⁻³	+ 10 ⁷ ..	6.00(14.18)	0.753(1.119)	0.676(1.084)
V ₂ B ₂	10 ⁻³	+ 10 ⁶ ..	10.00(18.20)	0.644(1.100)	0.549(1.024)
V ₂ B ₃	10 ⁻³	+ 10 ⁵ ..	13.33(20.95)	0.581(1.039)	0.497(0.998)
V ₂ B ₄	10 ⁻³	+ 10 ⁴ ..	12.66(20.76)	0.774(1.128)	0.669(1.081)
V ₂ B ₅	10 ⁻³	+ 10 ³ ..	24.00(29.21)	0.738(1.111)	0.628(1.060)
V ₃ B ₁	10 ⁻⁴	+ 10 ⁷ ..	28.00(31.91)	0.684(1.085)	0.593(1.043)
V ₃ B ₂	10 ⁻⁴	+ 10 ⁶ ..	30.67(33.58)	0.828(1.152)	0.732(1.109)
V ₃ B ₃	10 ⁻⁴	+ 10 ⁵ ..	32.00(34.35)	0.810(1.141)	0.699(1.092)
V ₃ B ₄	10 ⁻⁴	+ 10 ⁴ ..	32.00(34.37)	0.713(1.115)	0.657(1.075)
V ₃ B ₅	10 ⁻⁴	+ 10 ³ ..	40.67(39.61)	0.878(1.173)	0.771(1.127)
C ₁	Buffer control		84.00(66.53)	0.885(1.176)	0.758(1.121)
C ₂	Distilled water control		80.00(63.46)	0.913(1.188)	0.783(1.132)
C ₃	Untreated control		80.67(64.00)	0.885(1.176)	0.759(1.122)
S.E.M. ±			1.65	0.024	0.024
C.D. at 5%			4.464	0.0682	0.0682

* Figures in parentheses are angular transformed values

** Figures in parentheses are $\sqrt{x + 0.5}$ transformed values

Fig.7. Individual effects of infection with different concentrations of Kenohu virus and Staphylococcus aureus in third, fourth and fifth instar silkworms on extent of cocooning.



BACTERIA
 VIRUS
 FIGURE -7

Fig.8. Interaction effect of simultaneous infection with Kenchi virus and Staphylococcus aureus in fourth and fifth instar silkworms on extent of cocooning.

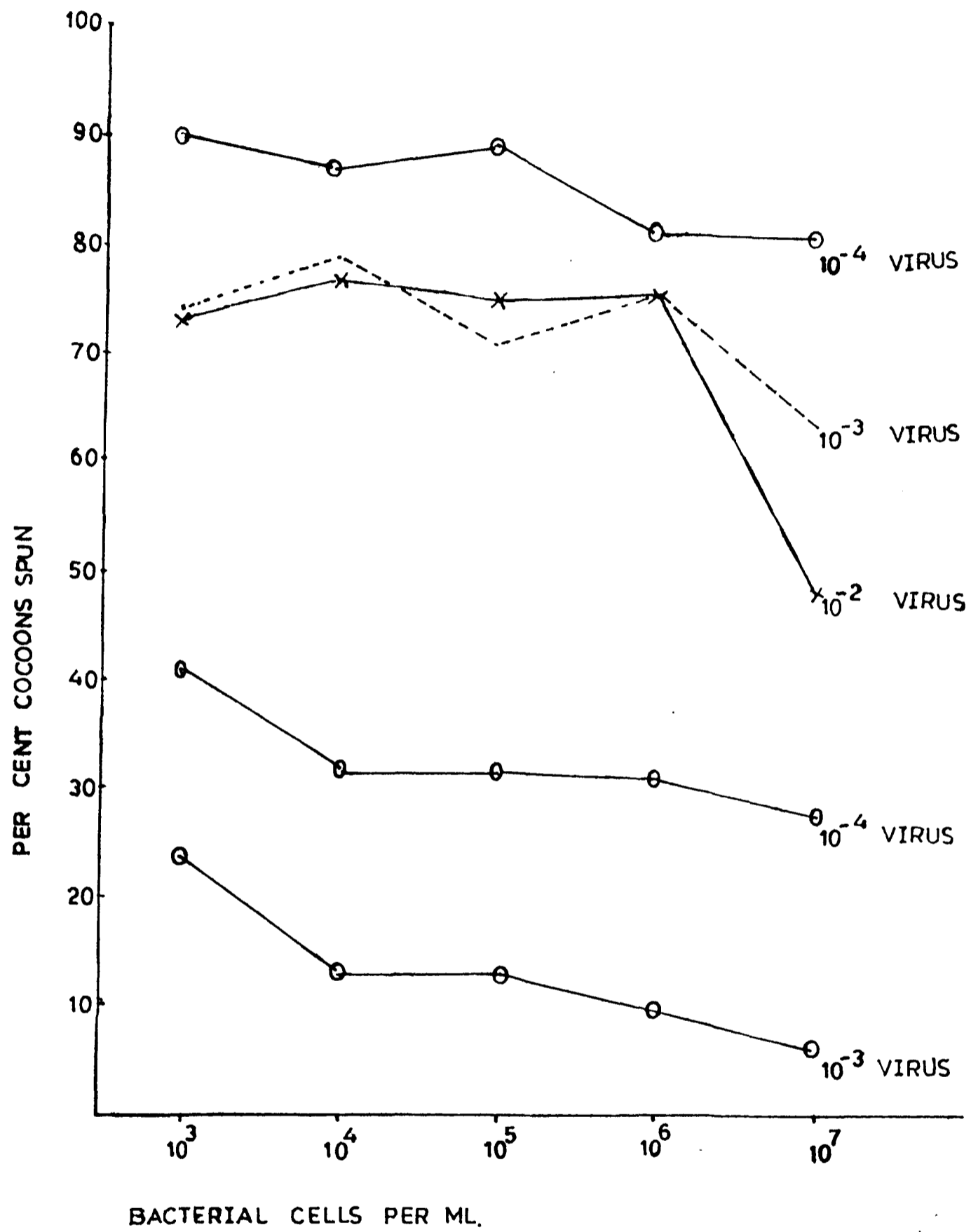


FIGURE -8

Table 45. Interaction effect of simultaneous infection with Kencha virus and *Staphylococcus aureus* in fourth instar on cocoon shell weight, cocoon shell ratio and moth emergence

No.	Treatments		Single cocoon shell weight (g)	Cocoon shell ratio (%)	Moth emergence (%)
	Kencha virus	<i>S. aureus</i>			
V ₁	10 ⁻²	-	0.000(0.707)*	0.00(0.00)**	0.00(0.00)**
V ₂	10 ⁻³	-	0.095(0.771)	12.78(20.95)	14.00(21.94)
V ₃	10 ⁻⁴	-	0.086(0.765)	13.35(21.41)	13.33(21.33)
B ₁	-	10 ⁷ cells/ml	0.080(0.762)	12.27(20.48)	23.33(28.88)
B ₂	-	10 ⁶ "	0.092(0.768)	12.12(20.28)	24.66(29.70)
B ₃	-	10 ⁵ "	0.085(0.765)	13.55(21.59)	31.33(34.02)
B ₄	-	10 ⁴ "	0.090(0.768)	14.71(22.52)	39.33(38.83)
B ₅	-	10 ³ "	0.087(0.766)	12.99(21.08)	37.33(37.66)
V ₁ B ₁	10 ⁻²	+	10 ⁷ "	0.000(0.707)	0.00(0.00)
V ₁ B ₂	10 ⁻²	+	10 ⁶ "	0.000(0.707)	0.00(0.00)
V ₁ B ₃	10 ⁻²	+	10 ⁵ "	0.000(0.707)	0.00(0.00)
V ₁ B ₄	10 ⁻²	+	10 ⁴ "	0.000(0.707)	0.00(0.00)
V ₁ B ₅	10 ⁻²	+	10 ³ "	0.000(0.707)	0.00(0.00)
V ₂ B ₁	10 ⁻³	+	10 ⁷ "	0.090(0.768)	12.11(20.33)
V ₂ B ₂	10 ⁻³	+	10 ⁶ "	0.083(0.764)	13.11(21.20)
V ₂ B ₃	10 ⁻³	+	10 ⁵ "	0.076(0.758)	13.33(21.37)
V ₂ B ₄	10 ⁻³	+	10 ⁴ "	0.095(0.771)	12.46(20.67)
V ₂ B ₅	10 ⁻³	+	10 ³ "	0.101(0.775)	14.15(22.01)
V ₃ B ₁	10 ⁻⁴	+	10 ⁷ "	0.081(0.759)	12.09(20.18)
V ₃ B ₂	10 ⁻⁴	+	10 ⁶ "	0.092(0.771)	11.10(19.42)
V ₃ B ₃	10 ⁻⁴	+	10 ⁵ "	0.100(0.774)	12.72(20.84)
V ₃ B ₄	10 ⁻⁴	+	10 ⁴ "	0.081(0.762)	10.95(19.30)
V ₃ B ₅	10 ⁻⁴	+	10 ³ "	0.096(0.792)	11.01(19.37)
C ₁	Buffer control		0.121(0.787)	13.67(21.69)	82.00(64.92)
C ₂	Distilled water control		0.123(0.789)	13.53(21.55)	78.00(62.05)
C ₃	Untreated control		0.133(0.796)	15.13(22.87)	78.00(62.13)
S.E.M. ±			0.005	0.76	1.36
C.D. at 5%			0.0142	2.162	3.869

* Figures in parentheses are $\sqrt{x + 0.5}$ transformed values

** Figures in parentheses are angular transformed values

Fig.9. Interaction effect of simultaneous infection with Kenchu virus and Staphylococcus aureus in fourth instar silkworm on extent of moth emergence.

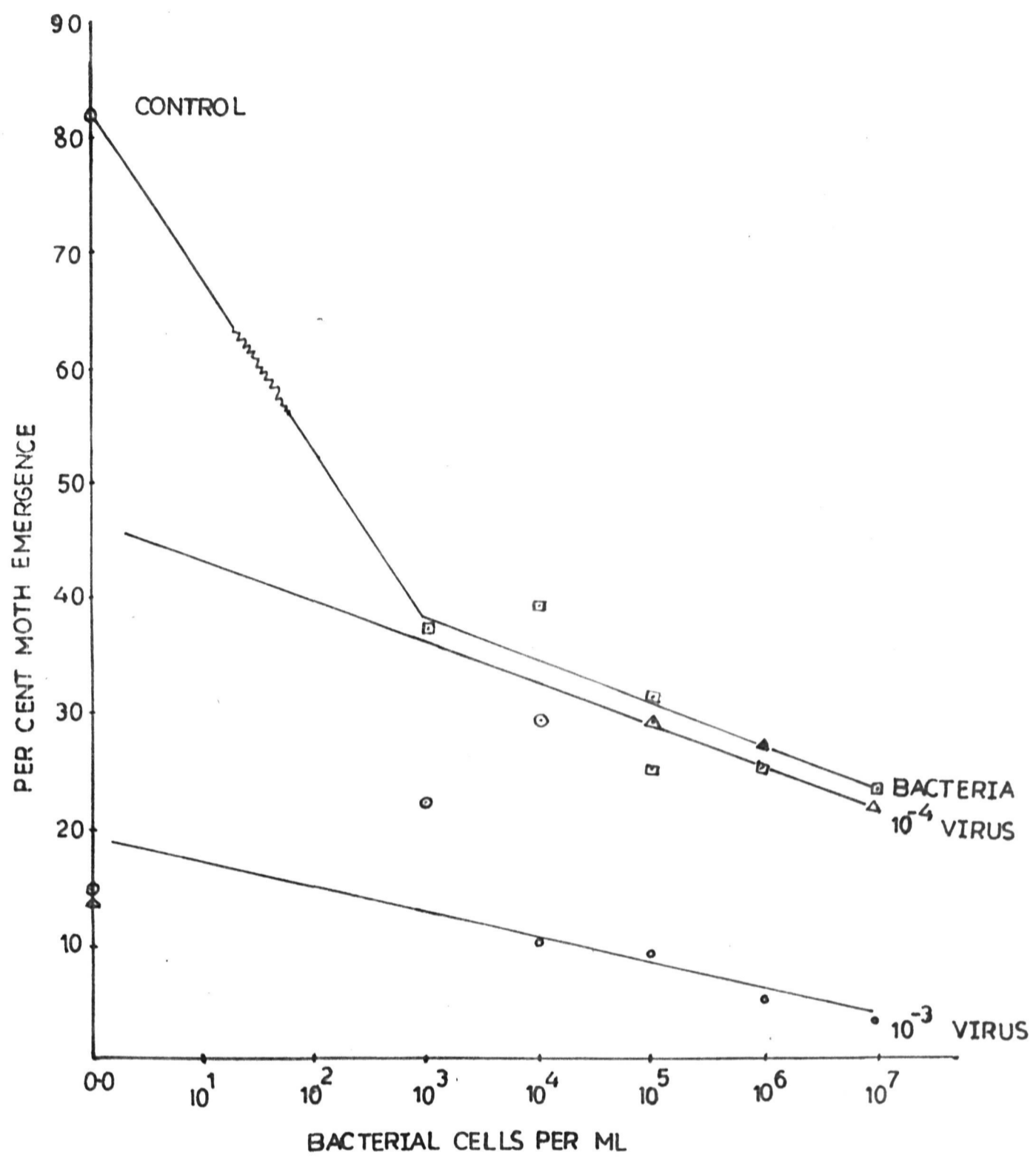


FIGURE-9

Table 46. Interaction effect of simultaneous infection with Kenchu virus and *Staphylococcus aureus* in initial fifth instar silkworm on maximum larval weight, extent of cocooning and cocoon weight

No.	Treatments		Maximum larval weight for 10 worms(g)	Per cent cocooning	Weight of 10 cocoons (g)	Weight of 10 pupae (g)
	Kenchu virus	<i>S. aureus</i>				
V ₁	10 ⁻²	-	12.703	73.33(58.92)*	7.413	6.153
V ₂	10 ⁻³	-	13.711	78.66(62.60)	7.527	6.530
V ₃	10 ⁻⁴	-	14.403	84.66(62.22)	8.890	7.653
B ₁	-	10 ⁷ cells/ml	14.797	66.66(55.44)	7.407	6.387
B ₂	-	10 ⁶ ..	15.077	76.66(61.29)	8.473	7.437
B ₃	-	10 ⁵ ..	13.389	85.33(67.77)	7.100	6.123
B ₄	-	10 ⁴ ..	15.241	78.66(63.20)	7.850	6.990
B ₅	-	10 ³ ..	14.577	80.66(64.35)	7.253	6.263
V ₁ B ₁	10 ⁻²	+ 10 ⁷ ..	12.691	48.00(43.85)	8.987	7.890
V ₁ B ₂	10 ⁻²	+ 10 ⁶ ..	14.333	76.00(60.72)	8.480	7.430
V ₁ B ₃	10 ⁻²	+ 10 ⁵ ..	13.343	70.66(57.37)	8.113	7.077
V ₁ B ₄	10 ⁻²	+ 10 ⁴ ..	12.945	78.66(62.83)	6.133	5.370
V ₁ B ₅	10 ⁻²	+ 10 ³ ..	14.389	74.33(59.01)	7.253	6.223
V ₂ B ₁	10 ⁻³	+ 10 ⁷ ..	14.828	64.00(53.24)	6.750	5.757
V ₂ B ₂	10 ⁻³	+ 10 ⁶ ..	14.060	75.33(60.37)	7.730	6.747
V ₂ B ₃	10 ⁻³	+ 10 ⁵ ..	15.782	75.33(60.30)	7.617	6.600
V ₂ B ₄	10 ⁻³	+ 10 ⁴ ..	14.357	76.66(61.17)	7.410	6.407
V ₂ B ₅	10 ⁻³	+ 10 ³ ..	14.140	73.33(59.35)	6.480	5.533
V ₃ B ₁	10 ⁻⁴	+ 10 ⁷ ..	13.873	81.33(64.84)	5.850	5.017
V ₃ B ₂	10 ⁻⁴	+ 10 ⁶ ..	14.777	81.33(64.45)	9.589	8.410
V ₃ B ₃	10 ⁻⁴	+ 10 ⁵ ..	14.572	89.33(71.05)	8.223	7.173
V ₃ B ₄	10 ⁻⁴	+ 10 ⁴ ..	14.291	86.66(68.61)	9.223	8.197
V ₃ B ₅	10 ⁻⁴	+ 10 ³ ..	14.973	84.66(66.97)	9.283	8.080
C ₁	Buffer control		16.030	92.66(74.67)	8.922	7.563
C ₂	Distilled water control		17.557	92.66(74.53)	9.048	7.827
C ₃	Untreated control		16.400	91.33(72.98)	9.105	7.833
S.E.M. ±			0.479	3.29	0.575	0.538
C.D. at 5%			1.362	9.361	1.636	1.530

* Figures in parentheses are angular transformed values

control it was 92.66 per cent (Table 46 and Figs. 7 and 8). Cocoon weight, pupal weight, shell weight and moth emergence was similarly affected (Tables 46 and 47). However, differences in the cocoon shell ratio remained non-significant, which may be due to the lesser weights of pupae as well as cocoons in the different treatments as compared to shell ratio with control where both shell as well as cocoon weights were higher, thereby compensating for any possible differences in shell ratio.

4.5.3.2 One day old fifth instar

The maximum larval weight was significantly less in infected worms (12.213 to 15.062 g/10 worms) compared to control (15.30 g). Even here the higher concentration of virus with bacterial dilutions gave less weight of 12.897 to 14.180 g/10 worms compared to that with more viral dilution with bacteria (12.985 to 15.062 g) (Table 48).

Extent of cocooning was very much affected in that it was 54.0 per cent with 10^{-2} virus and bacteria (10^7 cells/ml) as against 77.33 per cent with 10^{-3} and 10^{-4} virus and bacteria (10^7 cells/ml) compared to 92.00 per cent in case of control (Table 48). The cocoon weight, pupal weight, cocoon shell weight and moth emergence were also affected

Table 47. Interaction effect of simultaneous infection with Kenchu virus and Staphylococcus aureus in initial fifth instar silkworm on cocoon shell weight, cocoon shell ratio and moth emergence

No.	Treatments		Weight of 10 shells (g)	Cocoon shell ratio (%)	Per cent moth emergence
	Kenchu virus	<u>S. aureus</u>			
V ₁	10 ⁻²	-	0.877	11.96(19.93)*	58.66(50.01)*
V ₂	10 ⁻³	-	0.940	12.69(20.83)	64.66(53.54)
V ₃	10 ⁻⁴	-	0.987	11.12(19.43)	72.00(58.21)
B ₁	-	10 ⁷ cells/ml	1.000	13.53(21.61)	69.33(56.42)
B ₂	-	10 ⁶ ..	1.037	12.26(20.47)	73.33(59.06)
B ₃	-	10 ⁵ ..	0.913	12.93(21.06)	76.00(60.72)
B ₄	-	10 ⁴ ..	0.980	12.50(20.73)	73.33(59.23)
B ₅	-	10 ³ ..	0.950	13.07(21.13)	73.33(59.09)
V ₁ B ₁	10 ⁻² +	10 ⁷ ..	1.027	11.41(19.73)	44.00(41.54)
V ₁ B ₂	10 ⁻² +	10 ⁶ ..	1.033	11.80(20.06)	66.00(54.35)
V ₁ B ₃	10 ⁻² +	10 ⁵ ..	1.003	12.16(20.38)	63.33(52.79)
V ₁ B ₄	10 ⁻² +	10 ⁴ ..	0.743	12.25(20.45)	69.33(56.39)
V ₁ B ₅	10 ⁻² +	10 ³ ..	1.007	14.19(22.10)	69.33(56.45)
V ₂ B ₁	10 ⁻³ +	10 ⁷ ..	0.950	14.02(21.95)	60.66(51.24)
V ₂ B ₂	10 ⁻³ +	10 ⁶ ..	0.967	12.63(20.78)	58.00(49.66)
V ₂ B ₃	10 ⁻³ +	10 ⁵ ..	0.993	13.14(21.23)	65.33(53.98)
V ₂ B ₄	10 ⁻³ +	10 ⁴ ..	0.973	13.78(21.78)	68.66(56.02)
V ₂ B ₅	10 ⁻³ +	10 ³ ..	0.910	14.39(22.22)	74.00(59.39)
V ₃ B ₁	10 ⁻⁴ +	10 ⁷ ..	0.843	14.49(22.37)	66.66(54.75)
V ₃ B ₂	10 ⁻⁴ +	10 ⁶ ..	1.147	11.97(20.24)	67.33(55.46)
V ₃ B ₃	10 ⁻⁴ +	10 ⁵ ..	1.000	12.37(20.53)	77.33(61.88)
V ₃ B ₄	10 ⁻⁴ +	10 ⁴ ..	1.003	10.67(19.04)	81.33(64.61)
V ₃ B ₅	10 ⁻⁴ +	10 ³ ..	1.200	12.91(21.08)	86.00(68.06)
C ₁	Buffer control		1.293	14.54(22.43)	90.66(72.37)
C ₂	Distilled water control		1.300	14.35(22.27)	92.00(73.86)
C ₃	Untreated control		1.240	13.60(21.63)	89.33(71.19)
S.E.M. ±			0.059	0.740	2.470
C.D. at 5%			0.1678	2.105	7.021

NS

*Figures in parentheses are angular transformed values

NS = Non-significant

Table 48. Interaction effect of simultaneous infection with Kenchu virus and Staphylococcus aureus in one day old fifth instar silkworm on maximum larval weight, extent of cocooning, cocoon weight and pupal weight

No.	Treatments		Maximum larval weight for 10 worms(g)	Per cent cocooning	Weight of 10 cocoons	Weight of 10 pupae (g)
	Kenchu virus	<u>S. aureus</u>				
V ₁	10 ⁻²	-	12.708	75.33(60.23)*	6.667	5.743
V ₂	10 ⁻³	-	13.264	78.66(62.50)	8.110	7.017
V ₃	10 ⁻⁴	-	13.407	87.33(69.34)	9.060	7.970
B ₁	-	10 ⁷ cells/ml	12.213	84.00(68.60)	6.603	5.713
B ₂	-	10 ⁶	12.802	82.00(65.07)	7.657	6.757
B ₃	-	10 ⁵	13.763	86.66(69.05)	7.833	6.550
B ₄	-	10 ⁴	14.036	84.66(67.14)	6.597	5.343
B ₅	-	10 ³	14.172	85.66(67.34)	8.217	7.170
V ₁ B ₁	10 ⁻²	+ 10 ⁷	13.529	54.00(47.30)	8.160	7.260
V ₁ B ₂	10 ⁻²	+ 10 ⁶	13.589	76.00(60.72)	9.113	7.953
V ₁ B ₃	10 ⁻²	+ 10 ⁵	14.180	73.35(59.01)	7.607	6.653
V ₁ B ₄	10 ⁻²	+ 10 ⁴	12.897	80.66(63.96)	7.530	6.207
V ₁ B ₅	10 ⁻²	+ 10 ³	13.291	82.00(65.02)	6.123	5.257
V ₂ B ₁	10 ⁻³	+ 10 ⁷	13.713	77.35(61.64)	5.937	5.133
V ₂ B ₂	10 ⁻³	+ 10 ⁶	14.557	71.35(57.85)	6.167	5.323
V ₂ B ₃	10 ⁻³	+ 10 ⁵	14.343	78.00(62.16)	6.343	5.457
V ₂ B ₄	10 ⁻³	+ 10 ⁴	14.643	78.66(62.60)	7.320	6.330
V ₂ B ₅	10 ⁻³	+ 10 ³	13.622	76.00(62.72)	5.813	5.377
V ₃ B ₁	10 ⁻⁴	+ 10 ⁷	12.985	77.35(61.71)	6.633	5.693
V ₃ B ₂	10 ⁻⁴	+ 10 ⁶	14.753	78.00(62.59)	7.243	6.347
V ₃ B ₃	10 ⁻⁴	+ 10 ⁵	13.610	84.66(67.21)	8.190	7.227
V ₃ B ₄	10 ⁻⁴	+ 10 ⁴	15.062	85.35(67.63)	6.330	5.522
V ₃ B ₅	10 ⁻⁴	+ 10 ³	13.727	90.00(71.62)	6.673	5.723
C ₁	Buffer control		15.300	92.66(74.53)	9.165	7.830
C ₂	Distilled water Control		16.190	92.66(75.06)	8.748	7.555
C ₃	Untreated control		15.503	92.00(73.65)	8.805	7.560
S.Em. ±			0.441	2.37	0.485	0.457
C.D. at 5%			1.253	6.736	1.378	1.299

*Figures in parentheses are angular transformed values

significantly (Tables 48 and 49). But differences in cocoon shell ratio were not found to be significant.

4.5.3.3 Two days' old fifth instar

In case of infection with two days' old fifth instar silkworms, significant differences were noticed in maximum larval weight which were 11.456 to 14.179 g/10 worms in infected batches compared to 17.880 g in control (Table 50).

Differences in per cent cocooning and weight of cocoon and pupae were found to be non-significant between various treatments. Cocoon shell weight was significantly less in infected batches (0.687 to 1.273 g/10 cocoon shells) compared to control (1.187 g). However, cocoon shell ratio and moth emergence were not significantly affected by infection with Kenchu virus and/or bacteria (Table 51).

From the above data, it is clear that simultaneous infection of silkworm with Kenchu virus and bacterium or with either one of them individually was effective in causing reduction of many of the growth parameters studied in all the instars. Retardation in growth was also evidenced by premature mortality under heavy infection and significant prolongation in duration of instars and moults which was especially evident in infection in the early instars. The interaction effect of simultaneous infection was

Table 49. Interaction effect of simultaneous infection with Kenchu virus and *Staphylococcus aureus* in one day old fifth instar silkworm on cocoon shell weight, cocoon shell ratio and moth emergence

No.	Treatments		Weight of 10 cocoon shells(g)	Cocoon shell ratio (%)	Moth emergence (%)
	Kenchu virus	<i>S. aureus</i>			
V ₁	10 ⁻²	-	0.837	12.67(20.79)*	66.00(54.35)*
V ₂	10 ⁻³	-	1.020	12.60(20.75)	72.00(58.07)
V ₃	10 ⁻⁴	-	1.001	11.09(19.46)	82.00(64.94)
B ₁	-	10 ⁷ cells/ml	0.823	12.47(20.66)	80.33(63.77)
B ₂	-	10 ⁶ "	0.820	10.71(19.12)	76.33(61.07)
B ₃	-	10 ⁵ "	0.883	11.33(19.67)	80.33(63.84)
B ₄	-	10 ⁴ "	0.790	12.25(20.45)	78.00(62.10)
B ₅	-	10 ³ "	0.953	11.52(19.85)	81.00(64.39)
V ₁ B ₁	10 ⁻²	+ 10 ⁷ "	0.783	9.76(18.06)	49.66(44.89)
V ₁ B ₂	10 ⁻²	+ 10 ⁶ "	1.073	11.06(20.03)	68.33(55.84)
V ₁ B ₃	10 ⁻²	+ 10 ⁵ "	0.903	11.90(20.17)	67.33(55.20)
V ₁ B ₄	10 ⁻²	+ 10 ⁴ "	0.907	12.13(20.41)	74.33(59.61)
V ₁ B ₅	10 ⁻²	+ 10 ³ "	0.783	13.57(21.56)	74.66(59.88)
V ₂ B ₁	10 ⁻³	+ 10 ⁷ "	0.650	10.93(19.30)	69.00(55.86)
V ₂ B ₂	10 ⁻³	+ 10 ⁶ "	0.800	12.91(21.02)	66.33(54.28)
V ₂ B ₃	10 ⁻³	+ 10 ⁵ "	0.823	13.08(21.11)	71.66(58.02)
V ₂ B ₄	10 ⁻³	+ 10 ⁴ "	0.863	11.87(20.14)	73.00(58.75)
V ₂ B ₅	10 ⁻³	+ 10 ³ "	0.710	12.24(20.44)	67.66(55.36)
V ₃ B ₁	10 ⁻⁴	+ 10 ⁷ "	0.730	10.97(19.27)	67.00(54.97)
V ₃ B ₂	10 ⁻⁴	+ 10 ⁶ "	0.787	11.11(19.43)	68.00(55.66)
V ₃ B ₃	10 ⁻⁴	+ 10 ⁵ "	0.870	10.59(18.95)	79.66(63.29)
V ₃ B ₄	10 ⁻⁴	+ 10 ⁴ "	0.740	11.50(19.85)	80.33(63.72)
V ₃ B ₅	10 ⁻⁴	+ 10 ³ "	0.883	13.33(21.26)	85.33(67.53)
C ₁	Buffer control		1.290	14.05(22.03)	88.66(70.53)
C ₂	Distilled water control		1.152	13.13(21.24)	89.66(71.69)
C ₃	Untreated control		1.303	14.84(22.67)	88.33(70.17)
S.E.m. ±			0.065	0.82	2.10
C.D. at 5%			0.1847	2.330	5.969
				NS	

* Figures in parentheses are angular transformed values
NS = Non-significant

Table 50. Interaction effect of simultaneous infection with Kenchu virus and *Staphylococcus aureus* in two days old fifth instar silkworm on maximum larval weight, extent of cocooning, cocoon weight and pupal weight

No.	Treatments		Maximum larval weight for 10 worms (g)	Per cent cocooning	Weight of 10 cocoons (g)	Weight of 10 pupae (g)
	Kenchu virus	<i>S. aureus</i>				
V ₁	10 ⁻²	-	11.456	84.66(66.99)*	9.340	7.507
V ₂	10 ⁻³	-	13.850	84.00(66.45)	7.273	6.093
V ₃	10 ⁻⁴	-	14.139	84.66(67.98)	8.200	6.957
B ₁	-	10 ⁷ cells/ml	13.135	89.33(71.51)	7.883	6.770
B ₂	-	10 ⁶ ..	13.296	84.66(68.06)	6.910	5.847
B ₃	-	10 ⁵ ..	13.203	82.66(67.11)	7.137	6.067
B ₄	-	10 ⁴ ..	13.104	90.66(72.68)	5.890	4.963
B ₅	-	10 ³ ..	14.071	93.33(77.77)	8.263	7.147
V ₁ B ₁	10 ⁻²	+ 10 ⁷ ..	12.811	94.66(77.09)	7.300	6.513
V ₁ B ₂	10 ⁻²	+ 10 ⁶ ..	12.981	95.33(77.97)	6.747	5.807
V ₁ B ₃	10 ⁻²	+ 10 ⁵ ..	13.635	96.66(81.43)	6.950	6.013
V ₁ B ₄	10 ⁻²	+ 10 ⁴ ..	12.512	92.66(75.06)	6.440	5.530
V ₁ B ₅	10 ⁻²	+ 10 ³ ..	13.779	83.33(66.70)	7.820	6.843
V ₂ B ₁	10 ⁻³	+ 10 ⁷ ..	13.021	91.33(73.35)	7.510	6.383
V ₂ B ₂	10 ⁻³	+ 10 ⁶ ..	12.609	88.66(70.75)	6.703	5.640
V ₂ B ₃	10 ⁻³	+ 10 ⁵ ..	13.901	84.00(69.21)	9.683	8.500
V ₂ B ₄	10 ⁻³	+ 10 ⁴ ..	14.179	90.66(72.29)	6.070	5.127
V ₂ B ₅	10 ⁻³	+ 10 ³ ..	12.686	90.00(72.31)	7.367	6.280
V ₃ B ₁	10 ⁻⁴	+ 10 ⁷ ..	13.169	84.00(67.52)	8.087	7.010
V ₃ B ₂	10 ⁻⁴	+ 10 ⁶ ..	12.793	81.33(64.45)	8.620	7.173
V ₃ B ₃	10 ⁻⁴	+ 10 ⁵ ..	13.425	87.33(69.34)	6.693	5.577
V ₃ B ₄	10 ⁻⁴	+ 10 ⁴ ..	13.503	82.66(65.71)	7.620	6.493
V ₃ B ₅	10 ⁻⁴	+ 10 ³ ..	13.093	92.00(74.10)	7.503	6.450
C ₁	Buffer control		17.880	92.66(74.53)	8.588	7.365
C ₂	Distilled water control		16.593	92.00(74.39)	8.815	7.522
C ₃	Untreated control		17.060	94.66(77.30)	8.692	8.115
	S.E.M. ±		0.458	3.71	0.737	0.688
	C.D. at 5%		1.301	10.545 NS	2.094 NS	1.955 NS

*Figures in parentheses are angular transformed values

NS = Non-significant

Table 51. Interaction effect of simultaneous infection with Kenchu virus and *Staphylococcus aureus* in two days old fifth instar silkworm on cocoon shell weight, cocoon shell ratio and moth emergence

No.	Treatments		Weight of 10 cocoon shells(g)	Cocoon shell ratio (%)	Moth emergence (%)
	Kenchu virus	<i>S. aureus</i>			
V ₁	10 ⁻²	-	1.273	13.97(21.92)*	78.00(62.05)*
V ₂	10 ⁻³	-	1.083	14.82(22.68)	81.33(64.41)
V ₃	10 ⁻⁴	-	1.177	14.54(22.41)	81.33(64.75)
B ₁	-	10 ⁷ cells/ml	1.043	11.94(20.21)	86.66(68.80)
B ₂	-	10 ⁶ "	1.013	14.65(22.49)	83.33(65.91)
B ₃	-	10 ⁵ "	1.013	14.38(22.26)	79.33(63.87)
B ₄	-	10 ⁴ "	0.873	14.82(22.65)	89.33(71.27)
B ₅	-	10 ³ "	1.073	13.05(19.87)	92.00(74.39)
V ₁ B ₁	10 ⁻²	+ 10 ⁷ "	0.687	9.01(17.37)	93.33(75.28)
V ₁ B ₂	10 ⁻²	+ 10 ⁶ "	0.873	13.13(21.21)	92.66(74.40)
V ₁ B ₃	10 ⁻²	+ 10 ⁵ "	0.890	13.21(21.27)	94.66(77.30)
V ₁ B ₄	10 ⁻²	+ 10 ⁴ "	0.833	13.12(21.21)	92.00(73.92)
V ₁ B ₅	10 ⁻²	+ 10 ³ "	0.920	11.95(20.22)	82.00(65.46)
V ₂ B ₁	10 ⁻³	+ 10 ⁷ "	1.057	14.29(22.19)	90.00(71.80)
V ₂ B ₂	10 ⁻³	+ 10 ⁶ "	0.877	14.52(22.38)	86.66(68.83)
V ₂ B ₃	10 ⁻³	+ 10 ⁵ "	1.130	11.65(19.97)	82.66(65.68)
V ₂ B ₄	10 ⁻³	+ 10 ⁴ "	0.867	14.24(22.19)	88.66(70.52)
V ₂ B ₅	10 ⁻³	+ 10 ³ "	1.027	14.43(22.33)	89.33(71.64)
V ₃ B ₁	10 ⁻⁴	+ 10 ⁷ "	1.010	12.69(20.81)	82.66(66.17)
V ₃ B ₂	10 ⁻⁴	+ 10 ⁶ "	1.133	13.16(21.28)	80.00(63.46)
V ₃ B ₃	10 ⁻⁴	+ 10 ⁵ "	0.897	13.31(21.38)	85.33(67.68)
V ₃ B ₄	10 ⁻⁴	+ 10 ⁴ "	1.033	13.70(21.73)	81.33(64.53)
V ₃ B ₅	10 ⁻⁴	+ 10 ³ "	0.967	12.98(21.08)	89.33(71.05)
C ₁	Buffer control		1.187	13.86(21.84)	90.66(73.25)
C ₂	Distilled water control		1.202	13.59(21.66)	90.66(73.25)
C ₃	Untreated control		1.270	14.64(22.51)	93.33(75.55)
S.E.m. ±			0.088	0.86	2.93
C.D. at 5%			0.250	2.444	8.328
				NS	NS

* Figures in parentheses are angular transformed values
NS = Non-significant

more pronounced in third instar than in fourth instar and it was less pronounced in fifth instar. With advancement in age of fifth instar, the effect was less evident.

DISCUSSION

V. DISCUSSION

The results of the different experiments are discussed as follows under appropriate headings.

5.1 Testing of Kenchu virus isolates against antisera of infectious flacherie virus and densonucleosis virus of silkworm

Diseased silkworms showing Kenchu symptoms collected in Karnataka did not produce the precipitation bands in gel diffusion test with antisera of both infectious flacherie virus and densonucleosis virus (Ina strain) of silkworm. Also midgut cells of silkworms infected in the laboratory with Kenchu virus extracts did not produce specific fluorescence with antisera of both IFV and DNV, when examined by fluorescent antibody technique, thus indicating that the Kenchu virus preparations used in the present laboratory investigations are serologically quite distinct from silkworm IFV and Ina DNV existing in Japan.

Regarding the extracts of samples collected from different places, tests have been conducted only for serological evaluation for IFV and Ina DNV. Since Kenchu symptoms are known to be produced in infections other than viruses, especially bacteria such as Serratia marcescens (Vasantharajan and Munirathnamma, 1978), the viral etiology of these field collected samples is not established. The

negative serological response can only eliminate the presence of IFV or Ina DNV in the disease samples. The question of the actual causal agent for these different Kenchu epizootics encountered in the field is still open for investigation.

It is interesting to note in this connection that several small, non-occluded viruses of insects have not shared common antigens between them (Jukes *et al.*, 1973; David, 1975; Reinganum and Scotti, 1976). With the silkworm itself several strains of densovirus viruses which are not antigenically related have been reported from Japan (Seki, 1984; Watanabe *et al.*, 1986). Hence, it would not be surprising that investigations will reveal many other such non-occluded, small viruses of insects in general and the silkworm in particular.

5.2 Effect of refrigeration of silkworm eggs on the development and intensity of Kenchu disease

The results of the present investigations on egg refrigeration in the multivoltine breed, Pure Mysore have revealed a deleterious effect of consigning eggs on hatching percentages, larval growth, instar progression and mortality. The adverse effects of refrigeration were in evidence for either extended periods of refrigeration or later stages of embryonic growth or both. The inhibitory

influence of long duration refrigeration may also be partially due to a possible dehydration effect. In the present work, no specific effort was made to maintain the relative humidity at a high level within the refrigerator as such a practice is not common in commercial egg refrigeration. The detrimental influence of long duration refrigeration or refrigeration of later stage eggs on egg hatching was reported by earlier workers also (Narasimhamurthy, 1943a; 1943b; Devaiah and Thontadarya, 1975). However, adequate information is not available on the post-embryonic development of silkworms from refrigerated eggs. Even less is known about the response of such silkworms to artificial infection from known causative agents.

However, work has been done on the effect of low temperature on the susceptibility of silkworms to cytoplasmic as well as nuclear polyhedrosis in silkworm. Aruga *et al.* (1963) reported that cold treatment of silkworms at 5°C for 3 to 5 hours before or after per oral infection with cytoplasmic polyhedrosis virus enhanced the susceptibility of worms to the virus. Matsubara *et al.* (1984) investigated the effect of refrigeration of first instar silkworm larvae at 5°C for 24 hr on development of nuclear polyhedrosis. A 20 to 90 per cent mortality was recorded. Refrigeration of II, III and IV instars followed by infection resulted in

10 to 60, 70 to 90 and 100 per cent mortality when compared to 0 per cent in the respective controls. Sidhu and Singh (1968) observed 21.25 and 18.75 per cent of grasserie and flacherie, respectively, in silkworms refrigerated for 24 hours at 5°C and the corresponding figures for unrefrigerated larvae were 8.75 and 10 per cent respectively. The ability of silkworm larvae to resist infection of NPV was markedly reduced when they were subjected to low temperature of 5°C for 24 hours (Wu, 1983).

The results of the current investigations are not strictly comparable with the observations of the above workers since the present work involved cold treatment during embryonic growth. In the current work, no substantial accentuating effect of refrigeration of eggs on Kenchu virus infection could be noticed. Both larvae from refrigerated eggs or unrefrigerated control eggs were equally and severely affected by Kenchu virus infection and an additive influence was not apparent (Figs. 1 and 2). However, the present work does not throw light on the susceptibility of larvae resulting from cold treatment of eggs and exposed to different diseases over prolonged periods as would normally be prevalent in field rearings. This work is necessary before generalising on the lack of effect of refrigeration on incidence of Kenchu under field conditions.

5.3 Effect of releasing Kenchu virus infected silkworms into healthy populations in different proportions

The release of Kenchu virus diseased silkworms into healthy populations in different proportions resulted in a significant reduction in larval growth, moulting and instar duration, cocoon weight and percentage cocooning. This effect was seen when diseased individuals were released into healthy batches either in the II, III or IV instars. The adverse effects were more pronounced in the II instar treatment as compared to the III and IV instar treatments. The extent of damage caused to larval growth or percentage survival was dependent on the density of Kenchu virus infected individuals in the population, being higher at the higher levels of release. For example, releases of 4, 8, 12, 15, 20 and 24 per cent in the IV instar caused a reduction in percentage of worms spinning cocoons whose values were 82.67, 80.00, 76.00, 68.00, 61.33 and 51.33 per cent, respectively when compared to 97.00 per cent cocooning in control larvae.

The present work is an extension of the work conducted by Eswarappa (1983). Only first instar infection was used by him. The results had indicated 100 per cent mortality in all releases ranging from 4 to 40 per cent. However, the time required for 100 per cent mortality was reported

to be 35, 30, 25 and 20 days for 4, 10, 20 and 40 per cent releases, respectively.

In case of releases made during II, III and IV instars in the present investigations, the general tendency was that the per cent cocoons spun reduced with increase in the density of infected individuals released initially into healthy populations. The faecal matter of the infected individuals contaminated the beds as well as leaf material thereby the inoculum was more in batches released with more of infected individuals, creating more chance for healthy individuals to pick up the virus per os which resulted in high death rate of worms. These observations are similar to those of Bird (1953), Lower (1954), Jacques (1962), Stairs (1968) and Putman (1970) in case of virus diseases of some insects and mites.

The current work has revealed a better survival and performance with worms infected in the subsequent instars. This is in agreement with the observations of Hadimani (1980) who reported lesser mortality and higher weight gains in worms infected with Kenchu virus in later instars as compared to early instar-infected silkworms.

These investigations give ample support to the normal standard practice of giving extra care to young silkworm

rearing since Kenchu infection at early instars is bound to lead to a total loss of the crops whereas the loss is minimized if the infection occurs after the IV instar.

5.4 Influence of nutrition on intensity of Kenchu virus infection

The factors relating to nutrition which have been taken for the present research have been broadly placed under three headings—underfeeding, post-moult starvation and varying leaf quality by feeding leaves of different maturity (tender, medium and coarse leaves). These factors were chosen for investigations since these are the factors which are subject to much variation in commercial silkworm rearing.

In the underfeeding experiments, a pre-infection underfeeding regime was followed. Subsequent to infection, the standard schedule of feeding was adopted. The results indicated a slight reduction in larval and cocoon weights but a considerable, significant reduction in percentage of worms surviving to spin cocoons. Meth emergence also was drastically reduced. Weight of cocoon and cocoon shell also reduced with underfeeding. Muthukrishnan et al. (1978) also observed a reduction in the cocoon shell weight with worms allowed four hours feeding per day compared to that

of worms fed 24 hr per day in B. mori. Similarly, Srivastava et al. (1982-83) obtained a single cocoon weight of 2.001 and 3.031 g with 18 and 24 hr of feeding duration, respectively in eri silkworm, Samia cynthia ricini which result is comparable with the trend in the current results. In general, the data recorded for both IV and V instars indicated similar trends in results, especially with moth emergence. There was a significant reduction in moth emergence in both batches of infected larvae (IV and V instars).

Superimposition of underfeeding and Kenchu virus infection caused further reduction in moth emergence. This observation is supported by the findings of Szalay-Harzo (1957) according to whom there was severe defoliation by Lymantria dispar over a considerable area and the larvae were deprived of sufficient food thus resulting in heavy infection by a virus disease in that only 10 per cent of them only pupated, thus resulting in less moth emergence.

Post-moult starvation ranged from 12 to 48 hours. This was followed by a normal feeding schedule. Infections were effected immediately after the starvation period and starvation was not inflicted in the subsequent instars. The data recorded resembled those obtained with underfeeding

in that the influence of post-moult starvation on larval, pupal and cocoon weights was a moderate reduction whereas the influence was adverse on survival as revealed by percentages of spinning worms and emerging moths.

Superimposition of infection over post-moult starvation caused slight reductions in larval and cocoon weights but a marked decrease in percentage of spinning larvae and moth emergence. The effect of starvation or underfeeding in aggravating infections in insects is well established. Devaiah (1973) found the infection of Nosema bombycis to become severe in IV and V instar silkworms if starved for longer durations, and 48 hr starvation drastically reduced larval resistance. Repeated starvation and starvation for longer durations in all instars of silkworm rendered them susceptible to flacherie and grasserie (Samson et al., 1980). Wu (1983) observed the resistance to NPV to reduce very markedly in silkworms when they were starved for 24 hr at 25°C. In the present experiments, the effect of infection following starvation on survival was additive in respect of III and IV instar infections. The results were less drastic in respect of V instar infection.

Differences in leaf quality as determined by their maturity were not observed to influence appreciably the

larval and cocoon weights but significant effect was observed in the percentage of cocoon spinning and moth emergence. The latter two were significantly more with coarse leaf feeding. Infection with Kenchu virus followed a normal course causing reduction in body weight, prolongation of instars and moults and reduction in survival. These effects were not substantially affected by the quality of leaf. Slightly higher larval and cocoon weights in certain cases in respect of tender leaf and water treated leaves is, to some extent, in agreement with the findings of Krishnaswami et al. (1971) who obtained more weight of full grown worms (19.1 g/10 worms) on tender and middle leaves and 18.60 g with mature leaf in breed D₃C. Similarly, Sudo et al. (1979; 1981) observed a significant correlation between larval body weight and leaf order in that body weight was more with top leaves. Viability is one of the important considerations particularly in parental breeds of hybrids to be used for commercial rearing. In the present studies, the moth emergence was more on mature leaf and this is strongly supported by the observations of Marayanaprakash et al. (1985) who concluded that bivoltine larvae thrived well on tender leaves and the cross breed (L x KA) on mature leaves producing heavier cocoons.

The effect of either post-moult starvation or underfeeding is not appreciably affecting the body weights

is partially due to the subsequent normal feeding followed in the two investigations. The compensatory effect of normal feeding is, however, not extended to the percentage survival as reflected in the cocoon spinning and moth emergence. This might mean a lack of sufficient body reserves in these partially starved worms. Early instar starvation although followed by full feeding in later instars is unable to compensate the effects of malnutrition imposed in the earliest instars. The reasons for this may not be far to seek. The main reserve tissue in the insect is the fat body. In fact, in the honey bee the fat body was observed to constitute 65 per cent of the total body weight of full grown larva (Bishop, 1922; 1923). It is well known that fat body cells are limited in number immediately after hatching but their numbers increase during subsequent moults. The fat body cells begin to secrete reserve materials like glycogen and fat after feeding is resumed. Starvation prevents the enlargement and vacuolation of the cells (Wigglesworth, 1972). Since fat reserves are necessary for moulting also, starvation in early instars tends to have an effect on subsequent moulting and all processes accompanying it. In view of the fact that metamorphosis is entirely dependent on stored food, the limitation in the number of fat body cells and hence the

amount of reserve food, may have the effect of drastically reducing successful metamorphosis.

5.5 Interaction between Kenchu virus and *S. aureus*

Infection with either Kenchu virus or *Staphylococcus aureus* was effective in lowering many of the parameters used in the present investigation. Moderate body weight reduction was encountered with *S. aureus* infection whereas Kenchu virus infection led to a marked reduction in body weight. The same tendency was also seen in regard to cocoon parameters like pupal, shell weight and shell ratio. The parameter which was affected most was survival. With highest viral dose, percentage survival was zero when III instar silkworms were infected. Infection with *S. aureus* resulted in a lesser mortality which was dependent on the concentration. Retardation in growth was also reflected in the prolongation of moults and instars.

Simultaneous infection with Kenchu virus and *S. aureus* revealed the presence of interaction as judged by the effects on larval weight, percentages of cocooning and moth emergence. The mutual effect was one of the reinforcement in that the effect of the combination was more than that of either infective agent administered singly. This effect was more marked in III instar infected silkworms as

compared to infection in IV instar. In the V instar, hardly any additive effect could be detected although virus alone caused a slight lowering in the parameters. To quote one specific instance, combination of S. aureus with the three levels of virus resulted in no survival upto cocooning stage in the case of III instar infection. However, during IV instar infection one hundred per cent mortality was seen only with the combination of highest dose of virus with all concentrations of bacteria. It was also noticed that additive effect was generally present at all concentrations of virus and bacteria in combination during III instar infection. In IV instar as well as V instar infection, the additive effect was generally noticed with the combination of higher concentration of virus and high concentrations of S. aureus. In fact infection on the second and third days of V instar, especially the latter the individual effects of either virus alone or bacteria alone or the combination effects were minimal.

The decrease in the adverse effects of Kenchu virus infection in the silkworm with advancement in age was also reported by earlier workers on this disease (Nadimani, 1980 and Narayanaswamy, 1983). Reports on the decrease of susceptibility of insects to certain viral infections with increasing age are not rare (Vago and Cayrol, 1956; Hafez, 1958; Ignoffo, 1966; Atger, 1969; Neelgund and Mathad,

1974; Abul-Nasir et al., 1979).

Mutualism and interaction effects of different pathogens in the silkworm have been a subject of study (Kurisu, 1974; Vasantharajan and Munirathamma, 1978; Matsumoto et al., 1985; 1986). Kurisu (1974) observed highest mortality of silkworms in simultaneous mixed infection with infectious flacherie virus and bacteria as observed in the present studies. The current trend had been observed by Vasantharajan and Munirathamma (1978) even in case of bacterial interaction in that the associative effect between Staphylococcus sp. and Serratia marcescens was evident. Per oral infection of silkworms with both the species caused 48 per cent mortality while individually they did not cause any damage. The observations of Matsumoto et al. (1985) in that the infection of fourth instar silkworm with IFV (10^{-3}), Streptococcus faecalis (10^6) and Serratia marcescens (10^6) resulting in 20, 0 and 0 per cent mortality respectively while IFV + S. faecalis and IFV + S. marcescens causing 100 per cent mortality tend to support the current findings. The results of the present study showed the mortality rate to come down as evidenced by more cocooning in interaction treatments involving more diluted Kenchu virus and less bacterial (S. aureus) cells. This is in conformity with the findings

of Matsumoto et al. (1986) who observed larval mortality of 100, 100, 30 and 10 per cent respectively when fourth instar silkworms were fed with a mixture of IFV at 10^{-2} , 10^{-3} , 10^{-4} and 10^{-5} along with the bacterium S. faecalis (10^6).

The present investigations have revealed the importance of combination of pathogens in causing greater damage to the silkworm as compared to the individual pathogens. In commercial rearings, one is apt to encounter more than one pathogen in the rearing environment and hence an accentuation of loss due to disease.

Severe loss due to Kenchu disease is a common feature in silkworm rearings in Karnataka. The investigations by Vasantharajan and Munirathnamma (1978) revealed the presence of atleast three types of bacteria viz., red pigmented Serratia marcescens, non-pigmented Serratia and Staphylococcus sp. in Kenchu diseases encountered in the field and they observed the associative effect between Staphylococcus and Serratia marcescens. They also suggested a probable associative effect of Kenchu virus and bacteria. The present report definitely establishes the presence of such an interaction.

The present investigations have been concerned with simultaneous infection with Kenchu virus and S. aureus. The interaction effects, if any, of the bacterial infection preceding or succeeding the viral infection or vice versa are not known and need to be worked out. This is considered important in view of the likelihood of the occurrence of two types of infection at different points of time in large scale rearings of silkworms.

Further work on the mechanism of the interaction effect of high levels of bacteria with high levels of virus observed in the present case is necessary. The effect of a primary viral infection with IFV rendering the silkworm susceptible to bacterial infection has been discussed by Kurisu (1974). The interaction effect of Staphylococcus faecalis by facilitating the entry of Serratia piscatorum into the silkworm haemocoel was reported by Kodama and Nakasuji (1971). A similar effect of a Staphylococcus species on the mortality caused by Serratia marcescens VK1 was reported by Vasantharajan and Munirathamma (1978). The fact that interaction effect of Kenchu virus and S. aureus on growth and mortality are felt at higher concentrations of bacteria and virus, double infection only points to a greater tissue damage caused. Since the silkworm is known

to possess mechanisms for neutralising viruses as well as bacteria, it is interesting to speculate that an undue strain on these mechanisms may result in an interaction influence. The specific antiviral activity of red fluorescent protein was reported by Hayashiya *et al.* (1969; 1978). Lisuka (1985) also identified several antibacterial substances in the digestive juice of silkworm, B. mori. The lytic properties of haemolymph to some bacteria as also the non-specific antimicrobial activity of haemolymph obtained from silkworm larvae vaccinated with low doses of live or formalinised cells of Candida utilis was reported by D'Cruz (1982). He reported the bactericidal as well as bacteriostatic influence of immune haemolymph against S. aureus. Further work on these aspects may assist in elucidating the mechanism.

The study made currently has clearly revealed that the effect of simultaneous infection of Kenchu virus and S. aureus was more detrimental in third instar. Hadimani (1980) observed the early instar silkworms to be more susceptible to Kenchu virus compared to late instars. Rejection of rearings in early instar stage itself due to high incidence of Kenchu disease are not uncommon in Karnataka. Under such situations, it may be quite

probable that both virus and certain species of bacteria are involved. In view of this, lot of care may have to be exercised in young silkworm rearing to minimise the loss accruing due to Kenchu disease.

SUMMARY

VI. SUMMARY

The studies made on some aspects of Kenchu virus disease of mulberry silkworm, Bombyx mori L. during 1980-86 are summarised below:

1. Kenchu virus isolates collected from silkworm in Karnataka did not produce precipitation bands in double gel diffusion test with antisera of both infectious flacherie virus and densonucleosis virus (Ina strain) of silkworm. As well, the midgut cells of Kenchu virus infected silkworms did not produce specific fluorescence with antisera of the above two viruses when examined by fluorescent antibody technique thereby revealing that Kenchu virus of silkworm is serologically quite different from silkworm infectious flacherie virus and Ina densonucleosis virus existing in Japan.

2. With advancement in the age of the silkworm egg at the time of refrigeration or with increase in the duration of refrigeration of eggs or combination of both followed by Kenchu virus infection during first feeding caused less weight of worms, less number of worms entering subsequent instars and less LD_{50} for mortality.

at * Worms weighed less in infected batches of worms resulting from seven days old

eggs refrigerated for eight days and four days, five days old eggs refrigerated for eight days and three day old eggs refrigerated for eight days and six days. All the worms in refrigerated and infected batches died in third instar itself.

3. As the density of Kenchu virus infected individuals, initially released into healthy silkworm populations increased, there was reduction in larval weight, ET_{50} for Kenchu symptoms, cocooning and quantitative traits of cocoon, in respect of releases in second, third and fourth instars. Release of Kenchu virus infected worms in early instars had more drastic effect on parameters studied. Also increase in the density of released individuals caused the same effect.

4. Reduction in the number of feeds provided per day (underfeeding) to young silkworms associated with Kenchu virus infection in later instars resulted in less ET_{50} for Kenchu symptom manifestation and reduction in larval weight, cocooning and cocoon traits as well as moth emergence.

Starvation itself was found to have deleterious effect on silkworm and starvation for longer duration associated with Kenchu virus infection had more adverse effect on silkworm. Starvation combined with Kenchu virus infection resulted in slight reduction of larval and cocoon weights

and more reduction in the extent of cocooning and moth emergence. Additive effect of starvation and Kenchu virus infection was more apparent in third and fourth instar treatments compared to that of fifth instar.

Malberry leaf maturity did not have appreciable influence on Kenchu virus development and intensity. Coarse leaf feeding in general caused more cocooning and moth emergence.

5. Simultaneous infection of silkworm with Kenchu virus and the bacterium, Staphylococcus aureus was found to complement with each other. Interaction treatments involving higher dilution of Kenchu virus and less bacterial cells did not cause appreciable reduction in economic traits, while combinations with less dilution of Kenchu virus and more bacterial cells brought down the same. Combined infection with all dilutions of Kenchu virus and bacterial dilutions resulted in no cocooning in respect of third instar infection and that of 10^{-2} dilution of Kenchu virus with all bacterial dilutions in case of fourth instar infection. The interaction effect of simultaneous infection was more pronounced in third instar and it was less marked in fifth instar, especially with advancement in age.

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*Originals not seen.

APPENDICES

Appendix I

Effect of refrigeration of silkworm eggs on hatching, instar progression, maximum larval weight and extent of cocooning

Sl. No.	Treatments		Per cent hatching	Per cent of progression to IV instar	Per cent of progression to V instar	Maximum weight of 10 worms (g)	Per cent cocooning
	Age of egg (Days)	Duration of refrigeration (Days)					
1.	One day	Two days	83.65	94.66	92.66	12.4943	89.33
2.	"	Four days	95.16	84.00	78.00	12.4332	72.00
3.	"	Six days	92.95	79.34	72.66	11.0611	65.33
4.	"	Eight days	87.17	72.66	69.34	10.9948	60.65
5.	Three days	Two days	85.94	92.66	87.34	13.3984	86.00
6.	"	Four days	92.45	83.34	80.66	12.1347	69.33
7.	"	Six days	92.73	81.34	73.34	11.8524	68.00
8.	"	Eight days	94.42	70.00	64.00	10.6870	61.33
9.	Five days	Two days	89.34	93.34	90.66	14.9904	88.00
10.	"	Four days	91.32	74.00	74.34	13.4225	81.33
11.	"	Six days	91.60	71.32	68.34	11.2848	63.33
12.	"	Eight days	96.09	68.66	67.34	10.5106	38.00
13.	Seven days	Two days	95.81	92.66	90.00	13.9229	88.00
14.	"	Four days	92.86	90.00	86.66	12.1397	73.33
15.	"	Six days	95.61	78.66	76.66	12.0346	60.00
16.	"	Eight days	92.27	52.66	50.00	11.4290	46.67
17.	Unrefrigerated control		93.49	95.34	94.00	13.6420	90.00

Appendix II

Effect of refrigeration of silkworm eggs on weight of cocoon, pupa and cocoon shell and moth emergence

Sl. No.	Treatments		Weight of 10 cocoons (g)	Weight of 10 pupae (g)	Weight of 10 cocoon shells (g)	Per cent moth emergence
	Age of egg (days)	Duration of refrigeration (Days)				
1.	One day	Two days	7.1938	6.2292	0.9811	80.00
2.	"	Four days	6.6607	5.7383	0.8587	67.33
3.	"	Six days	6.5119	5.6333	0.8345	58.00
4.	"	Eight days	5.9672	5.1169	0.7637	48.00
5.	Three days	Two days	7.4865	6.5579	0.8795	68.00
6.	"	Four days	7.3173	6.3725	0.9134	68.00
7.	"	Six days	6.4290	5.5497	0.8340	68.00
8.	"	Eight days	6.4147	5.5144	0.8221	60.00
9.	Five days	Two days	7.5112	6.5469	0.8041	84.00
10.	"	Four days	7.2691	6.1992	0.9604	70.00
11.	"	Six days	6.7327	5.8353	0.8607	62.00
12.	"	Eight days	5.7522	4.9663	0.7377	36.00
13.	Seven days	Two days	7.2302	6.2198	0.9857	68.00
14.	"	Four days	6.7932	5.7149	0.8647	68.00
15.	"	Six days	6.7175	5.8834	0.8586	50.00
16.	"	Eight days	6.7412	5.8464	0.8447	42.00
17.	Unrefrigerated control		7.4904	6.4884	0.9457	88.00

Appendix III

Data on temperature and relative humidity of silkworm rearing room during the experimental period

Month	Temperature (°C)		Relative humidity (%)
	Minimum	Maximum	
1	2	3	4
September, 1981	19.8	27.9	95.0
October, 1981	19.4	27.8	91.0
November, 1981	18.9	26.7	88.0
December, 1981	14.7	25.1	87.0
January, 1982	15.0	26.0	94.0
February, 1982	15.3	30.0	82.0
March, 1982	19.4	33.0	96.0
April, 1982	21.8	34.0	78.0
May, 1982	21.7	33.1	85.0
June, 1982	20.2	29.2	94.0
July, 1982	19.5	29.2	90.0
August, 1982	20.0	28.9	91.0
September, 1982	19.1	29.5	90.0
October, 1982	19.1	29.7	91.0
November, 1982	21.4	27.3	91.2
December, 1982	18.6	27.0	90.0
January, 1983	12.7	28.3	81.0
February, 1983	17.0	32.7	70.0
March, 1983	20.0	34.6	66.0
April, 1983	22.3	35.2	75.0
May, 1983	21.8	34.5	80.0
June, 1983	20.0	30.7	83.0
July, 1983	19.8	29.8	84.0
August, 1983	19.4	27.8	91.0
September, 1983	19.4	27.3	91.0
October, 1983	18.4	28.3	88.0
November, 1983	15.0	27.8	79.0
December, 1983	16.5	25.7	85.0

Continued

Appendix III (Continued)

1	2	3	4
May, 1984	28.15	32.82	72.74
June, 1984	24.44	26.90	84.60
July, 1984	24.66	25.33	86.88
August, 1984	22.86	25.12	85.65
September, 1984	22.90	27.19	79.30
October, 1984	23.44	25.81	70.32
November, 1984	22.24	25.16	57.98
December, 1984	21.84	25.29	57.03
January, 1985	22.00	25.40	62.50
February, 1985	24.72	28.14	45.68
March, 1985	27.65	31.48	52.58
April, 1985	28.33	33.26	54.80
May, 1985	27.09	33.87	68.70
June, 1985	24.93	33.83	77.95
July, 1985	23.45	32.52	83.54
August, 1985	23.65	26.46	77.37
September, 1985	24.68	28.38	73.00
October, 1985	24.39	27.81	69.27
November, 1985	23.00	26.86	55.50
December, 1985	22.78	26.08	63.61
January, 1986	22.12	24.95	61.07
February, 1986	24.39	27.47	57.08
March, 1986	28.83	33.29	38.22
April, 1986	29.83	33.00	43.00
May, 1986	30.27	32.51	63.70

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