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INCIDENCE OF BACTERIAL AND VIRAL DISEASES OF SILKWORM,
BOMBYX MORI L. IN BANGLADESH AND THEIR CONTROL

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This is to declare that the materials embodied in this thesis are the original research works conducted on my part during the years 1985 to 1988, and have not previously been submitted for the award of any degree or diploma in anywhere. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except when due reference is made in the text of the thesis.

Bear

A. C. Barman.

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Section – A

General Introduction
Aim of Study
Literature Survey

CHAPTER 1.

GENERAL INTRODUCTION

Like other forms of life, the silkworm, Bombyx mori L. is also affected by various types of diseases. During the growth the silkworms come in contact with a number of pathogenic agents like virus, bacteria, fungus, protozoans etc. under natural environmental conditions. Life functions are thus interfered and a disease occurs. The ultimate result is the loss of worms and hence loss of cocoon production. The earliest scientific report on insect diseases was on silkworm ailments (Kirby 1826). Afterwards it was experimentally demonstrated that the micro-organisms are the causative agents for certain sickness in silkworms (Steinhaus 1963). A major breakthrough in our understanding of silkworm disease was achieved by Pasteur (1885) who identified that the protozoan, Nosema sp. was responsible for the devastating disease 'pebrine' of silkworm (Rene Dubos 1960).

Bacterial and viral diseases of silkworm are the most common diseases inhabiting all the silk producing countries and areas, although their severity varies with the consequent interaction of existing environment of different regions. Total crop losses due to these diseases are rarely known, but the cocoon yield is largely hampered affecting national plan as well as personal income.

In any of the cases of bacterial or viral diseases, starting point is the individual silkworm from which the disease is spread to the population. The occurrence of disease in a population is the consequence of interaction between pathogen, host and the environment.

But the major factor in disease development is the pathogen. The physiological status of the silkworm under certain circumstances enables the inhibition of the pathogenicity of the infecting agents, while the environmental conditions exert influence both on the viability and virulence of the bacteria or viruses as well as on the resistance of the worm host. In Bangladesh, sericulture industry has been in existence for a long time. Unfortunately, there had been no documentary report about different diseases affecting silkworm rearing in the country, except a cumulative report described by Barman and Pasha (1986).

Bacterial diseases are popularly knowns as 'flacherie' meaning flaccid condition. In Bangladesh the diseases are termed 'Kalshira' which means blackened dorsal vessel. In India also these are identified by their local names such as Sappe, Kenchu and Sarapikke (Chitra et al 1975). Originally, the term 'flacherie' was used to indicate a wide range of unhealthy conditions observed in the silkworms caused by microbial or nonmicrobial agents. Pasteur (1885) recognized the distinct etiology of flacherie and associated the disease with the intestinal microflora. Till todate, as much as 39 bacterial species are known to inhabit the silkworm, Bombyx mori gut or haemolymph, of which very few were found pathogenic under natural conditions (Afrikian 1960, Chitra et al, 1975).

It is established that all the bacteria present in the silkworm do not bear pathogenic properties and it could be that many bacteria

isolated from the diseased worms might be secondary invaders accentuating the disease caused by other agents. Many of the bacteria of the enteric cavity fall under this group (Chitra et al 1975). Bacterial diseases of silkworm are broadly classified into two classes:- 1) those that are restricted to the intestine, and 2) those that can also enter the haemolymph. The major contributions about bacterial pathogens and pathogenicity in silkworm come from the investigations of Nakasuzi and Kodama (1970), and Chitra et al (1975).

The association of bacteria for causing flacherie in silkworm was questioned in the early days. Because, conditions similar to bacterial flacherie were also noticed by feeding mulberry leaves of defective nutritional status and also a large number of organisms caused similar symptoms to that caused due to bacterial infection. However, it is now confirmed that pathogen is a must for creating a special type of disease symptom in silkworm. Artificial introduction of the pathogens with the consequent symptom has resulted this fact. Of course, there are other secondary factors which are responsible for bacterial propagation and spreading the disease. Feeding of contaminated leaves is the main source of this disease, which sometimes enter into the haemolymph through minute wounds.

Introduction of pathogenic bacteria in the larval body disrupt the normal physiological functions, which rapidly multiply in haemolymph. Disturbance of the metabolic processes leads to disease and death of the host. Some enteric bacteria like Streptococcus faecalis after introduction into the larval gut attach to the peritrophic membrane and multiply

into large colonies. As a result, the membrane degenerates completely, as such the function of nutrient absorption is blocked. Infection of the organism also resulted in the deterioration of the cytoplasm and nuclei. pH of the gut is markedly brought down when the worms are attacked by septicaemia disease (Chitra et al 1975), while in case of infection with Bacillus thuringiensis blood pH is increased progressively (Angus and Heimpel 1959), which is regarded as a consequence of delta-endotoxin damage to the midgut epithelium, more specially to effect on it's ion-permeability (Fast and Morrison 1972).

Viral diseases of silkworm are locally called as 'Rosa' (Nuclear polyhedrosis) and 'Salpa' (Gattine). Because of uncertain and infrequent occurrence cytoplasmic polyhedrosis is not known to the sericulturists.

Viral diseases of silkworm are widely classified into 1) Nuclear polyhedrosis, 2) Cytoplasmic polyhedrosis and 3) Infectious flacherie. Besides these, there is a separate disease called 'Intestinal nuclear polyhedrosis' in Japan (Anon 1975); while in India, sericulturists (Krishnaswami et al 1978) referred to a different disease of viral and bacterial association ' Gattine' as the commonest one. Nuclear polyhedrosis, Cytoplasmic polyhedrosis are named on the basis of the site of viral multiplication with the consequence of polyhedra formation. But the later two diseases are caused by noninclusion free viruses.

Through rate - zonal centrifugation it was found that nuclear polyhedra contained three different forms of virus particles (Kawarabata and Matsumoto 1973), which contains 80% single and 20% boundle

virus rods, each with a developmental membrane. They also noted almost equal infectivity in both the cases of single and bundle viruses. Studies on occluded and non occluded viruses revealed that the infectious unit causing per oral and haemocoelic infections differed from each other. The former was largely due to the viron with an envelop, and the haemocoelic infection was due to the viron without an envelop (Kawarabata and Aratake 1978).

The polyhedra is composed of 3-5% viral particles, the rest being protein. After ingestion of the polyhedra by the larva, the protein coat is dissolved in the gut to release the virus rods. These rods attach themselves to the midgut cells where infectious subunits are set free. The subunits after multiplication in different cells penetrate the epithelium and enter into the haemolymph. In case of cytoplasmic polyhedrosis, the major site of attack is the epithelial cells of the midgut from which the disease spreads to the other regions of the gut and to other cells. Aruga et al (1963) found that the cytoplasmic polyhedrosis virus multiplies mainly in the posterior part of the midgut. The polyhedra are found only in the cylindrical cells but not in the goblet cells (Tsugita 1955). But in case of infectious flacherie the virus is restricted to the anterior part of the midgut and not detected in any other tissues of silk gland, malpighian tubules, trachea, brain, gonad, fat bodies or integuments (Inoue and Aizawa 1972).

Infection with nuclear polyhedrosis virus stimulates nucleic acid and protein synthesis in different tissues of the silkworm, where

the rates of DNA, RNA and protein synthesis is higher than the normal worms. But this higher rate of synthesis lasted only for a short period, after which it slows down in all the tissues. The levels of various enzyme activities are also interfered. But the infection with cytoplasmic polyhedrosis enhances the protein metabolism in the midgut during the later stages (Watanabe 1970). It also accelerates nitrogen catabolism. Disturbance in nucleic acid and protein metabolism, free amino acid content of the haemolymph and digestive fluid are decreased to a large extent and leads to physiological disfunction and death of the infected larvae. On the other hand, multiplication of infectious flavo-cherie virus in the goblet cells disrupt the secretion of digestive fluids, at the same time bacteriostatic and virucidal properties of the goblet cells are lost. This permits proliferation of the enteric bacteria due to which death of the infected worms is accelerated.

Both the bacterial and viral diseases of silkworm are infectious in nature, where a number of predisposing factors or stressors are involved in their manifestation. Therefore, in controlling these diseases, the factors affecting the susceptibility need to be studied first, afterwards actions to be taken for their correction. However, in controlling insect diseases it is very often suggested to maintain and restore optimum rearing conditions which include proper condition of temperature, humidity, nutrition, sanitation etc. as important is freedom from overcrowding and predisposing factors.

viral diseases. The digestive juice of silkworm is strongly acidic, pH 2-10, which is unsuitable for growth and propagation of bacteria.

The principal reasons for accomplishing the control of micro-organisms can be summarized as :-

- 1) to prevent transmission of disease and infection,
- 2) to prevent contamination or growth by undesirable micro-organisms, and
- 3) to prevent deterioration and spoilage of materials by microorganisms.

Bacteria and viruses can be removed, inhibited or killed by physical agents, physical processes or chemical agents. A variety of techniques and agents act in different ways and each has its limits of practical application.

In controlling disease, although microorganisms are the physical target, there are many other biological characteristics which influence the rate at which they are killed or inactivated by various agents. As a fact, many factors must be considered in the application of any physical or chemical agent used to inhibit or destroy microbial populations, of which temperature, humidity, kinds of microorganisms, physical state of cells and environment are of major consideration.

Silkworm bears well resistance to diseases and unless its physiology is disturbed otherwise it is not subjected to attack by the pathogens by any means. That is why, in favourable seasons of prewinter and postwinter rearings, silkworms are hardly affected by bacterial and viral diseases. The digestive juice of silkworm is strongly basic, pH 9-10, which is unsuitable for growth and propagation of bacteria.

The red fluorescent proteineous substance formed by the digestive fluid has virus inactivating properties (Hayashiya et al 1968). Aizawa (1962) discovered antiviral unknown substance of high and low molecular weights in gut juice. Uchida et al (1984) isolated a protein that can precipitate NPV from the digestive juice of silkworm and had an additional activity to inactivate NPV, which is analogous to serological neutralization by serum proteins. Utsumi and Nishimura (1982) reported that some proteinous substance in the gut juice can inactivate bacteria. Many kinds of oxidases and lysozymes present in the haemolymph can inhibit bacteria, and the granulocytes possess powerful phagocytic action against foreign bodies. Utsumi et al (1987) found that there is an antistreptococcal character in the digestive juice of silkworm which might be controlled by genetic factors. Such resistance power is inherited which may be different in different races.

Resistance to viral diseases is related to the constitution of the silkworm, the variety, developmental stage and the physiological status. For instance, multivoltine are more resistant to viral diseases than bivoltines. In general, the races that can tolerate high temperature and humidity are more resistant to infectious flacherie. Again the hybrids are more resistant than their parental stock. Usually young silkworms tend to be more susceptible. Chinese workers (Anon - year not mentioned) found that the most susceptible is the newly moulted worm; the most resistant time is that of the most active feeding and that the resistance tends to fall as the time of moulting approaches. As the larva

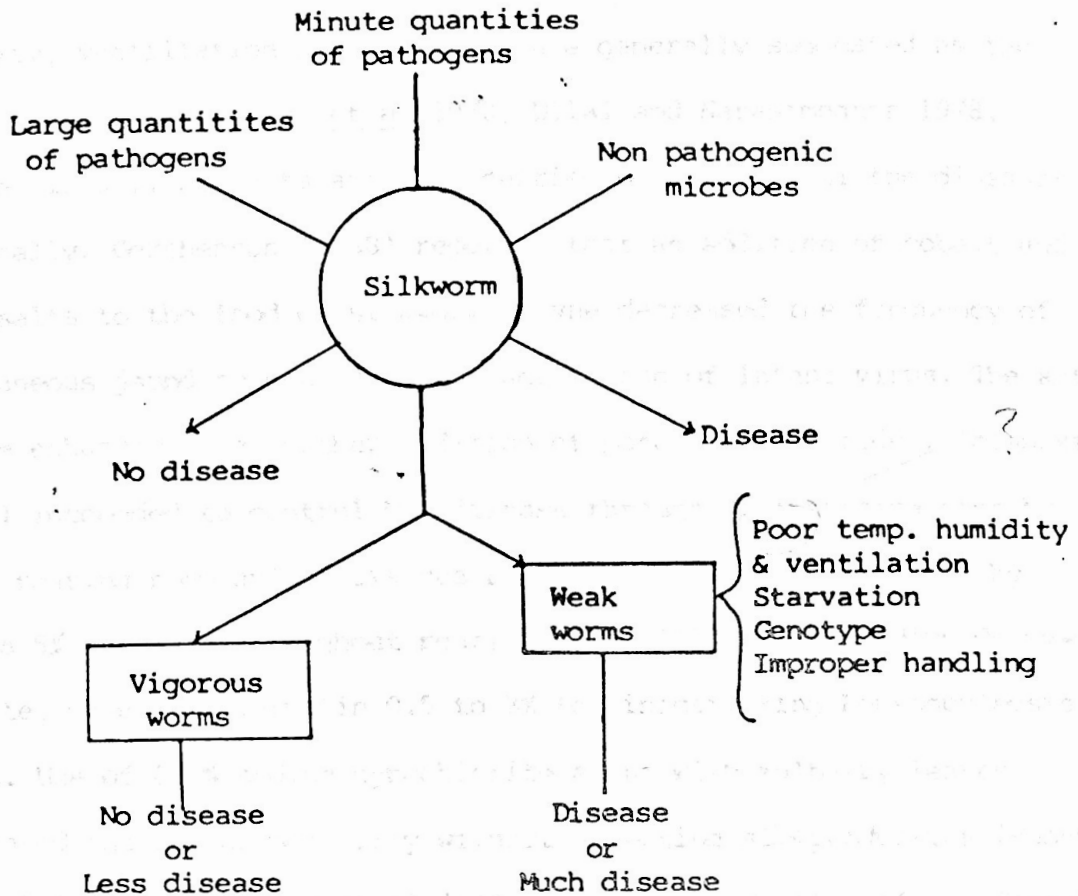


Fig. 1. The interrelationship of pathogen, silkworm and environment in regard to occurrence of infectious diseases (After Sengupta 1988).

developed in age, it increased in resistance to peroral infection of infectious flacherie virus and result in a prolonged period of lethal infection (Aratake et al 1973).

Scientists differently attempted to combat the malady in silkworms caused by the infectious bacterial and viral diseases. In most of the cases, prevention has been given more priority than controlling the disease. For this, maintenance of hygienic conditions, temperature,

humidity, ventilation and nutrition are generally suggested as the first step (Krishnaswami et al 1978, Ullal and Narasimhanna 1978, Samson 1983). Attempts are also carried out to control the diseases chemically. Gershenson (1958) reported that an addition of cobalt and zinc salts to the food of silkworm larvae decreased the frequency of spontaneous jaundice caused by the activation of latent virus. The action may be enhanced by a further addition of potassium chloride . Golanski (1961) succeeded to control NPV disease through disinfecting eggs by 2.5%, rearing room and appliances by 10% and spraying silkworms by 0.5 to 5% formalin throughout rearing. Seki (1986) used sodium deoxycholate, ether and formalin 0.5 to 3% for inactivating Densonucleosis virus. Use of 0.2% sodium hypochlorite along with mulberry leaves decreased the larval mortality without affecting silk production (Hosny et al 1986). Chinnaswamy et al (1986) found that dusting of paraformaldehyde + Benzoic acid + Lime from 3rd stage to cocoon formation was effective in controlling diseases and also improved the economic characters. Finally, the Indian Scientists formulated a chemical composite (Resham keet oushad) which, on spraying over silkworm, is claimed to minimize the viral and fungal diseases (Samson et al 1987). However, no systemic report is available on the chemical control of bacterial diseases.

In controlling infectious diseases of bacterial and viral origin, selection of resistant varieties has stood as a universal concept. A lot

of successful results on this line are available. Aizawa et al (1961) developed a virus - resistant strain through careful selection in thirteenth generation from the artificially induced diseased lines. Ugigawa and Aruga (1966) obtained a resistant strain to Infectious flacherie virus (IFV) after selection through five generations. Watanabe (1967) developed a CPV-resistant strain by selecting survivors from virus fed larvae for eight generations showing as much as sixteen fold resistance compared to unselected line. Hybrids from this parent also showed marked heterosis. Nomani et al (1979) also developed a comparatively NPV resistant line after selection through thirteen generations. Development of bacteria-resistant strain by feeding bacteria germs and selection through nineteen gens. has been reported by Barman and Pasha (1984). Lastly, Sengupta (1988) stressed on the judicious selection of breeds or crosses to suit different areas at different seasons. Races or combinations of multi x bivoltine or multi x multivoltine races would be the actual means for minimising diseases in commercial scale.

are many other bacterial and viral diseases. The infectivity is exaggerated by the inferior quality of the cocoons. This directly leads to increasing the rate of the pupation mortality in the course of the other bacterial diseases.

It is strongly felt that the kinds of bacterial and viral diseases, their nature and extent of damage and the proper etiological and remedial measures; followed under the prevailing conditions of rearing must be related to all the sericulturists engaged in the country to take timely action for their control. The lot of control methods developed by the workers

CHAPTER 2.

AIM OF THE STUDY

In Bangladesh, commercial silkworm rearing and cocoon production are limited to four major seasons:- Jaistha-comprising months of May-June, Bhaduri-comprising months of July-August , Agrahayani-comprising months of November-December and Chaitra-comprising months of February-March. Again in between two commercial rearings there are seed crops, which are conducted in the Government nurseries. As a matter of fact , silkworm rearing and cocoon production is continuously run throughout the year covering almost 9-10 months. The months from April to September are characterized by high temperature, while there added an extra monsoon weather of high humidity from July to October. Practically, the periods from April to September can not be regarded suitable for silkworm. Only because of leaf availability in large quantity, two commercial rearings and two seed rearings fall under this period. Average rearing performance recorded was only 40-50% cocoons posing almost 50% loss, which are mostly due to bacterial and viral reasons. Low productivity is accompanied by the inferior quality of the cocoons. This directly leads to unhealthy atmosphere for the sericulture industry in the country affecting national economy.

It is urgently felt that the kinds of bacterial and viral diseases, their nature and extent of damages and the major etiological and predisposing factors under the socioeconomic conditions of rearing must be cleared to all the sericulturists engaged in the country to take timely action for their control. The lot of control methods developed by the workers

from abroad have some limitations in their direct application. Because our environmental conditions and rearing practices differ largely from others. For instance, in Poland (Golanski 1961) formalin spray was recommended commercially for controlling jaundice disease of silkworm, which by repeated trial was found to have an adverse effect here (unpublished). Similar important is the selection of disease-resistant parental lines or hybrids from the locally available silkworm races. Thus, in the present study, emphasis was given firstly to detect the relationship between host, pathogen and the existing environment and secondly, to direct the possible change or addition of new agents or idiology to the above components to minimize the losses as imposed by bacterial and viral diseases.

CHAPTER 3.

BRIEF LITERATURE SURVEY

3.1. Causative agents for bacterial and viral diseases of silkworm.

The earlier reports on bacterial diseases of silkworm were available from the investigations of Sawamura (1903, 1905 and 1906), who recorded a number of pathogenic bacteria in silkworms causing disease. Then upto the middle of this century almost nothing was known in this concern except certain general observations on certain bacterial species (Stark and Sherman 1935 and Steinhaus 1943 & 1949). Angus (1954) discovered that the cause of paralysis in silkworm was due to presence of a bacterial toxin. During early sixtieth our knowledge on the bacterial disease of silkworm was greatly enriched by the investigations of Steinhaus and Elizabeth (1954). Steinhaus (1963) wrote a great book 'Insect Pathology' where he incorporated his valuable findings. Causal agents for Septicaemia disease in silkworm were investigated by Talalev (1956) and Ono et al (1965). On the other hand, comparative toxicology and characteristics of crystal forming bacteria causing toxicosis in silkworm was studied by Angus and Heimpel (1959), Angus (1967), Faust et al (1967), Ramakrishnan (1968), Fast and Morrison (1972) etc.

Chitra et al (1975) enlisted as many as 35 bacterial species which inhabitsilkworm and a number of them produce disease. Krishnaswami et al (1978) attempted to observe the effect of feeding certain bactericides to silkworm, while Janko and Yasuhisa (1980) tried to find out the mode of

action of the Bacillus thuringiensis delta-endotoxin in the silkworm larvae. Distribution and toxicity of B. thuringiensis in different sericultural farms (Ohba et al 1984) and it's toxicity on mosquito larvae (Padua et al 1984) were also tested. Iwanami et al (1985) observed that bacterial contamination caused death of larvae or pupae inside the cocoons. A mixed infection of bacteria with infectious flacherie was noticed by Matsumoto et al (1985).

The polyhedral disease of silkworm was earlier reported by Suzuki (1936 & 1941). At the same period studies were also carried out to observe the biochemical nature of the inclusion bodies and the virus causing jaundice disease (NPV) of silkworm (Glaser and Stanley 1943). During early sixtieth Tsujita (1955) discovered the cytoplasmic polyhedrosis virus infecting silkworms and Ooba (1956) attempted to induce these diseases through artificial means. Aizawa (1959 and 1967) had gone through further details where he studied the mode of multiplication of nuclear polyhedrosis virus inside the silkworm. Martignoni and Schmid (1961) observed that natural populations of lepidopterous larvae had variant degree of resistance to viral infections under natural conditions. The etiologic agents for causing infectious flacherie disease of silkworm were detected by Aizawa et al (1964), while Iwashita and Kanke (1969) discovered the histopathological diagnosis method of this disease. At the same time Tanami et al (1969) studied on the quantitative interactions between flacherie-virus and silkworm larvae for induction of the disease. In 1973, Kawarabata and Matsumoto detected the methods for isolation and purification of

nuclear polyhedrosis virus. Also the functional difference between occluded and non occluded virus of nuclear polyhedrosis were discovered (Kawarabata and Aratake 1978). Bell and Kanavel (1976) observed the effect of cytoplasmic polyhedrosis on larvae and pupae of pink bollworm. Hajime (1983) observed that nuclear polyhedrosis virus may be incorporated in the inclusion bodies formed by the cytoplasmic polyhedrosis viruses. Similarly, Matsumoto et al (1985) reported a mixed infection of infectious flacherie virus with bacterial disease.

A most scientific method, latex agglutination test, was discovered for the detection of flacherie virus of silkworm (Shimizu 1983). At the same time, an X-ray defraction method was suggested for the detection of polyhedrosis virus (Fujiwara et al 1984). Effect of dissolution conditions of polyhedral inclusion bodies in sodium carbonate solution on the yield of NPV was studied (Kobayashi and Nakagaki 1984). Lim et al (1984) in an attempt tried to induce nuclear polyhedrosis in the silkworm DNA. Recently, a new disease of virus origin, 'Densonucleosis' is detected by immunoperoxidase histochemical method (Guo et al 1985). Kobayashi et al (1985) observed that the protease activity was stimulated in the silkworm abdomen infected with NPV. Recently, Johannse et al (1986) found that follicular cells of the silkmoth are also affected by NPV. Shimugu and Arakawa (1986) could detect cytoplasmic polyhedrosis virus by the latex agglutination test, while the same technique was also applied for detecting DNV by Arakawa and Shimizu (1987).

3.2. Environments and diseases.

Watanabe (1951) studied the effect of some physical and chemical agents upon the silkworms in relation to the outbreak of grasserie disease, who found that the grasserie virus was relatively susceptible to heat but strongly resistant to low temperature. But Aruga and Arai (1959) discovered that polyhedrosis of silkworm could be induced by low temperature treatment. This view was experimentally supported by Hukuhara and Aruga (1959), while Karpov (1959 and 1960) observed that the disease could also be induced by X-rays.

Sharda et al (1956) found that enrichment of mulberry leaves with solutions of chloromycetin and glycine improved health of silkworms and also reduced larval mortality. Similar investigations with chloromycetin, glycine and molases were done by Verma and Atwa (1963) and with sugars, vitamins, proteins and aminoacides by Sengupta et al (1972) .

The influence of environment on the virus host relationship was studied by Pimentel and Shapiro (1962) and Ono et al (1969) who studied the bacteria-host relationship for their outbreak. Then Krishnaswami et al (1971) made special attention on certain ecological factors to prevent crop losses in adverse climatic conditions. The factors affecting the susceptibility of silkworm were also investigated by Aratake et al (1973). Samson et al (1981) studied the effect of starvation on the cocoon crop and incidence of diseases. The major factors affecting the susceptibility to nuclear polyhedrosis were studied by Kobayashi (1982). Wu (1983) observed the rela-

tionship of nuclear polyhedrosis to the proportion of mulberry leaf powder, vitamin C and water in the artificial diet, while Karaivanov et al (1985) attempted to rear silkworms at disturbed environmental conditions. Effect of thyroxine hormone on the growth and resistance was observed by Thyagraja et al (1985); similar effect on supplementation of glycine to the silkworm feed was observed by Shridhar and Radha (1986), with antibiotics by Murthy et al (1951), Murthy and Sreenivasaya (1953) and Tayade et al (1986). Zhang (1986)ⁿ studied on the time of last and first feeding and their relationship with some economic characters as well as sensitivity to virus.

3.3. Control measures: Chemical, Selection, defence mechanism.

Gershenson (1958) first reported the control of Spontaneous jaundice disease or nuclear polyhedrosis of silkworm by chemical means. Possibility of controlling insect viral disease by temperature treatment was also suggested by Thompson (1959). Afterwards antibiotics were effectively used for controlling bacterial diseases in silkworm by Afrikian (1960). At the same time Golanski (1961) demanded the control of silkworm jaundice by using different concentrations of formalin solutions. Then Krishnaswami et al ^{X Ghosh} (1963) preliminary tried some bed disinfectants against the same disease. King et al (1975) tried to control the bacterium, Serratia marcescens in an insect host-parasite rearing programme. Efforts on chemical control measures continued when Nomani and Mukherjee (1975) investigated the

efficacy of papzol against grasserie disease and also a number of chemical disinfectants were recommended by Anon (1983), Chinnaswamy et al (1986), Hosny et al (1986) and Rahman (1986). Baburashvilli et al (1984) used imanine as a feed additive against polyhedrosis disease. A number of workers again attempted to use therapeutic agents against silkworm diseases (Mladen et al 1984). Thus Miyajima and Kawase (1986) and Miyajima et al (1986) used several guanidine salts and derivatives against infectious flacherie. Seki (1986) tried to introduce a physico-chemical treatment for controlling densovirus of the silkworm. Finally, a group of workers formulated a composite bed disinfectant ' Reshamkeet Oushad' with a tremendous effectiveness against viral and fungal diseases (Samson et al 1987).

On the other hand, some workers stressed on the selection of resistant silkworm races for controlling the diseases. Aizawa et al (1961) selected a strain resistant to virus induction. At the same time, Aruga (1961) studied the induction and resistance to nuclear and cytoplasmic polyhedroses and Maudslayi et al (1961) studied the resistance to virus infections in the natural populations of lepidoptera. Resistance of F_1 hybrids and some common strains to virus induction was observed by Aizawa and Furuta (1962), while to per os infection with cytoplasmic polyhedrosis by Aruga and Watanabe (1964) and Watanabe (1965, 1967). Uzigawa and Aruga (1966) selected a resistant strain to infectious flacherie virus. Sidhu and Singh (1968) while studying resistance capability of certain mutant strains and breeds also observed the inducing factors leading to the development of both grasserie and flacherie diseases. Similar studies were also made by Sidhu and

in the silkworm which is lost the infection of nuclear polyhedrosis.

Kapila (1969). Kundu (1978) attempted to evolve a flacherie-resistant strains through selection. At the same time attempts were also tried to evolve a grasserie-resistant strain (Nomani et al 1979) and to observe the inheritance of tolerance to high temperature in some silkworm strains. Barman and Pasha (1984) evolved a bacteria-resistant silkworm strain through selection. Chernysh et al (1985) studied on the adaptogens and resistance of silkworms to stress-induced activation of latent virus infection. A silkworm strain was successfully selected resistant to nuclear polyhedrosis virus by Kawarabata et al (1985). Narayanswamy et al (1985) studied the reaction of different breeds of silkworm to Kenchu virus, Seki (1985) observed difference in resistance to the infection of denonucleosis virus among different strains of silkworms, while to both flacherie and denonucleosis virus by Kim. et al (1976). Also a low pathogenic variety to Bacillus thuringiensis was successfully selected (Kim et al 1986).

Aruga (1957, 1958) studied the mechanism of resistance of silkworms to cytoplasmic polyhedrosis in relation to different environmental factors. Aizawa (1962) detected an antiviral substance in the gut juice of silkworm. Recent workers differently attempted to find out the mechanisms which the silkworm bear themselves in defencing various diseases. Thus, Sekijima and Ohtsu (1976) detected a special antigen against infectious flacherie-virus, Utsumi and Nishimura (1982) detected an antistreptococcal protein (ASP) in the digestive juice, Qu et al (1984) found an antibacterial substance, Uchida et al (1984) isolated a virus inactivating protein from the digestive juice and Liu and Hou (1985) detected an ecdysteroid containing agent in the silkworm which inhibit the infection of nuclear polyhedrosis.

Bacterial & Viral diseases of Silkworm in Bangladesh.

CHAPTER 4

INTRODUCTION

5.1. Importance of diseases of silkworms

The subtropical environment of Bangladesh favours the growth and development of both the bacterial and viral pathogens of silkworm. Out of four commercial rearing seasons, three are largely apt to the infection of both types of diseases. It was earlier reported to affect as much as 40-60% during the summer and autumn rearings (Barman 1986). Now a days, viral diseases are considered as the most acute problem for silkworms in this country. According to survey figures from different countries, viral diseases account for about 70-80% of the total loss (Anon^{*}). In tropical region, however, an average annual crop loss due to diseases stands around 30% (Sengupta 1988). In the southern sericultural belt of India an annual damage caused by bacterial diseases is 20-40% (Chitra et al 1975).

clipping off the cocoon from and allowed to dry. Small amount of sugar III (Saturated solution in 7% alcohol), was added and dried. Two drops of distilled water was added for placing a cover slip and was observed under microscope. When I.I was used for observing fat bodies which sometimes are confused to be the polyhedra.

5.3. Examination of *Bombyx mori* polyhedra (P):

Excreta of the infected larvae were diluted in sterile distilled

* Book published by Regional Sericulture Training Centre, Guanzhou, China.
year not mentioned.

CHAPTER 5.

MATERIALS AND METHODS

5.1. Examination of diseased samples:

Diseased silkworms of 3rd to 5th instar were periodically collected from the continuous stock culture of silkworms at the Bangladesh Sericulture Research and Training Institute (BSRTI), Rajshahi, during the year 1985. Bacterial and viral diseases were detected firstly from the physical symptoms (Anon 1975, Krishnaswami et al 1978) and secondly, confirmed by microscopic examination with the aid of a bright field Olympus microscope at 600X after the specifications made by Regional Sericulture Training Centre, Guanzhou, China.

5.2. Examination of Nuclear polyhedrosis (NP):

Haemolymph of the infected silkworm was collected on a slide by clipping off the caudal horn and allowed to dry. Small amount of sudan III (Saturated solution in 70% alcohol), was added and dried. Two drops of distilled water was added for placing a coverslip and was observed under microscope. Sudan III was used for dissolving fat bodies which sometimes are confused to be the polyhedra.

5.3. Examination of Cytoplasmic polyhedrosis(CP):

Excreta of the infected larvae were diluted in sterile distilled water and examined under the microscope. Worms having physical symptoms of CP were dissected when a white patch of transverse fold was observed in the posterior part of the midgut.

5.4. Examination of Infectious flacherie (IF) or Gattine:

Silkworms having physical symptoms of IF were dissected. Absence of ingesta in the digestive tract and presence of only intestinal fluid in the thoracic region confirmed the disease. For further confirmation, a small portion of the midgut was dissected, crushed and examined under microscope when no polyhedra were detected.

5.5. Examination of bacterial diseases and pathogens:

Diseased silkworms having characteristic physical symptoms of bacterial flacherie were separately grouped and crushed in distilled water. After filtering through absorbent cotton the filtrate was centrifuged at 2500-3000 rpm for 10 minutes. The sediment thus obtained was examined several times under the microscope at 600X after staining in crystal violet and basic fuchsin solution. For further confirmation the isolates were subjected to culture in Brain heart infusion agar and Blood agar base media (Barman and Nahar, unpublished), purified through subcultures and finally identified through microscopic examinations after staining in Gram stain and following the specifications made by Regional Sericulture Training Centre, Guanzhou, China, for individual disease and pathogen. All procedures related to culture and isolation were conducted in aseptic conditions. Measurements were taken with the aid of micrometers.

in silkworms (Fig. 6). This disease takes only 2-3 days to kill the silkworms. Mortality to the host after the onset of disease is high. The larvae, pupae are also heavily infected with the disease during spinning.

CHAPTER 6.

OBSERVATIONS

6.1. Bacterial diseases:

During the study period from February, 1985 to November, 1985 four major types of bacterial diseases were detected. The characteristic symptoms and pathogens are described as follows:-

6.1.1. Black thorax Septicaemia.

This is an acute disease affecting mainly the 5th stage silkworms. The major symptoms of this disease are decreased appetite with sluggish movement, straightened body with swollen thorax, sometimes black spot in the thorax (Fig 2 & 3) with the increment of the disease, the body becomes very soft, discoloured and turn dark brown to black. The pulsation of the dorsal vessel becomes rapid and the haemolymph appears black apprehending death (Fig 4 & 5). After death the body undergoes putrefaction within six hours. The disease is prevalent in almost all the rearing months, but the frequency is more during high humid months.

The bacterium isolated from the infected silkworm was a Bacillus sp. belonging to the family Bacillaceae, Order Eubacteriales. The bacteria observed 2.5 x 1.5 microns with peritrichate flagella, gram-positive in staining with characteristic greyish-white translucent folded colony grown on blood agar base medium (Fig 6). This disease takes only eight-twelve hours for bringing mortality to the host after the onset of disease symptoms. Besides larvae, pupae are also largely infected with the disease during spinning.

FIG.2. Black thorax septicaemia affecting 5th
instar silkworm showing straightened
body with swollen thorax.



FIG. 3. Black thorax septicaemia in 5th instar silkworms.



FIG. 4. Prominent black spots in the thorax.



FIG. 5. Black thorax septicaemia infected silkworms
with blackened dorsal vessel.



FIG. 6. Causative bacteria (Bacillus sp.) of black
thorax septicaemia in culture.



6.1.2. Red-rose Septicaemia:

This is also acute in the mature silkworms. Primary symptoms are very much similar to that of black thorax septicaemia. The identifying symptoms are observed just prior to or after the death of the infected worms which turns slightly red in colour (Fig 7). This disease is not as much serious as in the case of black septicaemia, and frequency is also less, which is prevalent mostly during high humid months.

The isolated bacterium was a gram-negative Serratia marcescens Bizio. The bacteria are small rods measuring 1.5 x 0.5 micron having peritrichate flagella. They formed rose-red round colonies on brain heart infusion agar medium (Fig 8).

6.1.3. Bacterial intoxication:

This acute disease occurs among mature silkworms causing death within four to six hours of infection. Major symptoms observed were cessation of feeding, idle sitting with raised head, paralyzed prolegs and hook-shaped appearance of the head (Fig 9). The infected worm shortly becomes distressed and die. The dead worm gradually turns black (Fig 12) and exhibit a foul smell. This disease is more frequent in the seasons with high temperature and high humidity, resulting paralysis in silkworm (Fig.10).

The isolated bacterium was a Sotto bacterium or Bacillus thuringiensis var Sotto Ishiwata, belonging to the family Bacillaceae of the Order Eubacteriales. This bacterium forms small oval transparent white colonies on blood agar base medium (Fig 11).

FIG. 7. Silkworms infected with red-rose septicaemia.



FIG. 8. Causative bacteria (Serratia marcescens) of red-rose septicaemia in culture.



FIG. 9. Bacteria infected silkworms showing hook shaped appearance (bacterial intoxication).



FIG. 9. Bacteria infected silkworms showing hook
shaped appearance (bacterial intoxication).



6.1.4. Bacterial gastro-enteric disease:

This is a chronic disease, observed in all the larval stages caused by the propagation and multiplication of a number of bacterial species in the digestive tract. The major symptoms observed were: wrinkled body with stunted growth, paralysis, clear thorax, vomiting colourless fluid, sometimes diarrhoea with collapsed rectal end. The infected worms are not killed easily, but largely lag behind the development compared to healthy silkworms (Fig 13). The disease is more frequent in the climate with high temperature and humidity. This also occurs in other seasons, particularly when quality of mulberry leaves and management deteriorate.

A number of bacteria were found to be associated with this disease. The observed bacteria were: two types of Bacilli sp. one having transparent white colony and the other translucent round colony on blood agar base; one Micrococcus sp. (Fig 14); one Diplococcus sp. with translucent milky white serrated colonies and a Streptococcus sp. with irregular colonies (Fig 15).

6.2. Viral diseases:

6.2.1. Jaundice or Nuclear Polyhedrosis (NP):

This is commonly known as 'rosa' with characteristic swelling of the midgut and yellowing of whole larval body (Fig 16). This is the most frequent and serious disease which is supposed to be the main problem in silkworm rearing during three commercial crops of the country. The affected silkworms show shiny body wall, incessant movement around the edge of the rearing tray and abnormal swelling of the body segments (Fig 17).

FIG. 10. Bacillus thuringiensis infected silkworms
showing paralysed and distressed body.



FIG. 11. Bacillus thuringiensis in culture.



FIG. 12. Putrefied silkworms infected with
Bacillus thuringiensis.



FIG. 13. Silkworms with Symptoms of gastro-enteric
disease.



FIG. 14. Micrococcus sp. in culture.



FIG. 15. Streptococcus sp. in culture.



The body wall ruptures easily with the exudence of affected haemolymph containing huge number of polyhedra- the infecting agents for healthy silkworms. As the haemolymph passes out, the body shrinks and the worms die. Corpses of the worms blacken and rot away quickly.

Nuclear polyhedrosis is an acute disease which kills the infected worms within twelve to twenty hours after first appearance of the disease symptoms. This disease is prevalent in all the larval stages, but from 3rd stage onwards symptoms could easily be detected. Outbreak of the disease occurs mostly during the summer and autumn rearings comprising two major commercial seasons of Jaistha and Bhaduri. A reasonable incidence is also observed during prewinter rearing (Agrahayani) when there exists high relative humidity in the environment.

The infecting agent of Nuclear polyhedrosis is Borrelinavirus bombycis (Bucculoviridae) forming four to six micron octadecahedral hexahedron polyhedra of uniform shape and size.

6.2.2. Cytoplasmic Polyhedrosis (CP):

This is a chronic type of viral disease, less severe compared to other diseases. The infected worms show ill growth, stunted body, reduced leaf consumption and dull white colour of the body (Fig 18). It occurs in the worms from 3rd to last instar. The infected worms may or may not caste old skin, the thorax becomes slightly transparent (Fig 19), the posterior portion gradually atrophies and sometimes anal protrusion is observed.

Under Bangladesh climate this disease is seen only during autumn rearing season, but frequency largely varies from year to year.

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FIG. 16. Characteristic yellowing of jaundice (NPV)
infected silkworm.



FIG. 17. Characteristic swelling of midgut segments
in NPV infected silkworms.



FIG. 18. CPV infected silkworms showing stunted development.



FIG. 19. CPV infected 5th instar silkworms with slightly transparent body texture.



FIG. 20. Gattine infected 3rd instar silkworms.

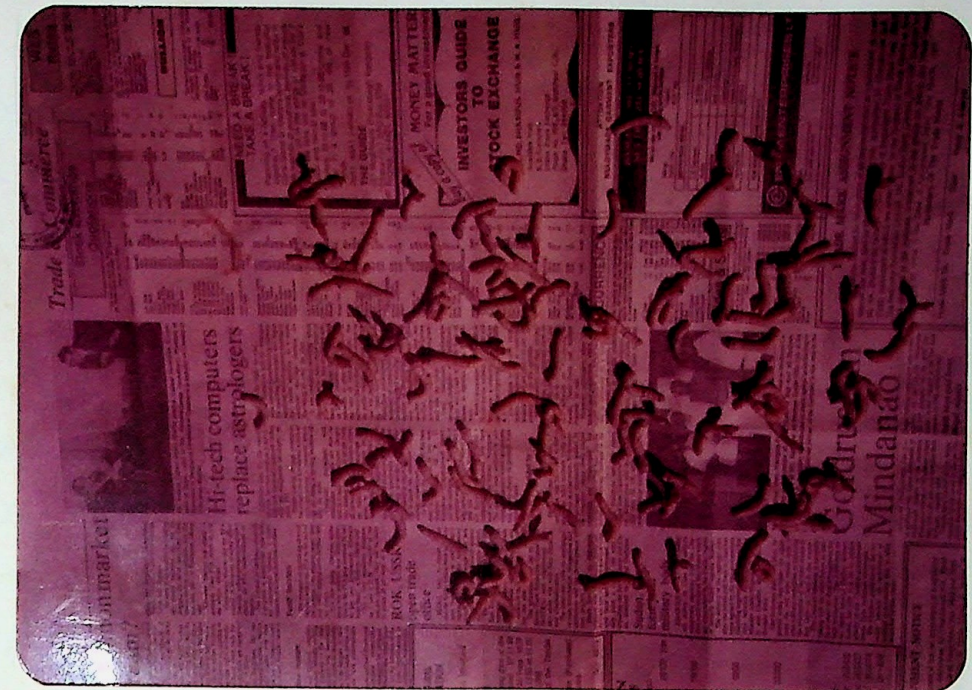


FIG. 21. Gattine infected 5th instar silkworms resemble ripen worms.





The etiological agent of cytoplasmic polyhedrosis is the Smithia-virus bombycis (Reoviridae) forming icosahedral hexahedron or tetragon one to ten micron polyhedra of ununiform size.

6.2.3. Gattine or Infectious flacherie (IF):

This is a chronic disease, under Bangladesh climate it incurs a reasonable loss in silkworm rearing. The major symptoms are - reduction in leaf consumption, sluggishness, pale white body colour with swollen thorax containing only digestive fluid, which finally turns transparent (Fig 20&21). The infected worms could be mistaken as ripen ones, but when mounted on the 'Chandrika' (bamboo mountage) they only move around and do not form cocoons. The infected worms may or may not complete moulting and individual disparity in size is great. 3rd instar to 5th instar silkworms are mostly infected, the severity increases with the age of the worms.

Under Bangladesh climate, gattine is seen more or less in all the commercial seasons. But the severity is observed only during autumn rearing when both the temperature and humidity remain high. The etiogenic agent is the Moratorvirus (Picornoviridae), a free virus which does not form polyhedra.

CHAPTER 7.

DISCUSSION

The bacterial diseases observed under the investigation are also reported to be common in other silk producing countries - the difference lies only in their degree of severity. Afrikian (1960) investigated the pathogenicity of some spore forming and non-spore forming bacteria, and found that among non-spore forming bacteria, the Proteus and Coli aerogenes groups and certain species of Streptococci affect silkworm larvae of 4th and 5th instars very frequently. While among spore forming bacteria, the most wide spread infection was caused by the representatives of the Bacillus cereus thuringiensis. Bacilli groups were also found to be more serious in this study compared to other groups. Of the Bacilli groups, Afrikian (1960) detected a number of species namely B. subtilis, B. C. mycoides and B. mesentericus which had good pathogenic properties on silkworm.

Ono et al (1965) confirmed four bacteria genera as the causal agents for septicaemia in silkworm, these are Serratia, Aeromonas, Pseudomonas and Proteus. Of them, Aeromonas at per os feeding was highly lethal for 4th and 5th instar silkworms when high temperature and high humidity existed in the environment. Iwanami et al (1985) supported this view and added a new bacterium Streptococcus faecalis as the causal agent of septicaemia. They found that Serratia marcescens was highly pathogenic to pupae and Serratia faecium to larvae when the silkworms are fed with contaminated mulberry leaves. In the present investigation, pathogenicity of Serratia marcescens was noted either through minute wounds or injection and not through per os inoculation.

Extensive studies were carried out to find out the cause of paralysis when silkworms are infected with Bacillus sotto. Angus(1954) found that a toxic substance present in B. sotto is responsible for paralysing silkworms when fed, but the crystal free spore were pathogenic when injected. It was further (Angus et al 1959) ascertained that delta-endotoxin of B. sotto was the main reason of paralysis which is remained associated with a progressive increase in blood pH from 6.8 - 8.1 . But Ramakrishnan (1968) was of the opinion that leakage of the midgut's high potassium content into the haemolymph was responsible for paralysis. However, the view of Angus et al (1959) is widely accepted to day suggesting that moist environment is the main predisposing factor for bacterial intoxication and favourable to the proliferation and spread of B. sotto. This supports the present investigation.

Nuclear polyhedrosis, cytoplasmic polyhedrosis and infectious flacherie occur more or less in all the silk producing countries during summer, autumn, as well as in spring rearing seasons. In China, cytoplasmic polyhedrosis and infectious flacherie occur in the summer and autumn crops in response to high temperature, while the greater incidence of nuclear polyhedrosis in the spring crop was due to high humidity. In this study, both nuclear polyhedrosis and infectious flacherie were observed when high temperature or high humidity existed in the environment.

Of the three viral diseases nuclear polyhedrosis is more common and severe than the other two. Chitra et al (1975) also found that 4th and 5th instar silkworms are mainly affected by nuclear polyhedrosis. The

authors also noted that the size of the inclusion bodies varies from 'very large' to 'very small' and is related to the time interval between inoculation and appearance of the symptoms. Larger is the interval longer is the size of polyhedra. Tsujita (1955) observed two types of virus infecting the silkworm, rod-shaped one and spherical one. The former usually invades the nuclear portion of the epidermic cells and some other tissues, while the later invades the cytoplasm of the cylindrical cells of the midgut epithelium. The view was unanimously supported by recent workers (Krishnaswami et al 1978, Ullal and Narasimhanna 1978). The incubation period for manifestation of the disease is largely dependent on the temperature. 'With constant temperature and smaller inoculation longer is the incubation period, but with constant inoculum and high temperature shorter is the incubation period' (Aizawa 1967). Kawarabata and Aratake (1978) studied that the per oral infection of silkworm nuclear polyhedrosis was due to viron with an envelop, but the haemocoelic infection was due to viron without an envelop.

The Moratorvirus, as the etiogenic agent for infectious flacherie was detected by Aizawa et al (1964). They found that young larvae were more susceptible to per os infection which was decreased in 4th and 5th stages. Further Matsumoto et al (1985) showed that the susceptibility of infectious flacherie virus-infected larvae increased in presence of Streptococcus faecalis and Serratia marcescens. The intestines of larvae infected with infectious flacherie contained large number of these bacteria. S. faecalis, which is the main component of intestinal microflora

of the healthy silkworms, showed higher virulence of infectious flacherie when associated with S. marcescens. Indian sericulturists (Krishnaswami et al 1978, Samson 1983) described a separate disease 'gattine' having relatively the similar symptoms of infectious flacherie. Gattine disease was claimed to be caused by a non occluded gattine virus combined with Streptococcus bombycis, the later being secondary invader. In the present study, infected worms having symptoms of gattine when examined, neither polyhedra nor Streptococcus could be observed. However, reports on this gattine disease from other countries dealing sericulture are not available. But the symptoms of infectious flacherie in many ways, show similarities with the gattine disease.

Viral diseases of Silkworm in Bangladesh.

Section - C

Incidence of Bacterial & Viral diseases of Silkworm in Bangladesh.

CHAPTER 8.

INTRODUCTION

The weather conditions of Bangladesh and the present rearing practices of silkworm largely favour the outbreak of both bacterial and viral diseases. Most of the private farmers do not have separate and ideal rearing house for silkworms. They are not trained and use the fore-father's age old techniques. As such reduction of average cocoon yield at 40-50% occurs during two seasons of Jaistha and Bhaduri (Barman 1986).

Silkworm crop losses occur in all the silkworm rearing areas of the world, but their kind and severity vary. The incidence of different diseases differs from region to region, crop to crop and even farmer to farmer in the same crop. Leaving aside minor variations it has been found that crop losses are generally more in the tropics than in the temperate region (Sengupta 1988), which showed an annual loss due to diseases at around 30% and another 5% to 10% due to pests. This is also not uncommon that out of four crop harvests during a year, a farmer may fully fail and another partly. Whereas in temperate region, the total crop loss during a year hardly exceeds 10%. For instance, in Japan current average annual loss is around 7.5% only (Sengupta 1988). In China it was estimated that about 70-80% of the total loss of silkworm was due to viral diseases. A laboratory investigation carried out during 1983 suffered about 21.3% larval mortality due to viral disease, 23.0% due to bacterial and 30.0% due to physiological ailments of the total mortality (Barman and Pasha 1986).

accounted in a year. A number of laboratory as well as field results on the incidence of bacterial and viral diseases of silkworms are available from India (Sidhu and Singh 1968, Chitra et al 1975, Anon 1983 and Saratchandra 1988) showing different degrees of frequencies in their individual investigation.

CHAPTER 9.

MATERIALS AND METHODS

Incidence of the bacterial and viral diseases was carried out under the laboratory conditions. In rearing silkworms, scientific methods for disinfection, sanitation, feeding, bed cleaning, spacing and care at moulting were strictly followed to the recommendation of Krishanaswami *et al* (1978). The experiment was conducted in two different sets:- 1) Monthwise covering 10 months of the year from February 1985 to November 1985 and 2) Commercial season wise- covering 4 seasons namely, Jaistha (May-June 1985, Bhaduri: (August-September 1985), Agrahayani (October-November 1985) and Chaitra (February-March 1986). For the first experiment a widely used popular local race, Nistari(M) was taken. But for the second experiment, five commercial multivoltine races namely, BSR-3, M_2P_2 , Nistari(S), Nistari (M) and BSRI-801 were employed for all the seasons so as to achieve an accurate effect of the concerned season.

Three replications with 300 silkworms in each were considered on the first day of 3rd instar for both the experiments. Observations on diseased worms were carefully recorded everyday at bed cleaning from 3rd instar to spinning, mainly on the basis of physical symptoms (only confused ones were tested under microscope at 600X). Both the experiments were conducted inside a brick made pakka building and hardboard bottomed wooden rearing trays (75 cm x 50cm dia) were used. Finally the data were processed and analysed for further interpretation. Statistical methods were followed from the procedures described by Shukla and Gulshan (1986) and Gupta (1986).

CHAPTER 10

OBSERVATIONS

10.1. Incidence under the influence of different months of the year:

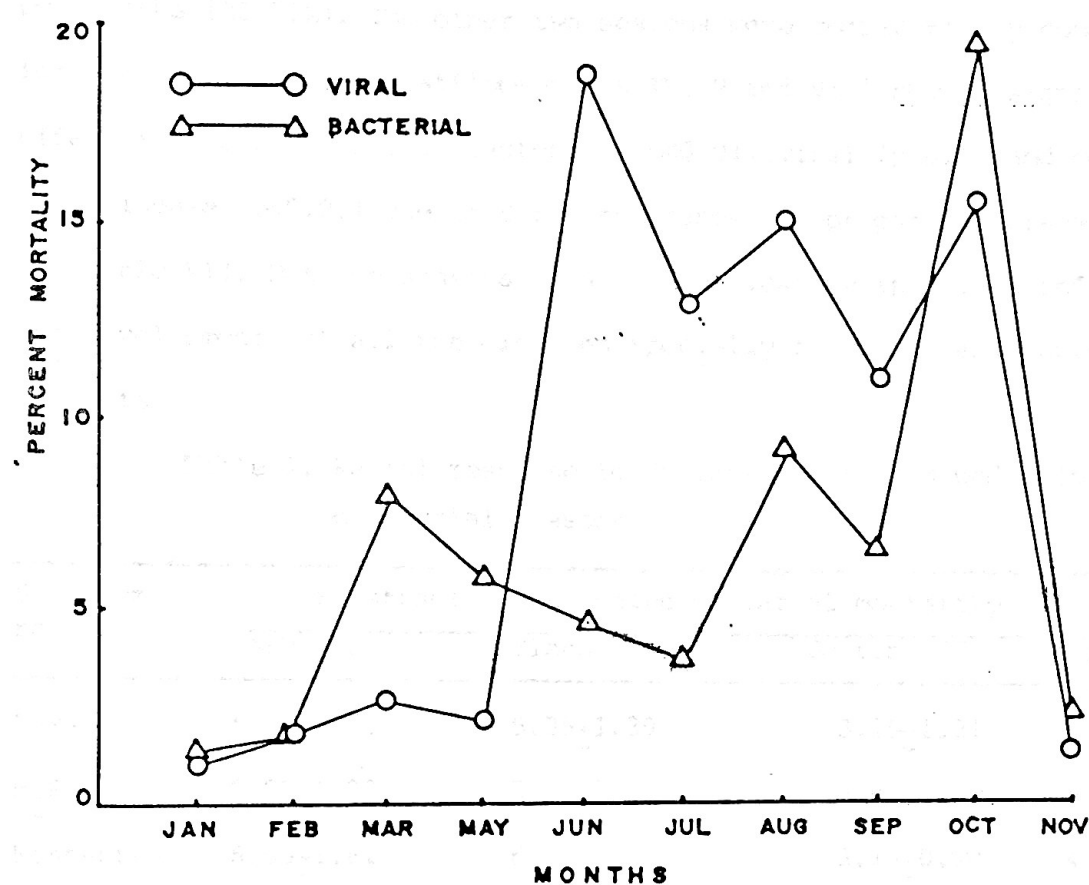
Monthwise incidence of bacterial flacherie and viral diseases could be seen in Fig 22 which reflects highest bacterial disease incidence during the month of October (19.8%) followed by August (9.2%), while the minimum incidence was observed during the period from November to February (1.3% to 2.3%). But the maximum incidence of viral diseases was observed in June (19.0%) followed by October (15.7%), August (15.2%) and July (13.0%). Again the period from November to May showed lower incidence of viral disease (1.0% - 2.7%).

Environmental effect of different months differed significantly ($p < 0.01$) for the incidence of both the diseases. The interaction between months and diseases were also highly significant ($p < 0.01$) as shown in Appendix table I.. Correlation and regression analysis of the two diseases on the prevailed temperature and relative humidity showed that the viral diseases are highly correlated (Appendix table III) with these two factors which are highly significant ($p < 0.01$) for their incidence. Although these two factors showed positive correlation for bacterial incidence, it was insignificant meaning that there might be some other factors responsible for the outbreak of bacterial disease (Appendix table II).

10.2. Incidence under the influence of different commercial seasons:

Mean larval mortality in five different silkworm races due to nuclear polyhedrosis, flacherie, gattine and deficiency diseases under

Fig. 22. Incidence of bacterial and viral diseases of silkworm under the environment of different months.



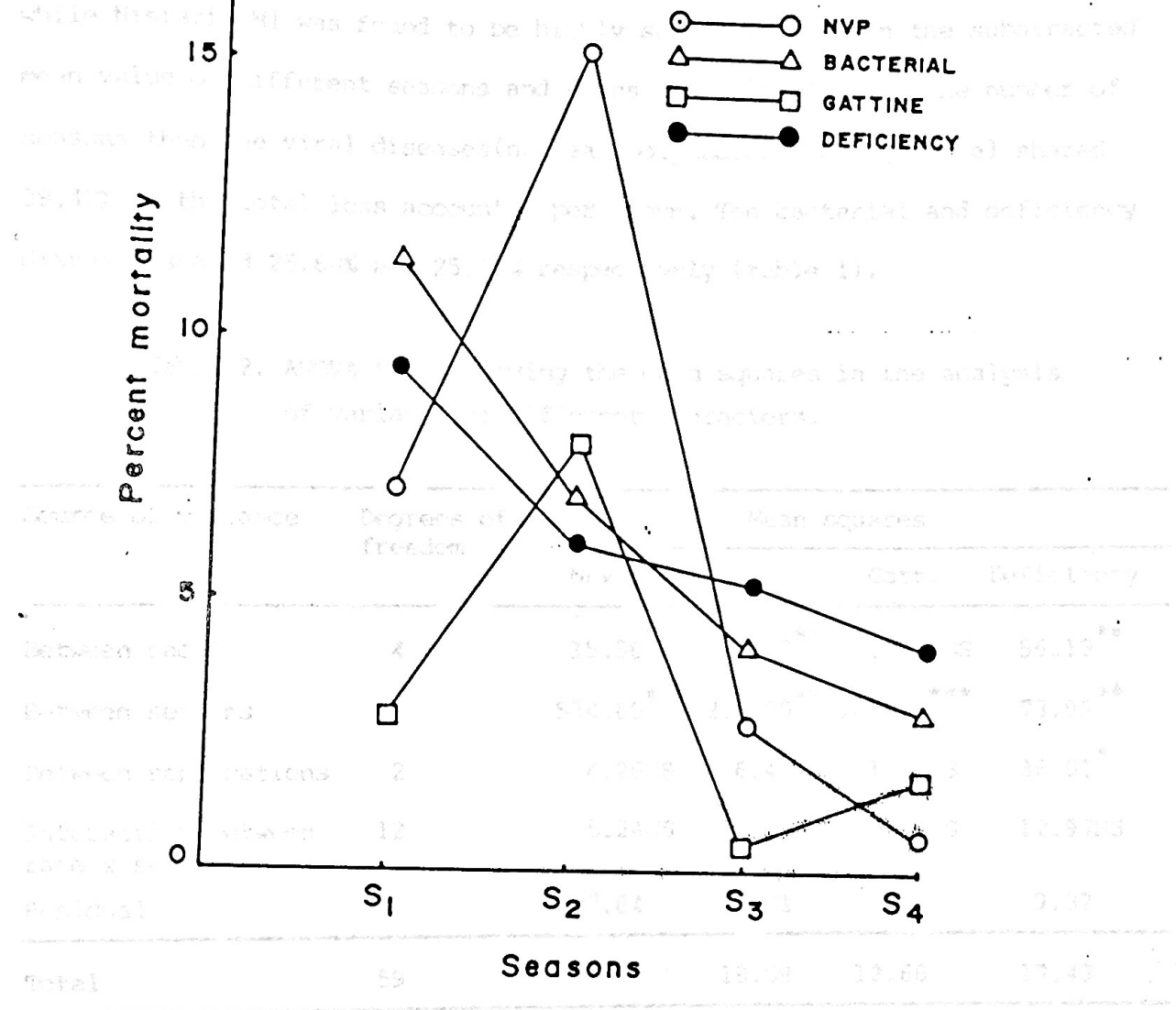
the influence of four commercial seasons of Bangladesh is presented in table I. As indicated in the Fig 23 : that Jaistha season induces higher mortality due to bacterial infection and deficiency disease. But Bhaduri was responsible for the highest incidence of nuclear polyhedrosis, this season also resulted highest total larval mortality (36.70%) followed by Jaistha (30.51%). The other two seasons were comparatively congenial for all the races. F- test (Appendices IV, V and VII) showed significant effect in the incidence of bacterial ($p < 0.01$), viral ($p < 0.01$) and deficiency disease ($p < 0.01$) due to different races except gattine disease (Appendix table VI). But the seasons always significantly ($p < 0.001$) influenced the development of all the diseases specially flacherie and nuclear polyhedrosis.

Table 1. Racial response to different diseases under four commercial seasons.

Silkworm race	Substracted mean value of larval mortality (%)			
	NPV+ SE	Flacherie+ SE	Gattine+SE	Deficiency+SE
BSR-3	5.26+1.70	5.39+1.39	3.16+1.31	6.14+1.08
M ₂ P ₂	5.89+1.92	5.42+1.19	2.91+0.96	4.25+1.11
Nistari(S)	6.03+1.81	6.07+1.08	3.19+0.90	4.63+0.92
Nistari(M)	8.26+1.79	8.10+1.51	4.50+1.22	9.68+1.25
BSRI-801	6.55+1.73	7.05+0.99	2.39+0.68	6.84+1.12
Mean	6.39	6.40	3.23	6.30
SEM	2.86	2.87	1.44	2.82
Total mor- tality/annum	25.56	25.60	12.92	25.20
Lsd($p < 0.05$)	1.61	1.58	1.64	2.51

Fig. 23. Incidence of bacterial and viral diseases of silkworm under different commercial rearing seasons.

Partial response to different diseases over of four commercial seasons is presented in Fig. 24 which showed that H₂P₂ and BSK-3 races were comparatively less susceptible to both the diseases caused by bacteria and viruses, while the H₁ was found to be highly susceptible to both the diseases. The mean values of different seasons and races are given in Table 1. The incidence of bacterial and viral diseases in the four seasons is shown in Fig. 23. The bacterial and deficiency diseases accounted for 75% of the total loss in the four seasons. The bacterial and deficiency diseases accounted for 75% of the total loss in the four seasons.

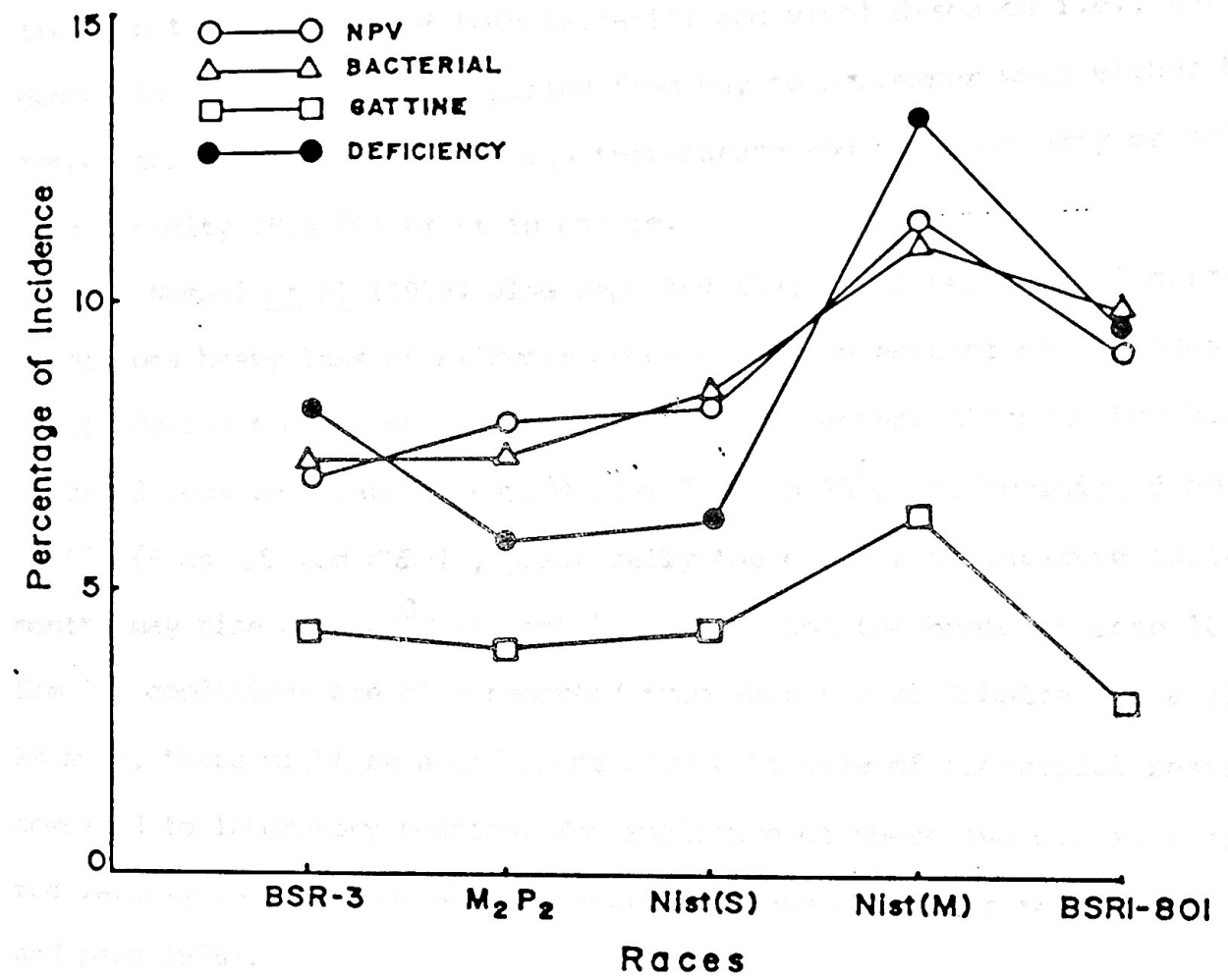


Racial response to different diseases out of four commercial seasons is presented in Fig. 24 which showed that M_2P_2 and BSR-3 races were comparatively less susceptible to both the diseases caused by bacteria and viruses, while Nistari (M) was found to be highly susceptible. When the subtracted mean value of different seasons and races was multiplied by the number of seasons then the viral diseases (nuclear polyhedrosis and gattine) shared 38.48% of the total loss accounted per annum. The bacterial and deficiency diseases shared 25.60% and 25.20% respectively (table 1).

Table 2. ANOVA table showing the mean squares in the analysis of variance of different characters.

Source of variance	Degrees of freedom	Mean squares			
		NPV	Bact.	Gatt.	Deficiency
Between races	4	15.56 ^{**}	16.19 ^{**}	7.32 NS	56.13 ^{**}
Between seasons	3	534.60 ^{***}	214.50 ^{***}	167.94 ^{***}	73.95 ^{**}
Between replications	2	4.26NS	6.43NS	1.12NS	36.01 [*]
Interaction between race x season	12	5.24NS	19.53 ^{**}	4.99NS	12.97NS
Residual	38	3.84	3.71	3.99	9.32
Total	59	37.01	18.58	12.66	17.43

Fig. 24. Racial response to different diseases under different commercial rearing seasons.



In Karnataka State, bacterial infection is reported to be responsible for a heavy toll in commercial rearing, the amount being largely varied in the observations of different workers. In the observations of Srinivas Rao (1950), this disease took about 30% of the total loss. In another investigation Srinivas Rao (1950) found 20-47 bacterial incidence, but Sankarshankar (1954) reported 15.5% incidence in commercial rearing.

CHAPTER 11

DISCUSSION

The experimental findings of both the experiments showed a definite trend in the incidence of both bacterial and viral diseases i.e., the incidence was higher during the period from May to September when either high temperature (Fig 25) or both high temperature and high humidity or only high humidity (Fig 26) exist in nature.

Nomani et al (1979) also reported that under West Bengal climatic conditions heavy loss of silkworm crops occurs on account of diseases specially during the summer months - March to September. Although the highest recorded room temperatures varied from 30°C to 33°C and humidity from 87% to 99% (Figs 25 and 26), practically the outdoor temperature during these months may rise upto 40°C in certain periods and the humidity upto 100%. Similar conditions are also reported from West Bengal (Bhattacharya 1988). As such, there might be a different result in case of commercial rearing compared to laboratory rearing. The incidence of these two diseases differed largely in different sericultural zones and also year to year (Barman and Pash 1986).

In Karnataka State, bacterial flacherie is reported to be responsible for a heavy toll in commercial rearing, the incidence largely varied in the observations of different workers. As per observations of Sidhu and Sing (1968), this disease took about 70% of the total loss. In another investigation Chitra et al (1975) found 20-40% bacterial incidence, but Saratchandra (1988) reported 55.52% incidence in commercial rearing.

Fig.25. Mean room temperatures of different months studied over three years (1985-87).

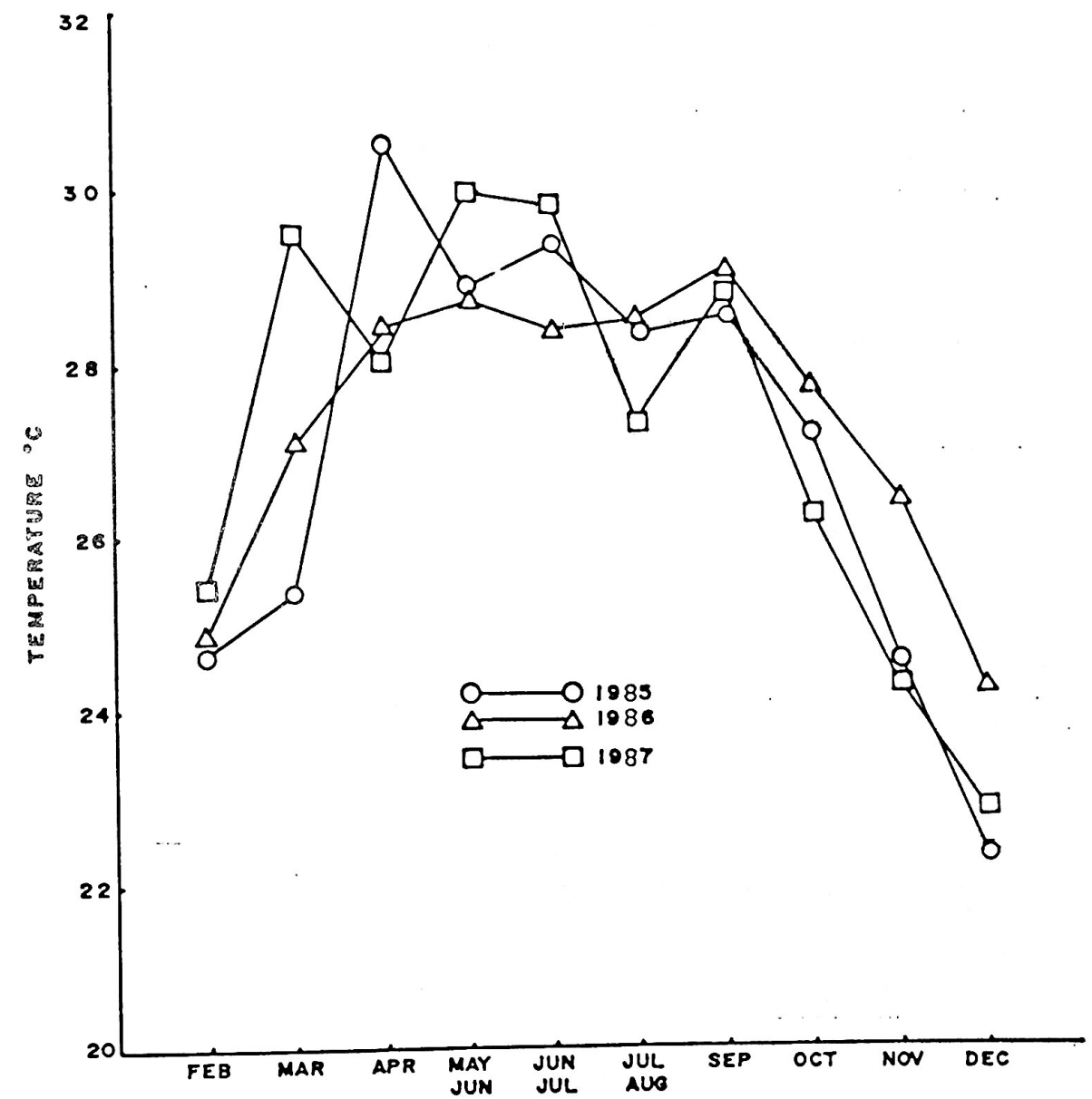
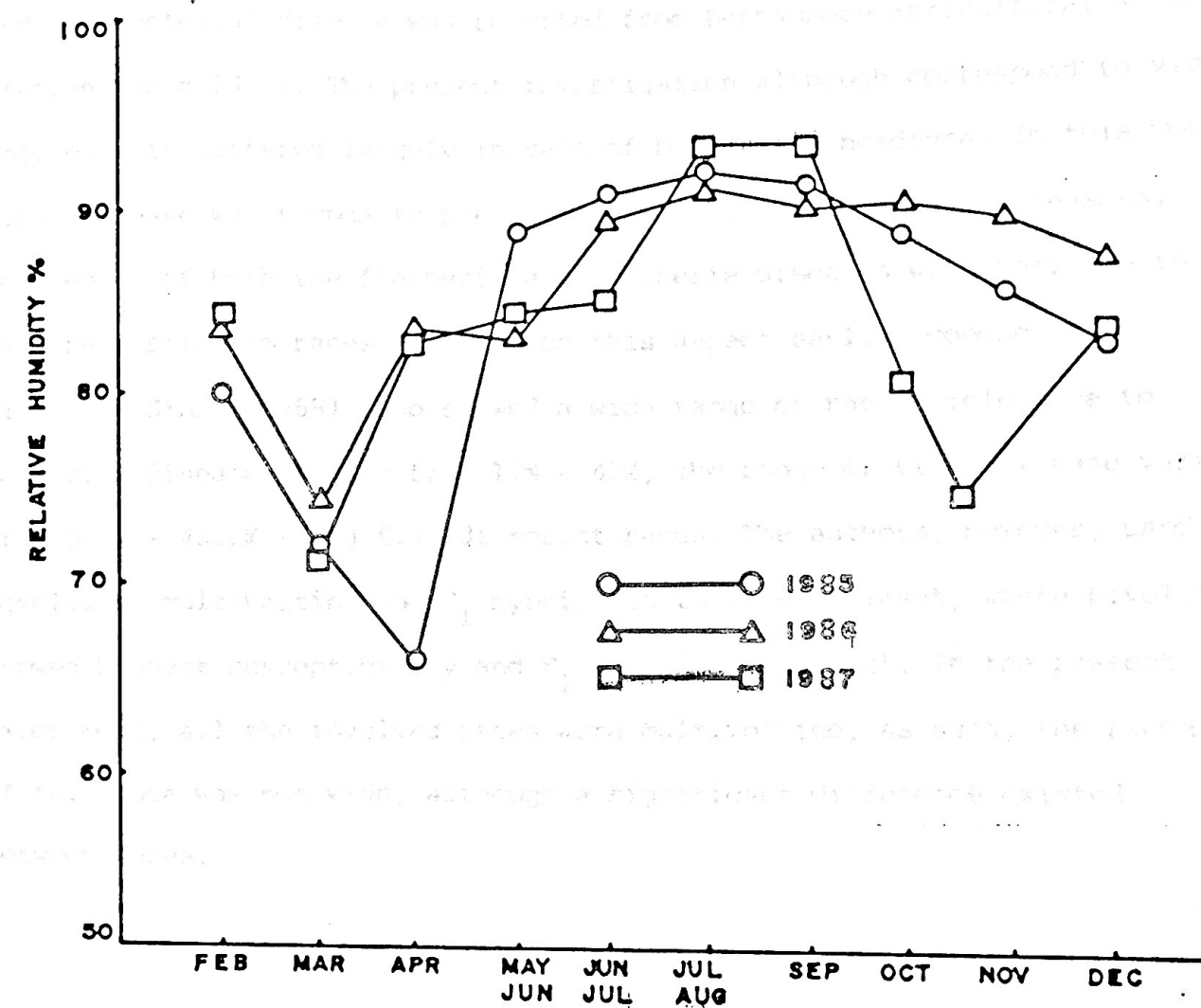


Fig.26. Mean room relative humidities of different months studied over three years (1985-87).



Similarly, a variable viral incidence of 15% (Sidhu and Singh 1968) and 27.75% (Saratchandra 1988) was also reported.

In laboratory conditions, a maximum incidence of 14% viral disease and 35% bacterial disease was reported from Berhampore Sericultural Research Station (Anon 1983). The present investigation although correspond to viral incidence it differed largely in case of bacterial incidence. In this work, viral disease was found to predominate in most of the rearing seasons. Occurrence of both the flacherie and grasserie diseases were variable in different silkworm races. A study on this aspect earlier conducted by Sidhu and Singh (1968) also showed a wide range of racial tolerance to bacterial disease ranging from 15% - 43%, the range of viral disease varied from 6.5% - 45.5% among five different races. The authors, however, used bivoltine, multivoltine and F_1 hybrids in their experiment, where bivoltine showed highest susceptibility and F_1 hybrids the lowest. In the present experiment, all the involved races were multivoltine, as such, the range of tolerance was not wide, although a significant difference existed between races.

Section—D

Effect of different Ecological factors for prevalence of Bacterial & Viral diseases.

Temperature of the rearing room plays the most vital role in the growth of silkworms. Because of cold blooded nature silkworm possesses a short tolerance range to temperature fluctuation. With the rise in temperature, higher degree of metabolism are accelerated, while they are slowed down as the temperature goes down. Similarly, higher temperature accelerates larval growth while low temperature slows down. Most of the silkworms rearing silkworms dial-out the optimum temperature range for maximum growth of silkworms between 22°C and 28°C (Yamagata 1932, Kishimoto et al 1970, Ullal and Katarikamma 1978 and Mahaveera 1981). The young silkworms (1st - 3rd instar) prefer comparatively higher temperature

CHAPTER-12

INTRODUCTION

Silkworms are, by nature, quite delicate and very sensitive to environmental conditions. The most important environmental factors influencing the cocoon crops are the atmospheric temperature and humidity prevailing at the time of silkworm rearing, quality of mulberry leaves fed and finally the overall management systems including method of feeding, bed cleaning, care at moulting, spacing etc. Silkworms of different races as well as developmental stages respond variously to different environmental factors; even in the same locality and from rearer to rearer. However, the adverse conditions always produce injurious effect on silkworm affecting physiology and growth and thereby incurring loss of cocoon crop.

Temperature of the rearing room plays the most vital role in the growth of silkworms. Because of cold blooded nature silkworm possesses a short tolerance range to temperature fluctuation. With the rise in temperature, the metabolic activities are accelerated, while these are slowed down as the temperature goes down. Similarly, higher temperature quickens larval growth while low temperature shortens. Most of the scientists dealing silkworms chalked out the optimum temperature range for the normal growth of silkworms between 20°C and 28°C (Yokoyama 1962, Krishnaswami et al 1978, Ullal and Narasimhanna 1978 and Mahadevappa 1986). The young silkworms (1st - 3rd instar) prefer comparatively higher temperature

(26 - 28⁰C) and the late age worms comparatively lower temperature (23-25⁰C). Temperature above 30⁰C is positively harmful when the temperature below 20⁰C considerably slows down the physiological activities rendering the silkworms weak and susceptible to diseases (Watanabe 1964, Ullal and Narasimhanna 1978). Sudden change of temperature is dangerous for silkworms, especially for late age worms.

Humidity of the rearing room is another important factor in silk-worm rearing. The combined effect of both temperature and humidity largely determines the satisfactory growth of the silkworms and also the success of cocoon crops. Humidity directly influences the physiological functions. The optimum room humidities detected are 80-85% for young stages and 70-75% for late ages (Krishnaswami et al 1978). Chitra et al (1975) observed that the bacterial diseases take a heavy toll specially under conditions of high humidity. Chinese Sericulturists (Anon) are of opinion that cytoplasmic polyhedrosis and infectious flacherie tend to occur in summer which is related to high temperature, but greater incidence of nuclear polyhedrosis in the spring has relations with the humidity. Environmental high temperature and high humidity are most favourable to the propagation of bacteria and viruses, and so these diseases occur chiefly in rainy and cloudy seasons.

The mulberry leaf is the only food of silkworm, Bombyx mori L. the quality of which is influenced by various factors such as nature of the soil,

* The book published by Regional Sericulture Training Centre, Guanzhou, China. Year not mentioned.

rainfall and cultural practices. The carbohydrate, protein, fibre and water content are varied in different ages of leaves. Accordingly tender leaves are recommended for the early ages, while mature leaves for late age worms. The quality of mulberry leaves is affected to a large extent under adverse climatic conditions and also due to improper package of practices for it's cultivation. Feeding of such leaves causes nutritional deficiencies in silkworm and thereby leads to disease outbreak. Aruga (1958) found that when environmental conditions are not disturbed, quality of mulberry leaves markedly influence the appearance of polyhedral disease in silkworm from place to place. Aruga (1957) also observed that the main factor controlling the occurrence of midgut polyhedral disease in silkworm is the quality of mulberry leaves affected by high temperature and high humidity. Ono et al (1969) found that under high temperature and high humidity silkworms, fed with water immersed leaves, are largely affected by flacherie disease. When silkworms are provided only with tender or medium or mature leaves throughout larval duration under high temperature and high humidity, the effective rate of rearing goes down to only 4.74% to 5.94% irrespective of the types of mulberry leaves (Krishnaswami et al 1971). Chinese workers (Anon - year not mentioned) found that when spring and autumn crop worms are given immature leaves, then the mortality due to nuclear polyhedrosis goes up.

The traditional silkworm rearers do not follow any routine frequency in feeding silkworms. Some practise to provide mulberry leaves as much as six to eight times while some others two to three times in 24 hours.

The former practice is labour consuming and leads to wastage of mulberry leaves, while the later although is economic, leads to undernourishment of the silkworms. According to recent findings of sericultural scientists, it should be suffice if the worms are fed four times a day (Ullal and Narasimhanna 1978, Krishnaswami et al 1978) by adopting special care for retaining freshness of mulberry leaves. In the present investigation attempts are taken to find out the relationship of bacterial and viral diseases when deviations (± one feeding) are considered from the recommended normal feeding frequency (four feedings/24 hours).

Adequate spacing especially during late age worms provide orderly growth and successful cocoon crops. Silkworms is a very fast growing animal and records a 10,000 fold increase in weight and about 7,000 fold increase in size during the short span of larval period (Ullal and Narasimhanna 1978). At present, most of the silkworm rearers in the country use to practise overcrowd rearing either for want of rearing equipments or for conservation of mulberry leaves. Crowded condition leads to undernourishment and thereby uneven development of the silkworms. Also the microenvironment of rearing bed is largely affected leading to the outbreak of diseases. In the present study, relationship of different spacings with the incidence of bacterial and viral diseases is investigated.

Low temperature or refrigeration of silkworm larvae has an adverse effect promoting the growth of latent microorganisms present in the silkworm causing a disease. Some workers (Arugal 1957, Aruga and Arai 1959, Hukuhara and Aruga 1959) observed that refrigeration activate the latent virus

through a cold shock and thus cause polyhedrosis disease in silkworm, while others (Aizawa et al 1961, Sidhu and Singh 1968, Sidhu and Kapila 1969) could observe both the viral and bacterial diseases as a resultant effect of refrigeration.

Bacteria and virus germs are randomly present in the environment which are mostly carried with the mulberry leaves for creating disease in silkworms. Contaminated mulberry leaves are the main infecting source of most of the diseases of bacterial and viral origin except septicaemia which occurs mainly through wound infection. Mulberry leaves may also be contaminated with the faeces, stools, urines, passes or haemolymph of the infected silkworms. A number of reports are available on the nature and effect of artificially induced grasserie and flacherie diseases in the larvae of silkworms (Coba 1956, Aizawa et al 1973, Krishnaswami et al 1979 and Satish and Govindan 1986). A worthmentioning contribution in this issue comes from the study of Aizawa et al (1973), who studied several factors affecting the susceptibility of the silkworm to the per oral infections with nuclear polyhedrosis virus, cytoplasmic polyhedrosis virus as well as infectious flacherie virus. In the present study, emphasis was given to observe the incidence quantum of germ fed silkworms under different seasonal influence.

CHAPTER-13

MATERIALS AND METHODS

13.1. Determination of the effect of high temperature and high humidity.

This experiment was conducted during the month of September (1986). The recorded average room temperature of the month was 29.26°C (Maximum 30.83°C and minimum 27.69°C) and the relative humidity was recorded as 90.56% (maximum 95.29% and minimum 85.83%). Ten silkworm races and breeds used for commercial cocoon production were considered for the experiment. These were BSRI-801, BSRI-802, BSRI-83/1, BSRI-83/2, BSRI-83/3, BSR-3, SRI-Oval, BR/84, Nistari(S) and NanNong. All the races were mass reared upto 2nd instar. On the first day of 3rd instar they were replicated into three with 300 silkworms each which were selected randomly from the mass bed. Rearing of each lot was maintained strictly followed by the methods recommended by Krishnswami (1978). Observations on the bacteria and virus infected worms were made everyday at bed cleaning upto the period of mounting. Finally the data were summed up and analyzed.

13.2. Determination of the effect of different quality mulberry leaves.

Four unusual qualities of mulberry leaves viz. tender (T_1), mature (T_2) watersoaked (T_3) and one day preserved (T_4) with a control normal leaves (T_5) were fed to the silkworm throughout 3rd instar to 5th instar. The test material was a local race Nistari(S) and the experiment was re-

peated twice during the month of May-June (Average room temperature $30.16^{\circ}\text{C} \pm 3.95$ and relative humidity $84.72\% \pm 3.36$) and June-July (Average room temperature $30.05^{\circ}\text{C} \pm 0.90$ and relative humidity $85.11\% \pm 20.76$), 1987. Three replications each with 300 worms were maintained for each treatment. Incidence of both the bacterial and viral diseases were seperately noted everyday at bed cleaning from 3rd instar to spinning. Bed cleaning, spacing and other routine managements were equally provided to all the treatments.

13.3. Determination of the effect of different spacings.

Hardboard bottomed wooden trays of 75cm x 50cm x 7.5cm were used for rearing silkworms. A local race, Nistari(S) was considered for the experiment. Four spacings used were 300 worms ($1250\text{cm}^2/100$ worms), 400 worms ($937\text{cm}^2/100$ worms), 500 worms ($750\text{cm}^2/100$ worms) and 600 worms ($625\text{cm}^2/100$ worms) per tray. All the treatments were replicated into three individual batches. Normal feeding and managements were provided to all the batches. The experiment was repeated twice under the influence of two different seasons of May-June (S_1) and June-July (S_2), 1987.

13.4. Determination of the effect of different feeding frequencies.

Two deviations, 3 feedings/24 hour (8.0 AM, 4.0PM and 10.0PM) and 5 feedings/24 hour (8.0AM, 12.0 Noon, 4.0PM, 10.0PM and 4.0AM) were considered from the normal practice of 4 feedings/24 hour (10.0AM,

4.0PM, 10.0PM and 4.0AM). The two deviations correspond to the practice of many of the silkworm rearers in the country. Three replications were maintained for each treatment. Similar managements were provided to each batches. Observations on bacterial and viral incidence were recorded everyday during bed cleaning. The experiment was repeated in two different seasons (May-June and June-July 1987) with a local multivoltine race Nistari(S).

13.5. Determination of the effect of refrigeration.

To determine the effect of refrigeration on silkworm larvae in respect to the incidence of bacterial and viral disease, the experiment was conducted on the 3rd, 4th and 5th instar silkworms of Nistari(S), which were subjected to refrigeration at $5^{\circ}\text{C} \pm 1$ for 24 hours (Aruga and Arai 1959) in a cooled incubator just after ecdyses. The treatments were replicated each into three small paper trays with 300 silkworms. The refrigerated larvae resumed feeding after one hour of removal from the incubator. The experiment was repeated under the natural conditions of the months of April and June, 1985.

13.6. Determination of the effect of NPV germ feeding.

3rd instar silkworms of Nistari (S) race were fed with the suspension of nuclear polyhedra along with mulberry leaves for six generations during the months of June'85 (G_1), August'85 (G_2), October'85 (G_3) January'86 (G_4), March'86 (G_5) and May '86 (G_6). Before

feeding, the polyhedra were extracted and purified from the heavily NPV infected silkworm by clipping of caudal horns and prolegs and then repeated centrifugation (at 1800-2000 rpm for 20 minutes) in 25% and 50% sucrose solutions (Kawarabata and Matsumoto 1973). Finally the sediments were resuspended in sterilized distilled water. The polyhedral suspension bearing a fixed load of 120-150 polyhedra per microscopic field at 600X was smeared on the mulberry leaves to be fed to the moultout silkworms of 3rd instar. Polyhedra smeared leaves were fed only for six hours after which the worms resumed normal fresh leaves throughout the larval period. For each generation freshly prepared suspensions were used. Three replications (300 worms each) were maintained and a control batch with same number of worms and replications was reared side by side to justify the experiment. Observations on the mortality due to NPV, total mortality, average cocoon weight and average shell weight were noted sperately and analyzed.

13.7. Determination of the effect of bacteria germ feeding.

Infected silkworms having symptoms of bacterial flacherie were crushed in mortar-pestle and inoculated in the culture plates of Brain heart infusion agar medium, from which transfered to a liquid broth containing heart infusion agar and nutrient agar. The bacterial inoculum of a mixture of Streptococcus sp., Micrococcus sp., Bacillus sp. and Serratia marcescens was washed repeatedly in distilled water by centrifugation at 2500-3000 rpm for 10 minutes, then smeared on mulberry leaves

which were fed to the worms of 3rd instar for six hours, afterwards resumed normal leaves. The experiment was tried on a local race, Nistari(S) and repeated in four generations during the months of May, July, August and October 1987. A separate control batch was also maintained. Along with bacterial mortality, also the total loss and average cocoon weight were individually noted.

CHAPTER - 14.

OBSERVATIONS

14.1. Effect of high temperature and high humidity on the incidence of bacterial and viral diseases.

The effect of high temperature and high humidity under natural conditions of rearing on the incidence of flacherie and grasserie diseases and also on total mortality is shown in table-3.

Table 3. Mean performances showing the effect of high temperature and high humidity in different multivoltine races.

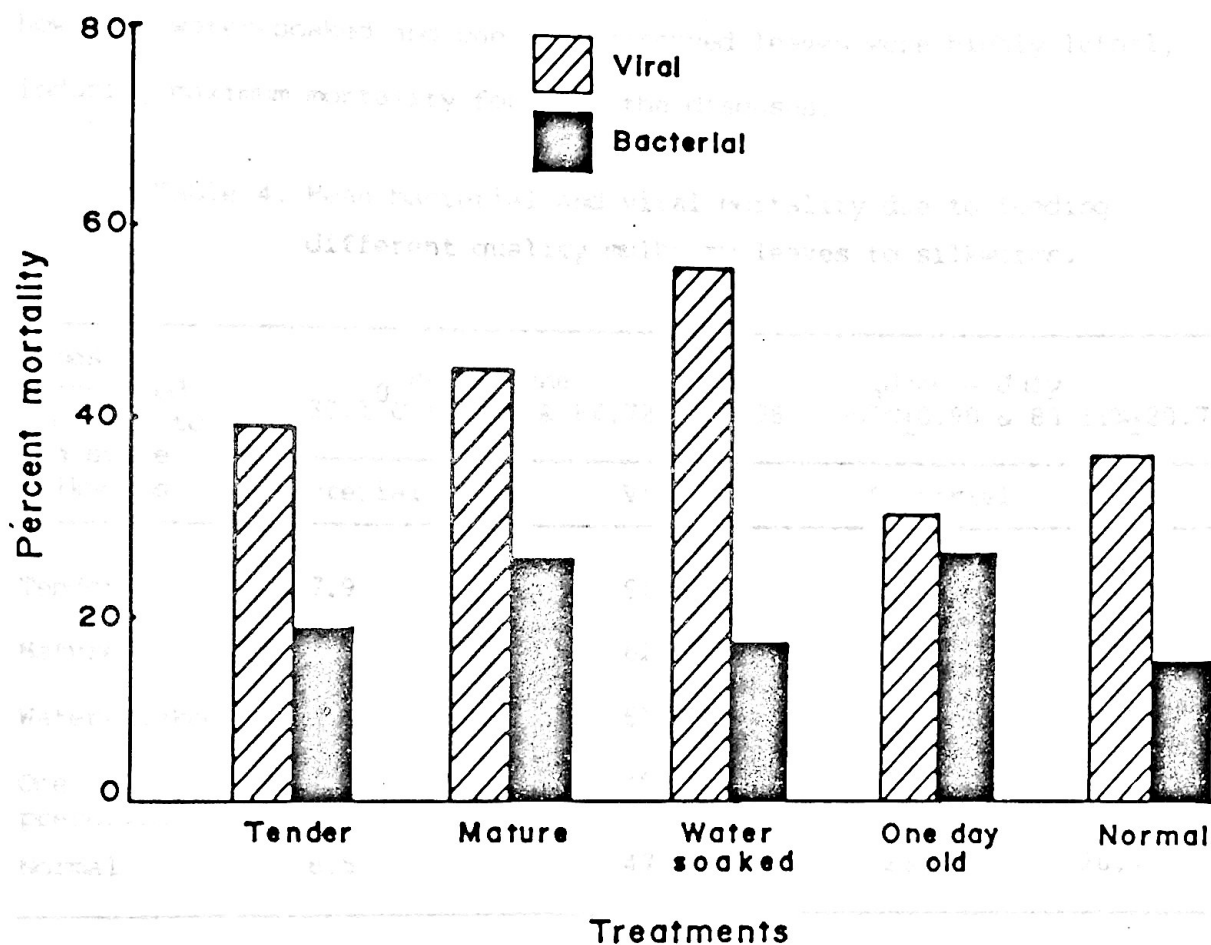
Silkworm races	Mean larval mortality %			Environmental index	
	Viral $\pm$ SE	Bacterial $\pm$ SE	Total $\pm$ SE	Av.Temp.	Av.R/H
BSRI-801	33.33 $\pm$ 1.51	5.00 $\pm$ 0.84	45.67 $\pm$ 1.25	29.26 ⁰ C $\pm$ 2.22	90.56% $\pm$ 6.68
BSRI-802	28.20 $\pm$ 0.58	6.57 $\pm$ 1.61	43.20 $\pm$ 2.39		
BSRI-83/1	20.20 $\pm$ 1.24	11.33 $\pm$ 3.70	35.43 $\pm$ 2.59		
BSRI-83/2	36.03 $\pm$ 5.73	15.20 $\pm$ 3.29	64.10 $\pm$ 9.45		
BSRI-83/3	29.10 $\pm$ 5.49	11.67 $\pm$ 1.62	54.53 $\pm$ 7.50		
BSR-3	14.20 $\pm$ 1.64	10.43 $\pm$ 0.58	36.77 $\pm$ 4.27		
SRI-oval	22.67 $\pm$ 4.46	9.77 $\pm$ 0.90	43.20 $\pm$ 2.54		
BR/84	26.23 $\pm$ 1.14	8.33 $\pm$ 0.49	41.43 $\pm$ 1.44		
Nistari(S)	19.00 $\pm$ 0.67	8.10 $\pm$ 1.24	36.00 $\pm$ 2.37		
NanNong	24.36 $\pm$ 5.10	30.33 $\pm$ 1.44	69.73 $\pm$ 6.77		
Mean	25.33	11.67	46.71		
Lsd(p<0.05)	8.86	4.49	11.85		

As it could be seen that both these factors affect differently on different silkworm races, which showed a highly significant difference in their mortality to both flacherie as well as grasserie diseases ($p < 0.001$). Mean viral mortality recorded from the silkworm races was 25.33% with the minimum of 14.20% in BSR-3 and maximum of 36.03% in BSRI-83/2. The resultant mean bacterial mortality was 11.67% with a minimum of 5.0% and maximum of 30.33%. The difference in the incidence of both the diseases ($p < 0.001$) and also the interaction between race and disease were also highly significant ($p < 0.01$) as shown in Appendix table VIII.

14.2. Effect of different quality mulberry leaves.

The effect of feeding tender, mature, water-soaked, one day preserved and normal mulberry leaves to the silkworm larvae and the resultant mortality due to bacterial and viral diseases are shown in Fig 27 & Table 4. Highest bacterial mortality was observed in case of feeding one day preserved mulberry leaves (26.46%) followed by mature leaves (24.95%). On the other hand, highest mortality due to viral diseases occurred due to feeding water-soaked leaves (56.16%) followed by mature leaves (45.60%). But the normal leaves and one day preserved leaves resulted the lowest bacterial (15.76%) and viral (30.45%) diseases respectively. In either of the cases, tender leaves had no significant effect. However, the effect of the treatments was highly significant ($p < 0.001$) for both bacterial and viral incidence (Appendices IX and X).

Fig. 27. Effect of different quality mulberry leaves on the incidence of bacterial and viral diseases of silkworm.



14.3. Effect of different spacings on bacterial and viral incidence.

The effect of different spacings on the incidence of bacterial and viral diseases is shown in Fig 28 which indicated that crowded spacing i.e., reduced spacing of $825\text{cm}^2/100$ worms caused highest mortality.

The treatments showed a highly significant ($p < 0.001$) influence under different seasons studied. Wide temperature fluctuations during May-June is supposed to accelerate viral mortality, while the wide humidity fluctuations during June-July is supposed to accelerate bacterial mortality when accompanied by the treatments. In both the seasons however, water-soaked and one day preserved leaves were highly lethal, inducing maximum mortality for both the diseases.

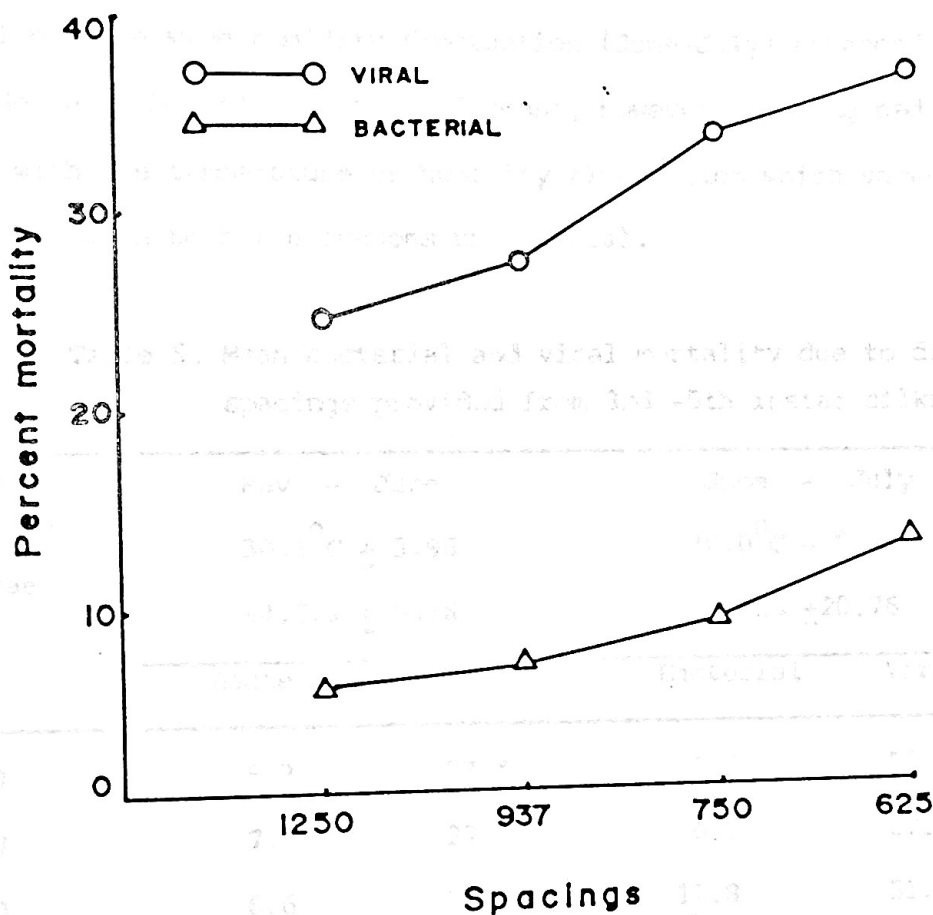
Table 4. Mean bacterial and viral mortality due to feeding different quality mulberry leaves to silkworms.

Types of leaves fed from 3rd to 5th stage silkworms	May - June $30.1^{\circ}\text{C} \pm 3.96$ & $84.72\% \pm 3.36$		June - July $30^{\circ}\text{C} \pm 0.90$ & $85.11\% \pm 20.76$	
	Bacterial	Viral	Bacterial	Viral
Tender	7.9	51.0	28.7	27.2
Mature	13.9	62.2	36.0	29.0
Water-soaked	9.4	61.3	24.3	51.0
One day preserved	17.4	46.6	35.5	14.3
Normal	8.5	47.7	23.0	26.7

14.3. Effect of different spacings on bacterial and viral incidence.

The effect of different spacings on the incidence of bacterial and viral diseases is shown in Fig 28 which indicated that crowded rearing i.e. reduced spacing of $625\text{cm}^2/100$ worms caused highest mortality

Fig. 28. Effect of different spacings on the incidence of bacterial
and viral diseases of silkworm.



Regression analysis between spacings and diseases showed highly significant ($p < 0.001$) positive correlations (Figs 2 and 3) for both the cases, i.e. higher is the spacing lower is the incidence and vice versa. The relationship was also significant.

both due to bacterial (13.05%) as well as viral (37.08%) diseases, while sparse rearing i.e. extended spacing of $1250\text{cm}^2/100$ worms resulted the lowest. Incidence of both the diseases significantly ($p < 0.001$) varied among different spacings (Appendices XI and XII). Seasons significantly influenced the bacterial mortality ($p < 0.001$), specially with reduced spacings of 750cm^2 and $625\text{cm}^2/100$ worms. That is, crowded rearing combined with wide humidity fluctuation (June-July) enhanced bacterial incidence (Table 5). For viral disease, however, spacing had no relationship with the temperature or humidity fluctuation which showed almost *similar* same value in both the seasons under trial.

Table 5. Mean bacterial and viral mortality due to different spacings provided from 3rd -5th instar silkworms.

$\text{cm}^2/100$ 3rd-5th instar larvae	May - June		June - July	
	$30.1^{\circ}\text{C} \pm 3.96$		$30.0^{\circ}\text{C} \pm 0.90$	
	$84.72\% \pm 3.36$		$85.11\% \pm 20.76$	
	Bacterial	Viral	Bacterial	Viral
1250	5.2	23.4	6.6	26.1
937	7.1	27.6	6.8	27.0
750	6.6	36.8	11.8	31.4
625	7.6	38.5	18.5	35.6

Regression analysis between spacings and diseases showed highly significant ($p < 0.001$) negative correlations (Figs 29 and 30) for both the cases, i.e. higher is the spacing lower is the incidence and vice versa. The relationship was almost linear.

Fig. 29. Regression line showing relationship of bacterial disease (Y) on different spacings(X).

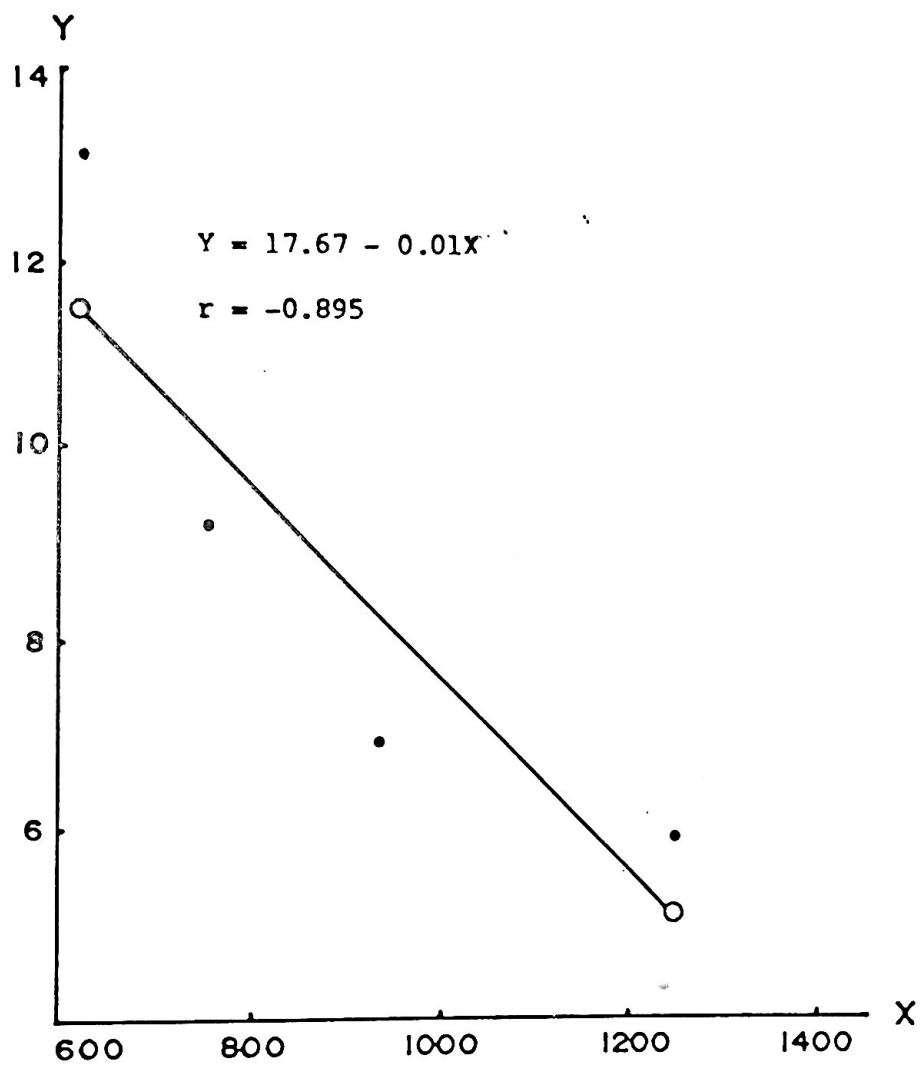
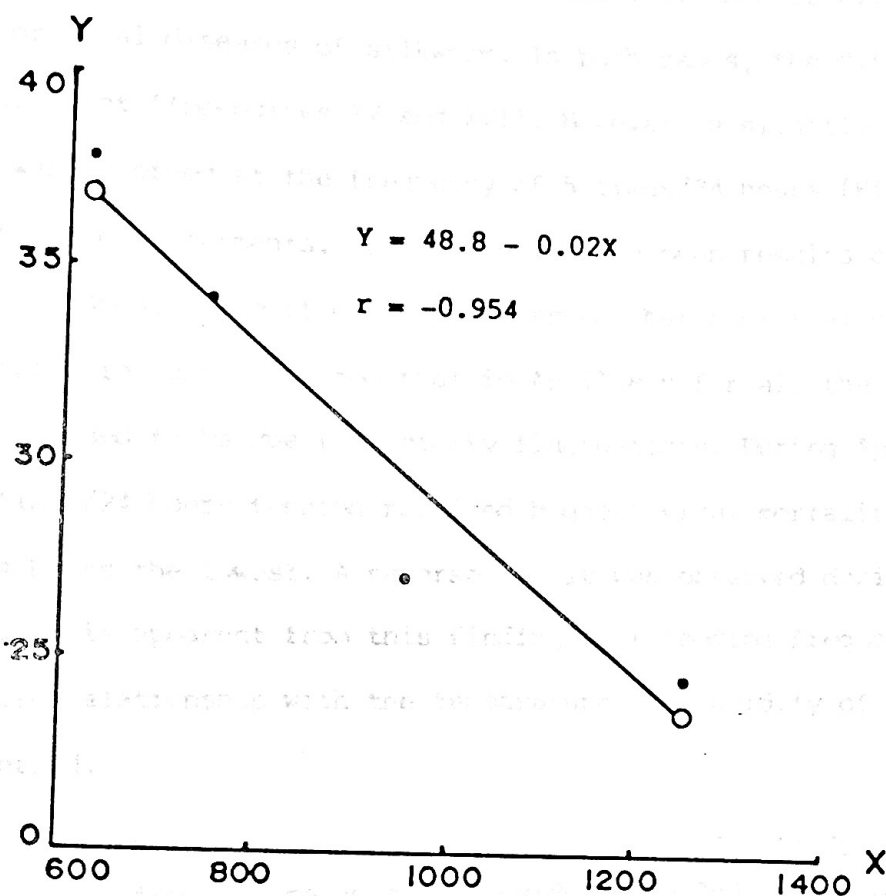


Fig. 30. Regression line showing relationship of viral disease(Y)
on different spacings(X).



Number of Insects	May - June		June - July	
	Mean	Std. Dev.	Mean	Std. Dev.
24 hours	30.1 ¹¹ ± 1.94	11.77 ± 1.94	37.0 ⁶ ± 1.70	12.16 ± 1.70
3	4.3	43.7	11.5	1.3
4	4.0	48.3	14.3	2.8
5	8.3	32.5	17.7	40.8

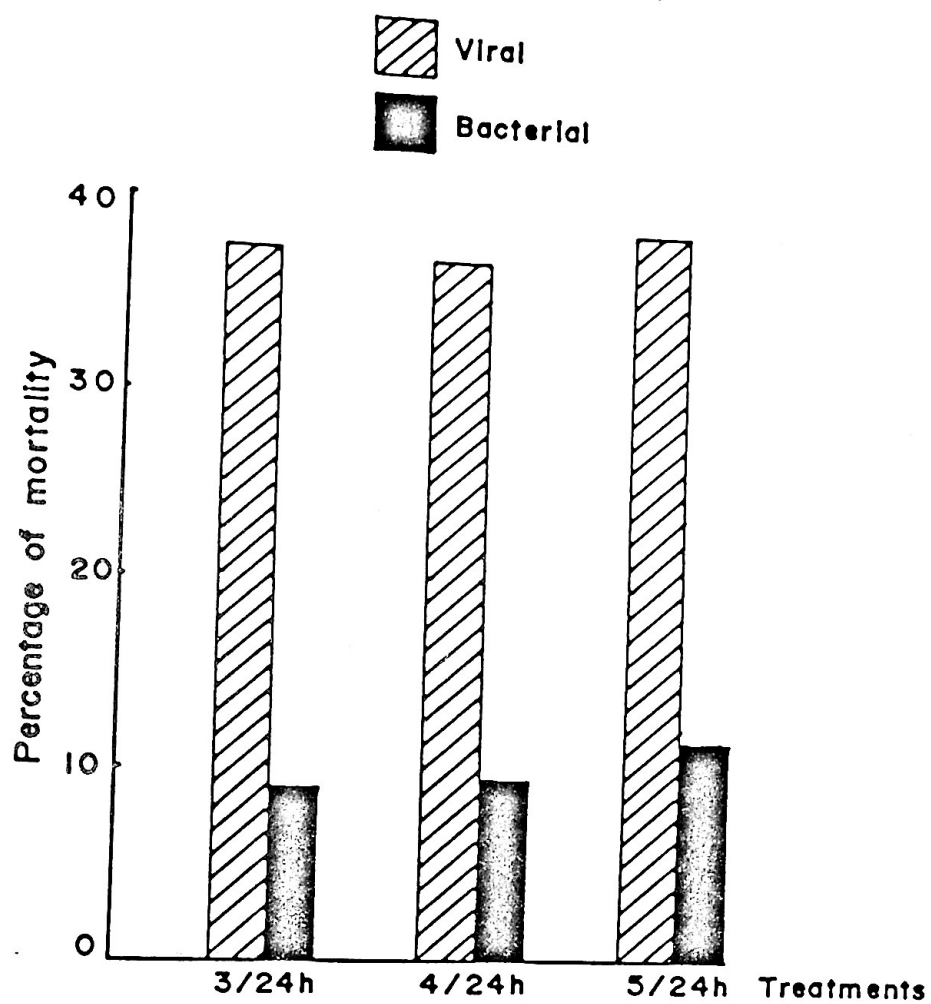
14.4. Effect of feeding frequencies on bacterial and viral incidence.

The deviation of feeding frequencies from the normal practice did not have any impact or relationship on the incidence of either bacterial or viral diseases of silkworm. In both cases, the difference was insignificant (Appendices XV and XVI). However, a slightly higher mortality was recorded at the frequency of 5 times/24 hours (Fig 31) compared to other treatments. Table 6 showed the mean results of two consequent seasons, where it could be observed that bacterial mortality largely varied in June-July from that in April-May for all the treatments, which is supposed to be due to humidity fluctuations. During April-May season 3 times/24 hours feeding resulted highest viral mortality and 5 times/24 hours the lowest. A reverse result was observed during June-July. It is apparent from this finding that feeding frequencies have a close relationship with the temperature and humidity of the rearing period.

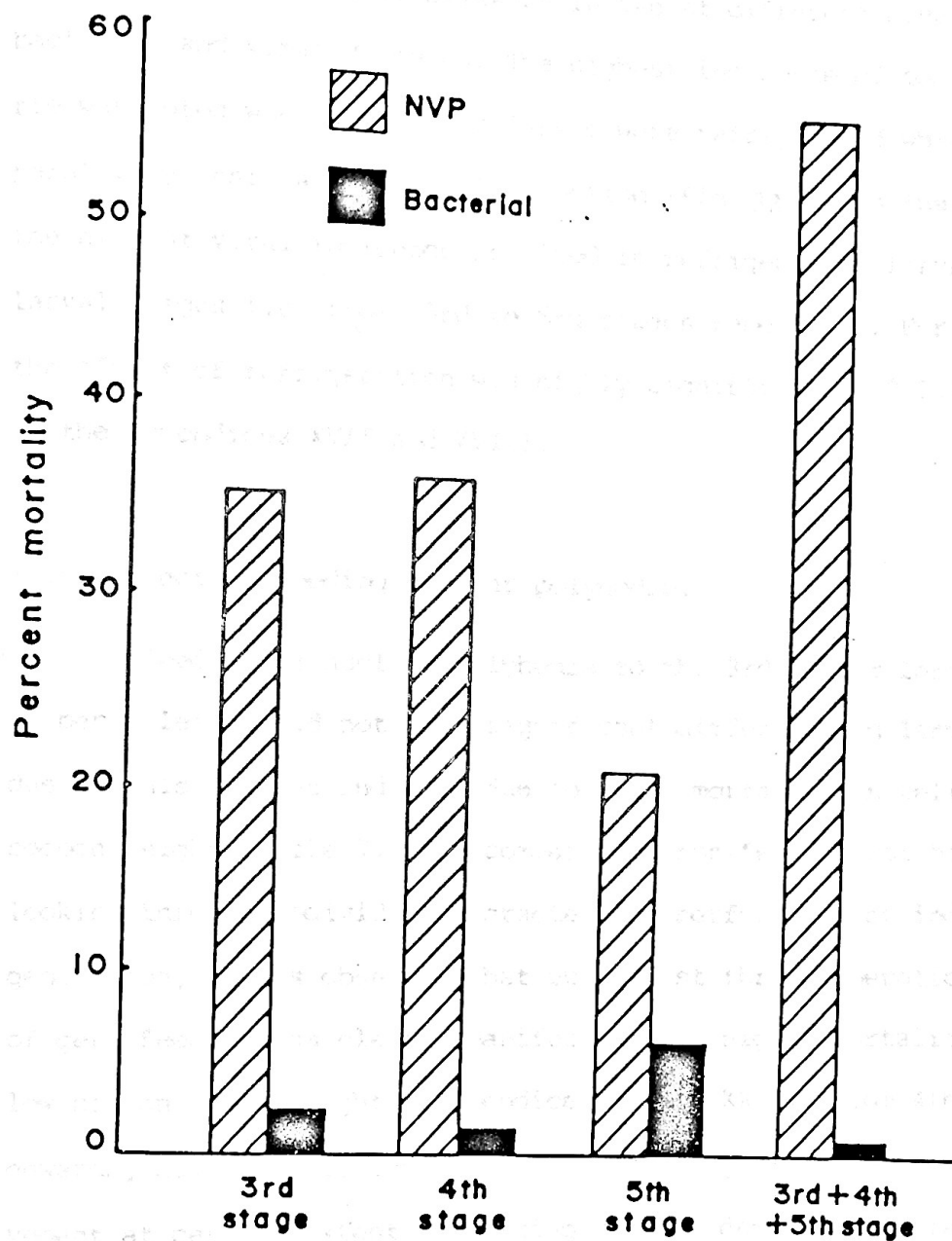
Table 6. Mean bacterial and viral mortality due to different feeding frequencies provided from 3rd - 5th instar silkworms.

Number of feeding/ 24 hours	May - June		June - July	
	$30.1^{\circ}\text{C} \pm 3.96$		$30.0^{\circ}\text{C} \pm 0.90$	
	84.72% $\pm$ 3.36		85.11% $\pm$ 20.76	
	Bacterial	Viral	Bacterial	Viral
3	6.3	43.1	11.5	31.5
4	4.0	38.5	14.3	35.8
5	8.3	32.5	14.2	44.5

Fig. 31. Effect of feeding frequency on the incidence of bacterial and viral diseases of silkworm.



.Fig. 32. Effect of refrigeration at different larval instars
on the incidence of bacterial and viral diseases.



14.5. Effect of refrigeration on the incidence of bacterial and viral diseases.

Refrigeration of silkworm larvae at different ages induced both bacterial and viral diseases. The highest incidence of bacterial flacherie was noted when 5th instar larvae were refrigerated which was comparatively resistant to viral induction (Fig 32). On the other hand, the highest viral incidence resulted in refrigerating larvae in all the larval stages i.e. from 3rd to 5th stages repeatedly. For both the cases, the effect of refrigeration was highly significant ($p < 0.001$) as analysed in the Appendices XVII and XVIII.

14.6. Effect of feeding nuclear polyhedra.

Feeding of nuclear polyhedra to the 3rd instar larvae along with mulberry leaves did not show significant difference in larval mortality due to this disease and also due to total mortality as well as average cocoon weight (Table 7) when compared to non-fed control batch. But looking through individual character and performance of individual generation, it was observed that upto first three generations the effect of germ feeding was clearly manifested with higher mortality rate and low cocoon shell weights (Appendices XIX to XXII). From 4th generation onwards, however, all the characters in the germ fed batch showed improvement at certain extent indicating certain degree of resistance to the disease. Both the treated and non treated batches were similarly influenced by the environmental conditions of each generation.

Total mortality 4.59 ± 4.06 31.75 ± 3.45

Avg. cocoon weight 218.79 ± 38.28 211.71 ± 31.26

Table 7. Effect of nuclear polyhedra feeding on different characters of silkworms.

Characters	NP Fed	Non - fed	Value of 't'
	Mean $\pm$ SE	Mean $\pm$ SE	
NPV mortality	12.95 $\pm$ 2.95	11.02 $\pm$ 2.41	0.83 NS
Total mortality	20.46 $\pm$ 4.13	21.83 $\pm$ 4.82	0.46 NS
Av. cocoon weight	955.05 $\pm$ 29.25	955.83 $\pm$ 25.00	0.105 NS
Av. shell weight	136.11 $\pm$ 5.50	134.66 $\pm$ 3.51	0.483 NS

14.7. Effect of feeding bacteria germs.

Feeding of bacterial pathogens along with mulberry leaves to 3rd instar silkworms did not induce the disease throughout the experiment in four different seasons. Mean larval mortality and total mortality of the germ fed batch (Table 8) showed insignificant difference (Appendices XXIII and XXIV) when compared to the nonfed control batch. Average cocoon weight, however, showed better value over the control and differed significantly ($p < 0.05$). Both the treated and non-treated batches were equally influenced by the climatic conditions of different seasons.

Table 8. Effect of bacteria germ feeding on different characters of silkworm.

Characters	Bacteria-fed	Non-fed	Value of 't'
	Mean $\pm$ SE	Mean $\pm$ SE	
Bacterial mortality	19.12 $\pm$ 4.90	18.95 $\pm$ 4.59	0.05 NS
Total mortality	34.54 $\pm$ 4.06	31.73 $\pm$ 3.60	1.01 NS
Av. cocoon weight	896.25 $\pm$ 38.92	851.33 $\pm$ 39.26	5.08*

CHAPTER 15.

DISCUSSION

Since silkworms are living organisms, the environmental conditions as well as nutrition are closely related with their growth and resistance. It is observed that the effect of different ecological factors were not the same to different races as well as different developmental stages of the larvae. Relationship between the environmental factors and the development of silkworms are prerequisite to control the microclimates of rearing rooms or rearing beds; which practically favours the development of silkworm diseases especially bacterial and viral. The effect was more serious for viral diseases when rearing was conducted under high temperature and high humidity alongwith feeding bad quality mulberry leaves (Aruga 1957). Ono et al (1969) observed that under high temperature and high humidity (30°C and 93% RH), the number of flacherie-infected worms were the highest (85%) among those fed with water immersed mulberry leaves; the incidence was comparatively less in worms fed with dried and normal leaves. Krishnaswami et al (1971) also recorded 31.5 - 80.0% loss of silkworms reared at $29-30^{\circ}\text{C}$ and 88-90% relative humidity. The authors also found that feeding of tender, medium and mature leaves alongwith high temperature and high humidity enhanced larval mortality 95.26-90.06%. In similar temperature ($28-29^{\circ}\text{C}$) Karaivanov et al (1985) noticed a remarkable reduction in viability (10-25%) along with some economic traits of cocoons. In the present experiment, feeding with oneday preserved leaves showed an increased outbreak of bacterial flacherie and that with water soaked

leaves caused increased viral grasserie. But feeding with mature leaves exhibited relationship for both the diseases irrespective of seasonal influences.

The area of the rearing bed influences remarkably the growth of silkworm larvae and also has an intimate connection with the economy of mulberry leaves, labour, rearing tools etc. A dense or crowded rearing as practised by the country-rearers can save the above materials at reasonable extent compared to a thin or sparse rearing. But owing to small amount of these requisites the saving has economically very little effect upon the rearing. On the other hand, it causes shortage of leaves and heating of rearing beds which makes larvae unhealthy and finally decreases resistance capability. Ullal and Narasimhanna (1978) recommended the optimum spacings for mature Univoltine or Bivoltine worms of 50 layings (20,000 worms approximately) to be 20 square meters or $1000\text{cm}^2/100$ larvae. For multivoltine and tropical bivoltine the recommended spacing is $900\text{cm}^2/100$ larvae. The view was supported by Krishnaswami et al (1978). The above mentioned spacings were claimed to be highly congenial for health of the silkworms. But their relationship with the disease outbreak were not mentioned. However, it is observed in this investigation that decreased spacings below $937\text{cm}^2/100$ worms significantly enhanced the development of bacterial and viral diseases within the silkworm population. However, in this study too, the disease

The rational feeding must be in harmony with the eating habit and appetite of the larvae which depends on the environmental temperature

and humidity. It increases with the rise of temperature and vice versa. Except extremely high humidity, the relation between humidity and appetite resembles the relation between temperature and appetite (Yokoyama 1962). Under humid condition the freshness of the mulberry leaves is retained for longer duration, hence feeding frequency may be lengthened, but under less humid condition this should be shortened. The finding of this investigation revealed that deviation of one feeding from the normal four feedings has no effect on the incidence of these diseases. But complete starvation of 12, 24, 36 and 48 hours resulted an increase in larval mortality due to flacherie and grasserie as observed by Samson et al (1981). Karaivanov et al (1985) also found that the decreased feeding frequencies under disturbed environmental conditions led to a marked reduction in viability (10-25%), the yield of cocoons (39.92-42.47%) and also the weight of cocoons by 25%. In this experiment, however, the cocoon characters were not taken into consideration, but total loss (viability) exceeded the above findings.

Refrigeration (5°C for 24 hours) of silkworm larvae at 3rd, 4th and 5th instar induced both flacherie and grasserie diseases, although most of the earlier workers (Aruga and Arai, 1959; Hukuhara and Aruga, 1959; Aruga and Watanabe, 1960 and Aizawa et al 1961) used this technique for artificially inducing nuclear and cytoplasmic polyhedrosis diseases. However, in this study too, the viral diseases dominated over bacterial diseases as an aftereffect of the refrigeration. Among others, Sidhu and Singh (1968) reported the outbreak of both flacherie (31.5%)

and grasserie (52.0%) due to refrigeration. Early age worms (1st to 3rd instar) were found comparatively resistant to refrigeration than those of late age worms (Aruga and Arai 1959) and that the induction of viral disease was more when the worms were refrigerated after 6-48 hours of ecdysis. But the early age worms when repeatedly refrigerated for 9-12 days, resulted in a mortality upto 50.68% along with deterioration of the commercial traits of cocoons, moth emergence and also the fecundity (Govindan and Narayanaswamy 1986). After refrigerating larvae of 2nd - 5th instars Aizawa et al (1961) noticed the incidence of nuclear polyhedrosis, cytoplasmic polyhedrosis as well as flacherie diseases. They also found that refrigeration accompanied with feeding of harder or rotten mulberry leaves, killed all the larvae.

Refrigeration reactivates the latent virus present in the gut of silkworm, thereby causing the disease (Hukuhara and Aruga 1959). But the incidence of flacherie due to refrigeration is not yet explained. It may be plausibly explained that flacherie disease occurs due to physiological disorder caused by refrigeration and is influenced by the environmental conditions.

Feeding of nuclear polyhedra contaminated with mulberry leaves upto three successive generations showed higher percentage of the disease than that of control. But during the later generations, the treatment practically showed no effect, resulting similar incidence of nuclear polyhedrosis in both the groups. It is assumed that the environmental conditions during 4th to 6th generations (January to May) suppressed the

incidence. It was experimentally proved that a slightly elevated as well as lowered temperatures (20°C & 30°C) from the normal (25°C) for several days before virus administration promoted the susceptibility to virus infection (Watanabe 1964). Similar result was also obtained in another lepidopterous larvae. Galleria mellonella (Pimental and Shapiro 1962), when low temperatures of $7-8^{\circ}\text{C}$ and high temperatures over 27°C largely affected the mortality of virus treated larvae. But Aratake et al (1973) observed that rearing of larvae inoculated with a NPV at 15°C resulted in much more frequency of disease and longer period of lethal infection than rearing at $30-35^{\circ}\text{C}$. They also suggested that a high temperature treatment (35°C for 12 to 24 hours) reduced the occurrence of the disease in the virus inoculated larvae. Crowding and malnutrition on the other hand, influenced the larval mortality in the inoculated population. Nomani et al (1979) obtained a significantly higher mortality in the NPV inoculated lot than the control. However, they could successfully bred the treated line for thirteen generations suggesting that resistance to NPV infection can be developed in silkworm through artificial inoculation coupled with selection.

Feeding of mulberry leaves contaminated with bacterial pathogens could not significantly increase bacterial infection compared to that of control lot irrespective of the seasonal effects. It could be explained that the bacteria germs alone can not develop the disease unless the physiological status of the larvae is disturbed by other stressors. Modern workers stressed on the presence of antibacterial substance in the digestive juice of silkworm which act as the defence mechanism to flacherie disease. Nakano et al (1987) isolated such a substance, 'Caffequinone'

which had antibacterial properties. Utsumi et al (1987) also isolated an antistreptococcal substance which is controlled genetically, being more in hybrids than pure lines. The present investigation indirectly supports the presence of antibacterial substance in the silkworm race (Nistari-S) dealt with, which resisted the development of the disease through per os introduction.

Control measures against Bacterial and
Viral diseases of Silkworm.

Section - E

Control measures against Bacterial and Viral diseases of Silkworm.

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CHAPTER 16

INTRODUCTION

Control of disease virtually refers to the inactivation of the causative agents. In case of silkworm where biological agents are not solely responsible for the disease and a number of predisposing factors are involved, killing of the causative microorganisms alone would not be effective for the purpose. In both the cases of bacterial as well as viral diseases, no curative measures are known so far, although it was attempted by a large number of workers. This is because of the fact that, once the disease has set in, the worms become impatient and stop feeding and thus the application of curative drugs becomes useless. As such, several workers primarily suggest the preventive or corrective measures so as to minimise the population of pathogenic microorganisms by disinfection, at the same time to restore sound health of the silkworms by providing optimum environmental conditions, suitable leaves and spacings (Golanski 1961, Krishnaswami et al 1978, samson 1983). Yet a lot of attempts have been made to develop therapeutic measures to achieve an effective control against these diseases.

The pathogenicity of bacteria largely depend upon the environmental factors, especially the quality of mulberry leaves and high temperature or humidity. Therefore, in controlling bacterial diseases, correction of the above factors is a must prior to taking other measures. Food, contaminated with bacteria, constitutes the main source of infection for silkworm larvae and the immunity of infection is

related to the microbial population in the larval intestine. Reports on the effective control measures against bacterial diseases of the silkworm are very limited. Earlier it was tried to inhibit the disease caused by Bacillus thuringiensis group by feeding the larvae with antibiotic soaked leaves (Streptomycine, aureomycine and tetracycline) which maintained the bacteriostatic status of the intestine (Afrikian 1960). Use of chloramphenicol for the same purpose was also advocated by some workers (Anon^{*}, Mladen et al 1984). Chloramphenicol and tylosine were also suggested for controlling spontaneous jaundice of silkworm by the authors. Besides killing of bacterial population, oral administration of different antibiotics along with mulberry leaves resulted in heavier caterpillars with increased nitrogen metabolism and higher silk yield, as studied by a number of workers (Murthy et al 1951, Murthy and Sreenivasaya 1953, Barman and Pasha 1985, Tayade et al 1986).

Certain chemicals other than antibiotics are also known to cause similar effect upon silkworms, such as chloromycetin and glycine (Murthy et al 1954, Verma and Atwa 1963, Sridhar and Radha 1984), thyroxine (Majumdar and Medda 1975 and Thyagraja et al 1985) etc. Certain bactericides like bactospeine, dipel and thuricides are tried with limited succes (Krishnaswami et al 1979 and 1981).

* Book published by the Regional Sericulture Training Centre, Guanzhou, China, but year not mentioned).

Selection and development of bacteria - resistant silkworm races has been given much more stress by some workers to achieve success against the disease. Sidhu and Singh (1968), Sidhu and Kapila (1969) succeeded to select a number of silkworm races relatively resistant to flacherie disease through artificially creating the disease. Kundu (1978) studied the possibility of evolving flacherie-resistant strain through selection from the survived individuals pre-inoculated with the flacherie-live germs. Through the same procedure Barman and Pasha (1984) was able to develop a bacteria-resistant strain. Selection of comparatively low pathogenic races to bacterial diseases also have been achieved by Kim et al (1986) and Zhang (1986) as a check over the disease.

The control of viral disease of silkworm has meant largely to combat the polyhedrosis. Scientists from the very early days tried variously to combat the malady created by different types of viral diseases. A large number of chemicals have been identified which proved to be effective in suppressing the polyhedrosis. Earlier workers recommended to disinfect the silkworm eggs with 1% sodium hydroxide solution (Bergold & Schramm (1942), 2.5% formalin (Letje 1939) and with hydrogen peroxide (Pospielow 1947). Watanabe (1951) found that 2% phenol and 0.1% sublimate inactivate the virus in one hour, while 1% formalin, 0.5% potassium permanganate and 0.1% actinoformine destroy it in half an hour, and 2% sodium hydroxide, 2% potassium hydroxide, 2% sodium carbonate, 0.5% tartaric acid, and 2% citric acid destroyed the virus within ten to thirty minutes. Golanski (1961) tested a number

of chemicals in an effort to control the jaundice (NPV) by disinfection. His results favoured the use of formalin solutions for the disinfection of eggs, caterpillars as well as food and equipments. Now a days, formalin, as a disinfectant, is widely recommended by the Indian Sericulturists (Krishnaswami et al 1978 and Samson 1983). Formalin 3% was also effective for inactivating Densonucleosis virus as observed by Seki (1986). On the other hand, Gershenson (1958) suggested the use of cobalt and zinc salts to the food of silkworm which decreased the frequency of spontaneous jaundice (NPV) caused by the latent virus.

Recent workers emphasized on the chemotherapeutic methods to control the viral diseases. Baburashvilli et al (1984) found that solutions containing 0.1% imanine reduced the frequency of polyhedrosis by 100% within three hours of normal feeding. A chemical 'Mayuran' containing ecdysterone when fed to the silkworms after soaking in mulberry leaves effectively suppressed the nuclear polyhedrosis (Liu and Hou 1985). Other workers (Miyajima and Kawase 1986, Miyajima et al 1986) used several salts and derivatives of guanidine for controlling the incidence of infectious flacherie virus.

The ageold concept of breeding resistant silkworm races or strains to nuclear and cytoplasmic polyhedroses is widely supported by the modern workers dealing sericulture. It is a known fact that different races vary in their resistance to various diseases. Pure lines are usually more resistant to polyhedral diseases compared to their hybrids and the hybrids show a medium degree of resistance

between two parental lines (Aruga 1961). Aruga concluded that "both types of polyhedral viruses are pre-existing in a non-infective state and inducing stressors disturb the physiological conditions culminating in the development of polyhedrosis". There is reason to believe that genetic variability exists between various races and stocks which is supposed to be controlled by Mendelian polygenes.

A number of workers have demonstrated several natural strains of silkworms resistant to both type of polyhedroses (Aruga and Watanabe 1960, 1964, Watanabe 1965, Sidhu and Singh 1968, Sidhu and Kapila 1969, Huang et al 1979 and Seki 1985), while some others tried to find out resistant hybrids to the polyhedral diseases. Umeya (1930) suggested that the high vigor of hybridization in silkworm comes due to reaction of the dominant allelomorphs as well as due to after reaction of the stimulus exerted upon the developing embryos by the ooplasm and nutrients. Most of the workers observed that the F_1 hybrids were comparatively resistant to virus induction than the common parental strains (Aruga and Watanabe 1960, Aizawa and Furuta 1962, Aruga and Watanabe 1964). Watanabe (1954) suggested that the manifestation of heterosis on the resistance to virus infection might be partly controlled by temperature conditions - when F_1 hybrids were stressed by low temperature showed greater resistance than their parental inbred lines; but none of them, stressed by the elevated temperature, was more resistant compared to the parental lines.

Attempts have been carried out to develop resistant silkworm strains to viral diseases through artificially inducing the disease.

Thus Aizawa et al (1961) succeeded to obtain a resistant strain to nuclear polyhedrosis after thirteen generations of treatment at 5°C for 24 hours followed by selection pressure. Nomani et al (1979) used the same method and also succeeded to develop a resistant race. On the other hand, by selecting survivors from the virus fed larvae for eight generations Watanabe (1967) was able to develop a resistant strain to cytoplasmic polyhedrosis.

In recent years some Indian sericulture scientists (Chinnaswamy et al 1986 and Samson et al 1987) claimed that composite bed disinfectants in the form of dust powder when applied on the silkworms largely reduced the larval mortality caused by viral diseases. But Chinese workers (Anon) hold the opinion to use integrated control measures for the viral diseases. The suggested main points are:-

- 1) Strict disinfection, destruction of the pathogen and breaking the transmission cycle,
- 2) strict separation of the moulting silkworms from those not yet in moult to prevent spread of infection in the rearing tray,
- 3) improve feeding and management to increase vigor and heighten disease resistance, and
- 4) selection of disease - resistant silkworm varieties.

heavily infected silkworms were done firstly by collecting in distilled water and then by repeated centrifugation at 1500-2500 rpm in 20% and 50% sucrose solutions (Chinnaswamy and Samson 1987). Polyhedral suspensions of different concentrations viz 1×10^6 in the first series,

CHAPTER 17

MATERIALS AND METHODS

Both the mechanical and chemical control methods were tried in the present investigation to assess the suitability of commercial application for controlling the two major types of diseases of bacterial and viral origin.

17.1. Mechanical control measures:

17.1.1. Selection of less susceptible silkworm races to nuclear polyhedrosis.

In this experiment, four multivoltine mutant strains namely NanNong, SRI(oval), BR/84 and Nistari(s); and six multivoltine improved acclimatized breeds namely BSRI-801, BSRI-802, BSR-3, BSRI-83/1, BSRI-83/2 and BSRI-83/3 were taken from the germ plasm bank of Bangladesh Sericulture Research and Training Institute, Rajshahi. All the races (except NanNong) are commercially used for cocoon production in the private sector in different seasons. The mutant strains were extremely homozygous while the breeds were little heterozygous.

Extraction and purification of the nuclear polyhedra from the heavily infected silkworms were done firstly by collecting in distilled water and then by repeated centrifugation at 1800-2500 rpm in 25% and 50% sucrose solution (Kawarabata and Matsumoto 1973). Polyhedral suspensions of different concentrations viz; 1×10^4 in the first season,

5×10^3 in the second season, 5×10^4 in the third season, 5×10^5 in the fourth season and 1×10^7 polyhedra per milliliter in the fifth season were used as the inductive factor for nuclear polyhedrosis.

Mulberry leaves smeared with polyhedral suspension were fed to the newly moulted larvae of the third instar for six hours. The treatment was always replicated into three consisting of 300 larvae each, taken randomly from the mass bed. Treatments and rearings were conducted under natural environmental conditions of five seasons namely, S_1 - November '85 ($24.0^\circ\text{C} \pm 1.08$, R/H $85.45\% \pm 5.27$), S_2 - January-February '86 ($24.77^\circ\text{C} \pm 1.24$, R/H $83.44\% \pm 4.80$), S_3 - April '86 ($28.01^\circ\text{C} \pm 1.24$, R/H $84.10\% \pm 5.78$), S_4 - June '86 ($28.48^\circ\text{C} \pm 1.29$, R/H $89.63\% \pm 4.03$) and S_5 - August-September '86 ($29.26^\circ\text{C} \pm 1.57$, R/H $90.56\% \pm 4.73$). Observations on the mortality due to nuclear polyhedrosis as well as due to other factors were recorded everyday during bed cleaning. The survived larvae were allowed to spin cocoons and the effective rate of rearing per 10,000 larvae and the yield per 100 layings were calculated from the total number of cocoons harvested and the average weight of single cocoon respectively.

17.1.2. Screening relatively resistant multivoltine hybrids to nuclear polyhedrosis.

A total of twenty F_1 crosses were made before each rearing season among the following multivoltine silkworm races, obtained from the germplasm bank of Bangladesh Sericulture Research and Training

Institute, Rajshahi:

A. Local strains

Nistari(S)

Nistari(M)

BR/84

SRI-(oval)

B. Improved breeds

BSRI-83/2

BSR-3

BSRI-801

BSRI-83/1

BSRI-802

The races of the group A are strictly indigenous and homozygous having capacity to resist high temperature and, to some extent, high humidity, but poor in silk content; while those of the group B are evolved acclimatized multivoltine races, slightly heterozygous, relatively susceptible to both high temperature and humidity but are comparatively rich in silk content. The following crosses were made:

Code number as
used in the exp.

Hybrids

C₁

BSRI-83/2 x Nistari(S)

C₂

BSRI-83/2 x Nistari(M)

C₃

BSRI-83/2 x BR/84

C₄

BSRI-83/2 x SRI(O)

C₅

BSR - 3 x Nistari(S)

C₆

BSR - 3 x Nistari (M)

C₇

BSR - 3 x BR/84

C₈

BSR - 3 x SRI (O)

Code number as
used in the exp.

Hybrids

C ₉	BSRI-801 x Nistari(S)
C ₁₀	BSRI-801 x Nistari(M)
C ₁₁	BSRI-801 x BR/84
C ₁₂	BSRI-801 x SRI(O)
C ₁₃	BSRI-83/1x Nistari(S)
C ₁₄	BSRI-83/1x Nistari(M)
C ₁₅	BSRI-83/1x BR/84
C ₁₆	BSRI-83/1x SRI(O)
C ₁₇	BSRI-802 x Nistari(S)
C ₁₈	BSRI-802 x Nistari(M)
C ₁₉	BSRI-802 x BR/84
C ₂₀	BSRI-802 x SRI(O)

The experiment was carried out under the natural environment of the three seasons namely, a) S₁ (Agrahayani) - comprising months of October-November '86 ($26.69^{\circ}\text{C} \pm 1.11$, R/H $90.22\% \pm 2.91$), b) S₂ (Jaistha) - comprising months of April-May '87 ($28.16^{\circ}\text{C} \pm 2.42$, R/H $83.56\% \pm 10.07$) and c) S₃ (Bhaduri) - comprising months of July-August '87 ($27.51^{\circ}\text{C} \pm 1.12$, R/H $93.43\% \pm 2.99$).

17.2.1. Feeding of antiviral chemicals.

Parental stocks were maintained separately and ten F₁ layings were prepared for each cross before each season, examined and brushed the first day of 3rd instar to the day before spinning were fed with mul-

for rearing. After second moult, 900 larvae were taken from each cross randomly which were subjected to low temperature (5°C for 24 hours) treatment for reactivation of the latent virus, the technique used by Aruga and Arai (1959), Aizawa et al (1961). The treated larvae were resumed feeding after one hour of removal from the incubator. All the treated batches were reared under similar conditions of the concerned seasons. Observations on the mortality due to nuclear polyhedrosis, total mortality, percent cocoon harvest and average cocoon weight (g) were separately recorded for each cross.

Evaluation and selection of less susceptible combination were made on the basis of least NPV mortality, maximum cocoon harvest and maximum estimated cocoon yield per 100 disease free layings (dfls).

The estimated yield was calculated by the following formula:

$$Y = \frac{30,000 \times \text{percent cocoon harvest}}{100} \times \text{Single cocoon wt(g)}$$

where Y = estimated yield in gram.

30,000 = average number of larvae/100 dfls

in case of multivoltines.

17.2. Chemical control measures:

17.2.1. Feeding of antiviral chemicals.

In this experiment, silkworm larvae of the race Nistari(S) from the first day of 3rd instar to the day before spinning were fed with mul-

berry leaves along with different concentrations of potassium permanganate (0.01%, 0.03%, 0.05%, 0.08% and 0.1%), phenol (1.0%, 1.5%, 2.0%, 2.5% and 3.0%) and formalin* (0.5%, 0.8%, 1.0%, 1.5% and 2.0%) every-day at 10.00 A. M. All the treatments consisted of three replications with 300 larvae each and a non-treated control batch with same number of larvae in three replications. Observations only on the mortality due to nuclear polyhedrosis were noted right from the next day of first treatment to see the consequent after-effect in disease control. The same experiment was repeated during two seasons of June 1985 and August 1985. Rearing was scientifically monitored to avoid unwanted errors due to other factors.

17.2.2. Spraying of antiviral chemicals.

Two antiviral chemicals, phenol (Watanabe 1951) and hydrogen peroxide (Pospielow 1947) each at the dosages of 1.0%, 3.0% and 5.0% were taken for spraying the caterpillars to kill the virus germs adhered to the body surface and feeding trays. For convenience, the treatments were specified as - Phenol 1% (T_1), 3% (T_2), 5% (T_3) and hydrogen peroxide 1% (T_4), 3% (T_5), 5% (T_6), while the control as (T_0). Silkworms from 3rd instar to 5th instar before spinning were thoroughly sprayed with the above ^{chemicals} treatments once in everyday (except moulting period) by a hand sprayer after bed cleaning. The silkworms resumed feeding just after spraying. Observations on the viral mortality were re-

* 40% formaldehyde was used as the basic solution. (1%, 2%, 3%,

corded from the next day of first treatment at bed cleaning, while that of effective rate of rearing, average cocoon weight and shell weight were taken after cocoon harvest to assess the consequent post-treatment effect. The experiment was repeated in two successive seasons of July-August '86 and September-October '86.

17.2.3. Use of selected bed disinfectants.

Selected dose of five disinfectants viz; formalin 2%, phenol 3%, potassium permanganate 0.1%, bleaching powder 5% and slaked lime dust were used to disinfect the rearing bed which is one of the most possible source of disease contamination. The liquid disinfectants were first soaked in coarse cotton cloth or pad and uniformly smeared on the rearing trays after removing the larvae at another pre-disinfected tray for that moment. Lime dust was uniformly dusted through fine cotton duster. All the disinfectants were applied only once a day from the 3rd instar to 5th instar. Three replications each with 300 silkworms were maintained for each treatment and two separate controls, one only water sprayed and the other non-sprayed were also maintained to study the comparative effectiveness.

Observations on the larval mortality due to viral, bacterial and deficiency diseases and total mortality were separately recorded.

The cocoon characters were also analysed after the cocoon harvest.

17.2.4. In vitro control of pathogenic bacteria.

Aqueous solutions of six chemicals viz; phenol (1%, 2%, 3%), silver nitrate (0.1%, 0.5%, 1%), bleaching powder (1, 2%, 3% active

chlorine), formalin (0.5%, 1%, 1.5%), potassium hydroxide (2%, 4%, 6%) and ammonium chloride (1%, 2%, 3%) were taken for the treatment. These chemicals are claimed to possess antibacterial properties (Michael *et al* 1977). Four bacteria pathogenic to silkworm namely, Streptococcus SP., Micrococcus sp., Bacillus thuringiensis var sotto and Serratia marcescens were used as the test pathogen which were collected from the stock culture of the Seri-pathology section, Bangladesh Sericulture Research and Training Institute, Rajshahi.

Brain heart infusion Agar medium and Trypticase soy broth enriched with nutrient agar were used for culturing the bacterial species. 5 ml of the mentioned treatments were taken in sterilized disposable test tubes along with control tubes of only distilled water. Half ml. of one day old broth culture of the respective bacteria were poured separately in the test tubes and mixed thoroughly by rolling over two palms. After ten to twenty minutes, the treatments were inoculated into petridishes having solid media. The inoculated plates were incubated at 37°C for eighteen hours. All these operations were conducted in aseptic conditions. Observations on the bactericidal effectiveness were noted separately on the basis of number of bacterial colonies grown in each plate, i.e.; +++ (more than 10 colonies), ++ (6-10 colonies), + (1-5 colonies) and - (no growth).

17.2.5. Application of antibiotics.

Five antibiotics, Terramycine, Chloramphenicol, Tetracycline (in the form of capsules), Streptomycine (in the form of injection)

and Sulfathiazole (in the form of tablet), used for human consumption were used in this experiment. For all the antibiotics three doses of 1000 ppm, 2000 ppm and 3000 ppm solutions were prepared separately and fed to the silkworms of 3rd instar to 5th instar twice in everyday soaking with mulberry leaves and fed for twelve hours. The treatments were replicated into three batches with 300 silkworms each. Also a separate non-treated control batch was maintained. Observations on the bacterial and viral diseases were recorded everyday. The experiment was repeated in three seasons, May, July and September, 1986.

(after second moult) twice in 4th instar (once after third moult and the other on the third day of 5th instar).

17.2.6. Dusting of formulated chemical composites.

Ten chemical composites (C) in the form of dusts were formulated with the following ingredients:

- CA : Lime powder 97%, paraformaldehyde 3%.
- CB : Lime powder 94%, paraformaldehyde 3% and Sodium benzoat 3%.
- CC : Lime powder 95%, paraformaldehyde 3% and benzoic acid 2%.
- CD : Lime powder 92%, paraformaldehyde 3% and Sodium benzoat 5%.
- CE : Lime powder 92%, paraformaldehyde 3% and Boric acid 5%.
- CF : Lime powder 89%, paraformaldehyde 3%, Sodium benzoat 3% and Sodium carbonate 5%.
- CG : Lime powder 90%, paraformaldehyde 3%, Benzoic acid 2% and Boric acid 5%.
- CH : Lime powder 89%, paraformaldehyde 3%, Sodium benzoat 3% and Boric acid 5%.

CI : Lime powder 90%, paraformaldehyde 3%, Benzoic acid 2%, Sodium carbonate 5%.

CJ : Lime powder 87%, paraformaldehyde 3%, Sodium benzoate 3%, benzoic acid 2% and Sodium carbonate 5%.

The ingredients were thoroughly mixed and the prepared composites were preserved in airtight polythene packets before and after use. After bed cleaning the silkworms (3rd instar to 5th instar) and rearing beds were uniformly dusted with respective composites through fine cloth duster. The application was done once in 3rd instar (after second moult), twice in 4th instar (once after third moult and the other on the third day) and thrice in 5th instar (once in every alternate day). All the treatments were replicated into three individual lots with 300 silkworms. Observations on the viral and bacterial mortalities, effective rate of rearing and average cocoon weight were separately recorded. The experiment was repeated in two seasons, March 1987(S_1) and June 1987(S_2).

The total average mortality in the five seasons due to NPV worm feeding also showed variations among different seasons. It varied from 19.8% in S₁(1987) to 40.4% in S₅(1991).

All the races studied, performed relatively better during November '85. But their performance with respect to cocoon weight and shell weight deteriorated during April '86 (Table 4). But the characters, however, remained more or less steady in BHR-3 and S₁ (oval) in later seasons.

CHAPTER 18.

OBSERVATIONS

18.1. Selection of less susceptible silkworm races to nuclear polyhedrosis (NP).

The comparative mortality due to NP, total mortality and the effective rate of rearing as a result of NPV germ feeding under different seasons could be seen in Fig. 33 . A F-test reveals highly significant difference in the racial mortality due to NP ($p < 0.01$) and also total mortality ($p < 0.01$), as shown in Appendices XXVI and XXVII. Results also indicate that seasons play a major role in the activation of the inductive factors. The months of January-February and April responded negatively to the development of NP disease. Of the tested ten silkworm races, SRI (oval) showed the lowest mortality (13.5%) due to NP followed by BSR-3 (14.6%) and BSRI-83/1 (15.3%), while BSRI-802 (20.9%) and BSRI-801 (20.8%) showed the highest. The total average mortality in the five seasons due to NPV germ feeding also showed variations among different races. It varied from 19.8% in SRI (oval) to 40.4% in NanNong.

All the races studied, performed relatively better during November '85. But their performance with respect to cocoon weight and shell weight deteriorated during April '86 (Table 9). Both the characters, however, remained more or less steady in BSR-3 and SRI (oval) in later seasons.

Fig. 33. Comparative performance of different multivoltine
races under different seasons fed with NPV germs.

Fig-33.

Comparative Viral mortality , Total mortality , And effective rate of rearing  of different Silkworm races after adminstering NPV germs.

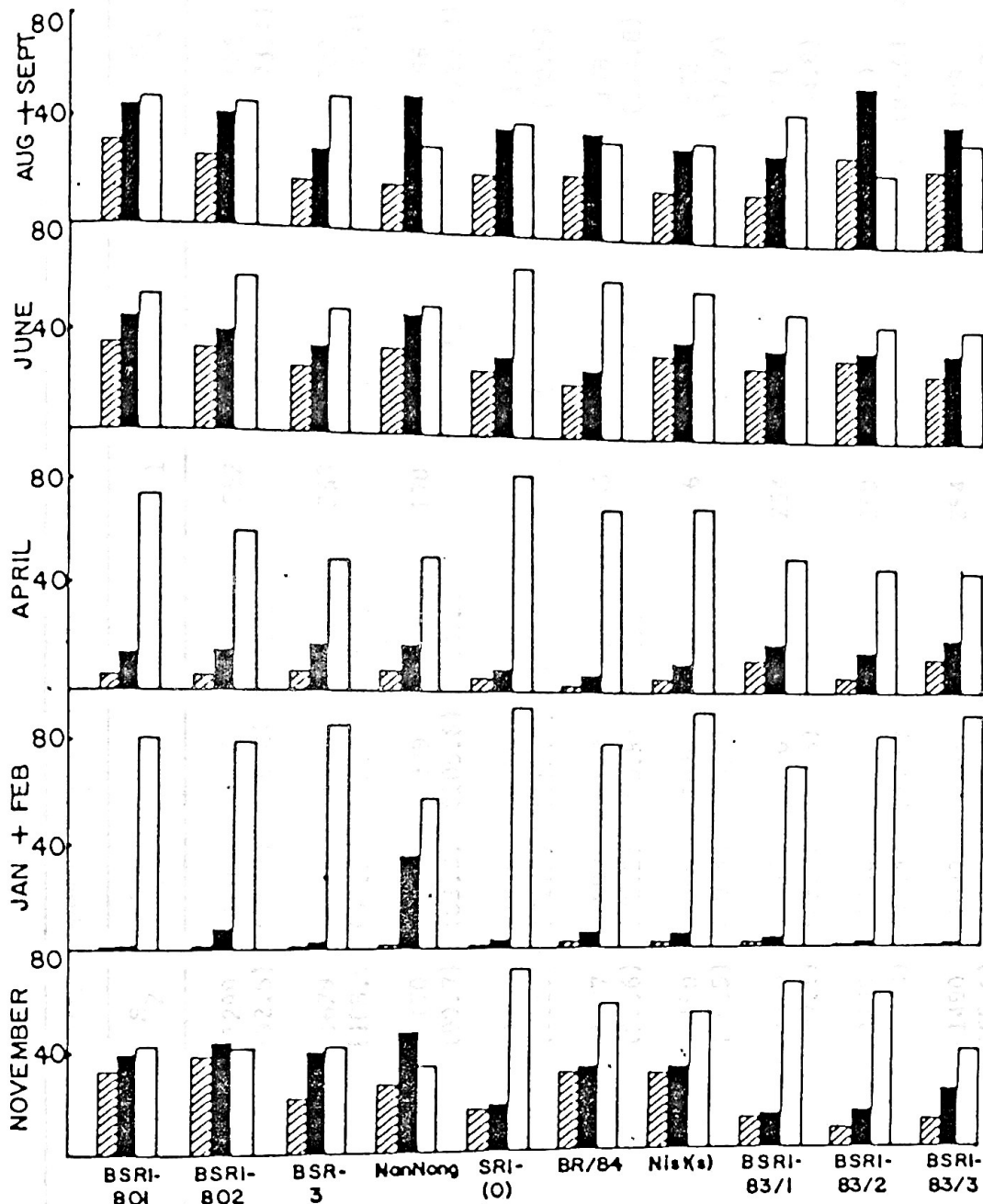


Table 9. Effect of feeding NPV germs on the cocoon characters of different silkworm races.

Silkworm races	Average cocoon weight (mg)					Average shell weight (mg)				
	S ₁	S ₂	S ₃	S ₄	S ₅	S ₁	S ₂	S ₃	S ₄	S ₅
BSRI-801	1404	1298 (92.5)	1008 (71.8)	1255 (89.4)	1187 (84.5)	252	190 (75.4)	149 (59.1)	185 (73.4)	170 (67.5)
BSRI-802	1501	1629 (108.5)	968 (64.5)	1288 (85.8)	1130 (72.3)	253	219 (86.6)	140 (55.3)	202 (79.8)	182 (71.9)
BSR-3	1173	1170 (99.7)	974 (83.0)	1339 (105.6)	1222 (104.2)	170	172 (101.2)	160 (94.1)	196 (115.3)	163 (95.3)
NanNong	1153	1175 (101.9)	820 (71.1)	1286 (111.5)	1046 (90.7)	248	174 (70.2)	130 (52.4)	176 (70.9)	134 (54.0)
SRI(oval)	1166	1127 (96.6)	892 (76.5)	1130 (96.9)	1156 (99.1)	155	166 (107.1)	135 (66.2)	178 (114.8)	145 (93.5)
Nistari(S)	1271	1150 (90.5)	895 (70.4)	1086 (85.4)	1046 (90.2)	176	188 (108.8)	140 (79.5)	172 (97.7)	155 (88.1)
BSRI-83/1	1401	1220 (87.1)	892 (63.7)	1156 (82.5)	1022 (72.9)	264	227 (85.9)	150 (56.8)	171 (64.8)	164 (62.1)
BSRI-83/2	1484	1149 (77.4)	1044 (70.4)	1241 (83.6)	1177 (79.3)	250	207 (82.8)	165 (66.0)	209 (83.6)	184 (73.6)
BSRI-83/3	1581	1490 (65.6)	1037 (65.6)	1262 (79.8)	1102 (69.7)	254	214 (84.2)	140 (55.1)	199 (78.3)	179 (70.5)
BR/84	1318	1159 (87.9)	890 (67.5)	1100 (83.4)	1045 (79.3)	204	196 (96.1)	135 (66.2)	182 (89.2)	170 (83.3)

Recorded values are mean of three observations. Data in the parentheses indicate the growth index.

Table 10. Effective rate of rearing per 10,000 larvae and yield per 100 disease free layings of the three topmost resistant races.

Silkworm races	Experimentation seasons				
	November '85	January- February '86	April '86	June '86	August- September '86
BSRI-801	-	-	-	-	-
BSRI-802	-	7800 (38.11)	-	-	-
BSR-3	-	8600 (30.18)	-	4300 (15.98)	4900 (19.96)
NanNong	-	-	-	-	-
SRI(oval)	7100 (18.95)	9400 (31.78)	8400 (22.47)	5500 (19.00)	-
Nistari(S)	-	-	7200 (19.33)	-	3900 (13.40)
BR/84	-	-	7100 (18.95)	4200 (13.86)	3300 (10.35)
BSRI-83/1	6550 (22.47)	-	-	-	-
BSRI-83/2	6200 (19.33)	-	-	-	-

Data in the parentheses indicate the yield per 100 dfls in kg.

When three topmost resistant races based on mortality due to NP disease in each season are selected, SRI(oval) - a mutant strain was found best for four seasons and was followed by BSR-3 and BR/84 which qualified each for three seasons (Fig 33). But when seasonwise production per 100 dfls was calculated, then BSRI-83/1 was found best for November '85 (22.47 kg), BSRI-802 for January-February '86 (38.11kg), SRI(oval) for April '86 and June '86 (22.47 and 19.0 kg respectively) and finally BSR-3 for August- September '86 season (19.96 kg). SRI (oval) showed the highest effective rate of rearing for all the seasons, followed by BR/84, Nistari (S) and BSR-3 (Table 10).

18.2. Screening relatively resistant multi x multivoltine F_1 hybrids to nuclear polyhedrosis.

The analysis of variance presented in Table 12 suggests that the hybrids ($p < 0.01$) as well as seasons ($p < 0.001$) differ significantly with respect to all the studied characters of mortality due to NP, total mortality, percent cocoon harvest and average cocoon weight. Also the interaction between hybrids and seasons was highly significant ($p < 0.01$) for all the characters tested. The mean performance of the twenty hybrids studied over three seasons for four different characteristics is summarized in table 11. and a comparative account of the performances under the three seasons are shown in Figs 34 to 38.

Fig. 34. Mortality of different crosses due to artificial induction of Nuclear polyhedrosis under different seasons.

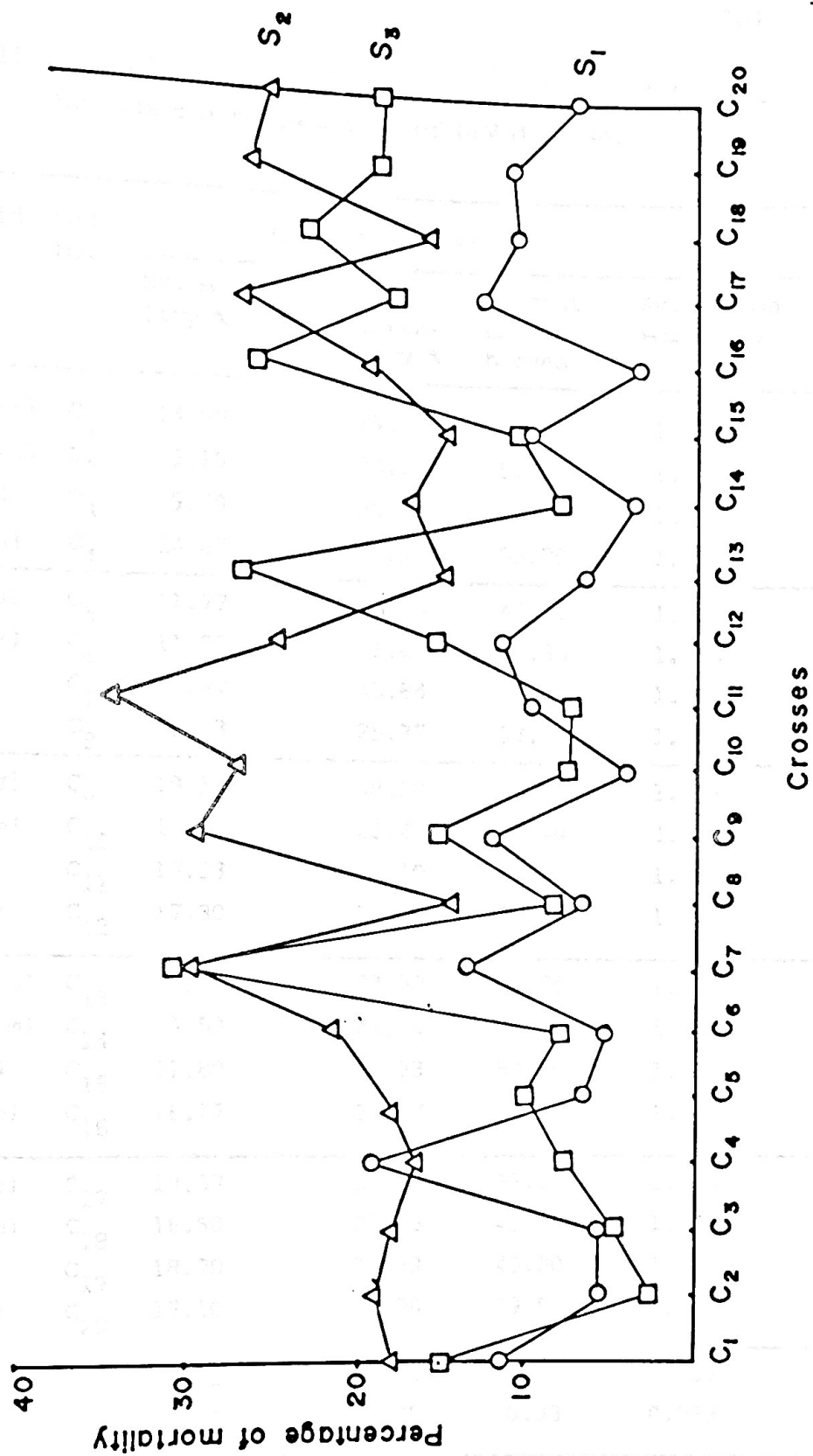


Table 11. Mean of different characters resulted in different hybrids due to induction of NPV disease.

Name of the hybrid	Code No.	Characters studied			
		NPV mortality %	Total mortality %	Percent cocoon harvest	Av. cocoon weight(g)
BSRI-83/2 x Nist(s)	C ₁	14.90	39.73	41.50	1.137
BSRI-83/2 x Nist(m)	C ₂	9.10	24.37	55.80	1.206
BSRI-83/2 x BR/84	C ₃	9.69	28.23	53.70	1.100
BSRI-83/2 x SRI(o)	C ₄	14.67	32.12	53.20	1.198
BSR-3 x Nistari(s)	C ₅	11.77	30.14	47.10	1.124
BSR-3 x Nistari(m)	C ₆	11.73	31.43	55.30	1.063
BSR-3 x BR/84	C ₇	24.77	45.84	33.50	1.164
BSR-3 x SRI(o)	C ₈	9.53	25.37	53.30	1.137
BSRI-801 x Nist(s)	C ₉	19.13	38.90	38.70	1.034
BSRI-801 x Nist(m)	C ₁₀	12.90	28.44	51.40	1.249
BSRI-801 x BR/84	C ₁₁	17.23	35.10	53.00	1.138
BSRI-801 x SRI(o)	C ₁₂	17.30	35.07	33.70	1.102
BSRI-83/1 x Nist(s)	C ₁₃	16.17	28.37	42.70	1.233
BSRI-83/1 x Nist(m)	C ₁₄	9.53	21.70	57.70	1.095
BSRI-83/1 x BR/84	C ₁₅	11.80	32.33	50.50	1.097
BSRI-83/1 x SRI(o)	C ₁₆	16.27	28.57	46.90	1.227
BSRI-802 x Nist(s)	C ₁₇	19.37	32.31	43.20	1.104
BSRI-802 x Nist(m)	C ₁₈	16.50	29.63	42.90	1.173
BSRI-802 x BR/84	C ₁₉	18.70	36.43	45.00	1.126
BSRI-802 x SRI(o)	C ₂₀	17.00	30.90	39.50	1.195
Lsd ($p < 0.05$)		3.38	5.13	5.89	0.058
($p < 0.01$)		4.80	7.29	8.38	0.083

Fig. 35. Total larval mortality of different crosses due to artificial induction of Nuclear polyhedrosis under different seasons.

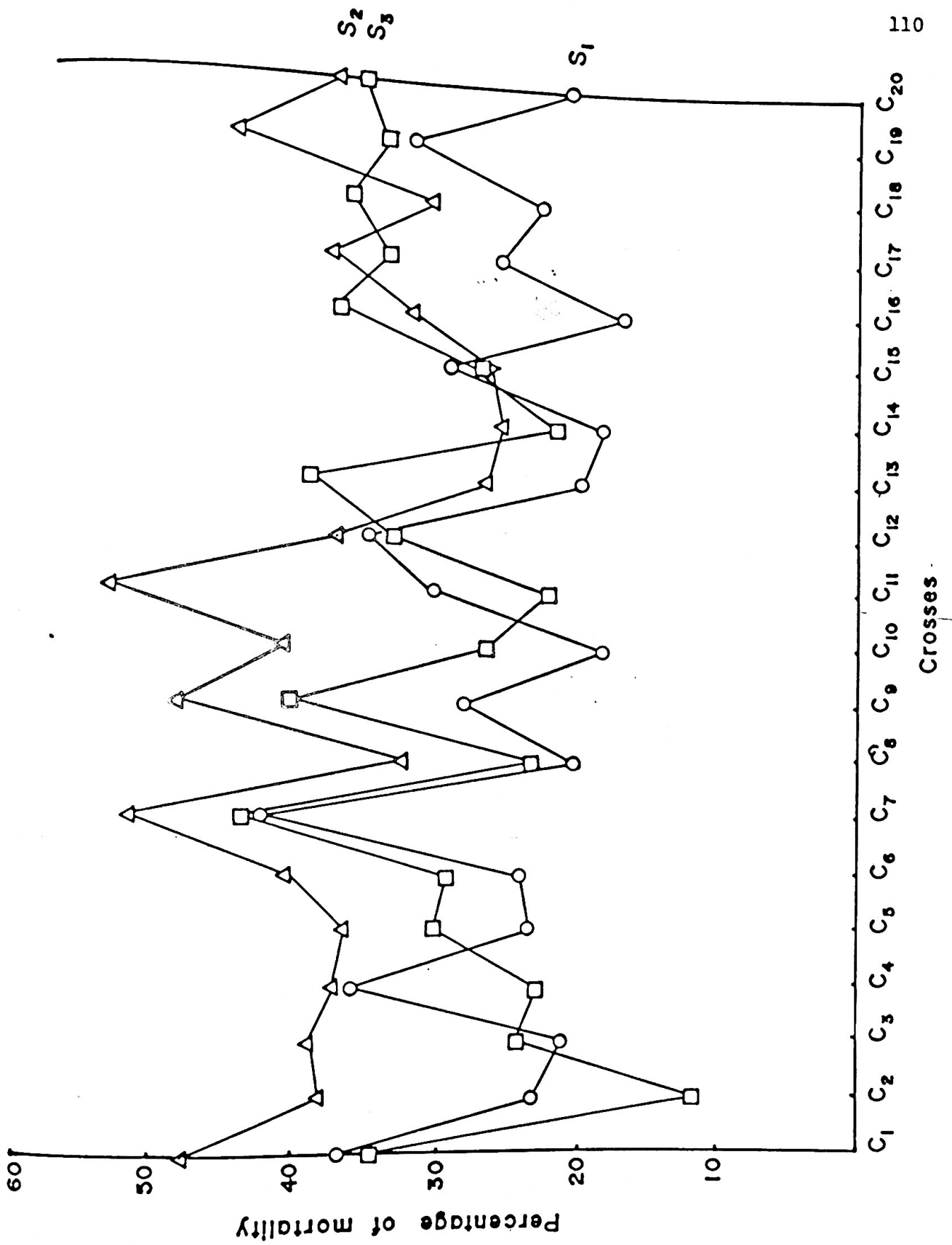
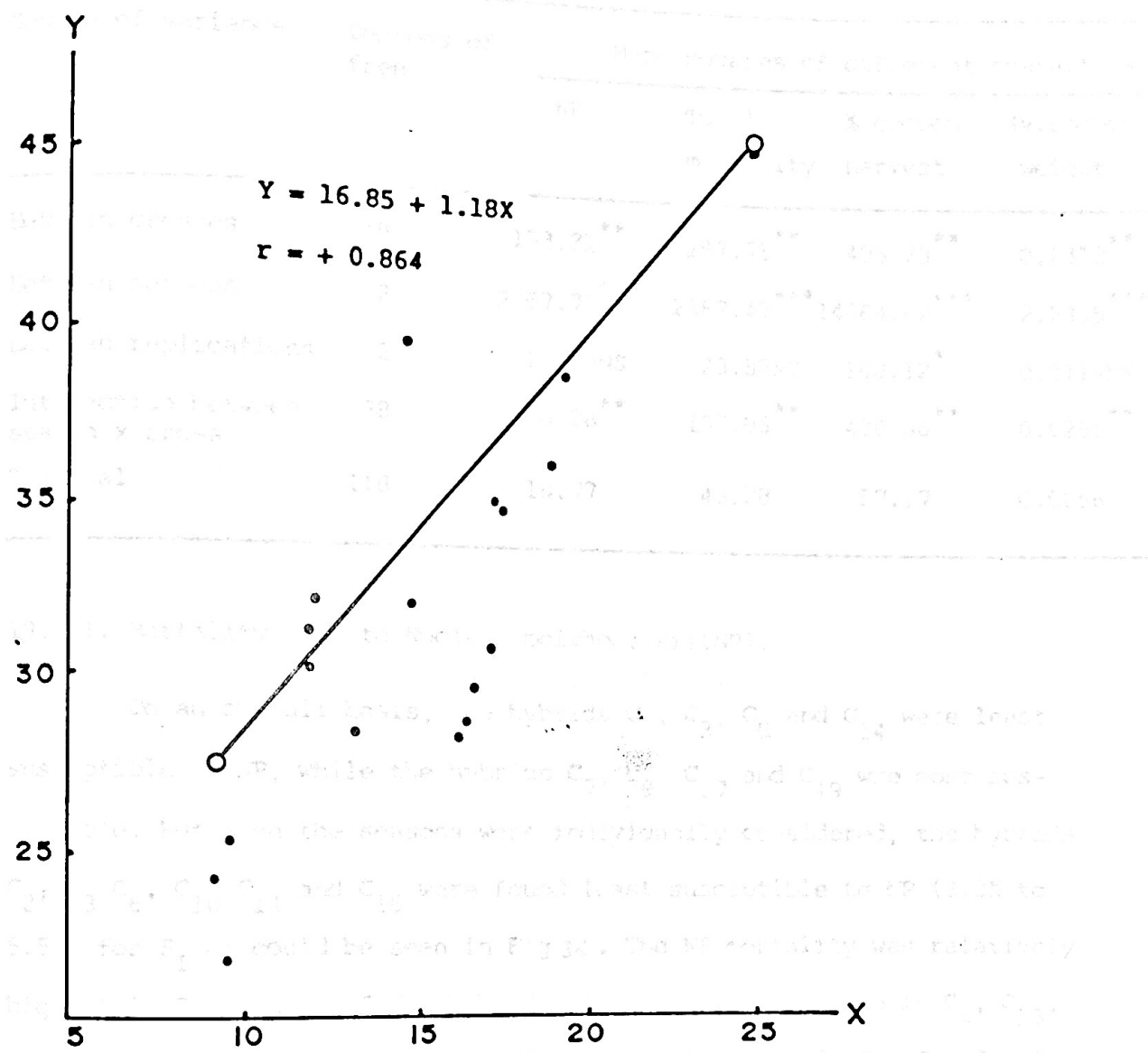


Fig. 36. Regression line showing relationship of total mortality
(Y) on mortality due to NP (X).

Table 17. Effect of temperature on the rate of infection of cotton by the cotton bollworm, *Heliothis virescens* (L.).



On all of the above, the hybrids C_1 , C_2 , C_3 and C_{14} were least susceptible to IP, while the hybrids C_7 , C_8 , C_9 and C_{19} were most susceptible. On the same basis, the hybrids C_1 , C_2 and C_{14} were found least susceptible to IP (1.25 to 1.5 for S_1), while the hybrids C_7 , C_8 , C_9 and C_{19} were found most susceptible to IP (14.38 to 15.38). For S_2 season, the hybrids C_1 , C_2 , C_3 , C_4 , C_7 , C_{10} , C_{11} and C_{14} showed resistance to IP (1.58 to 1.68). On the other hand, the hybrids C_5 , C_6 , C_{12} , C_{13} and C_{15} were completely susceptible to IP (14.38 to 15.38).

Table 12. ANOVA table showing mean squares of different characters analysed for twenty different crosses.

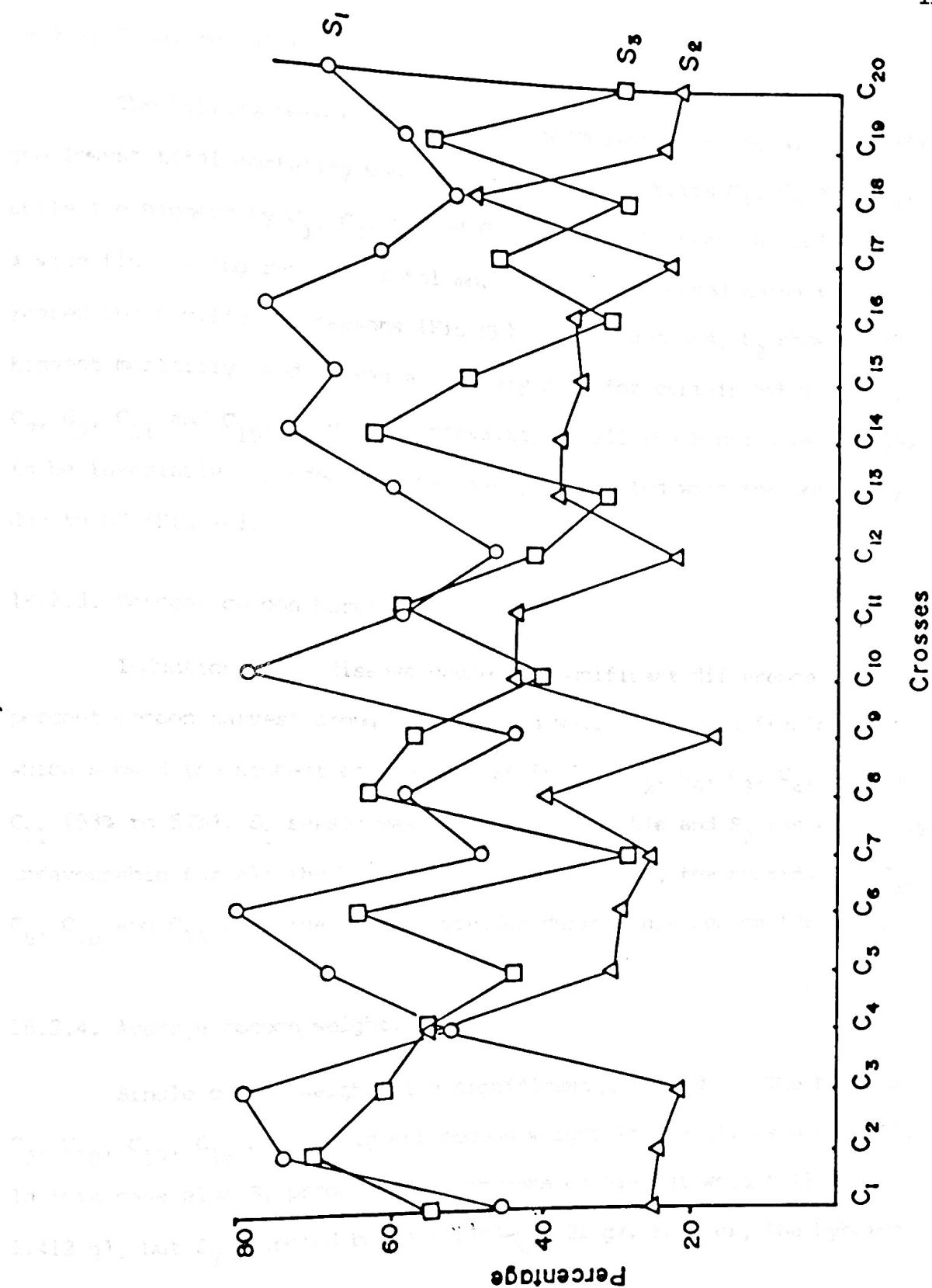
Source of variance	Degrees of free	Mean squares of different characters			
		NP	Total mortality	% cocoon harvest	Av.cocoon weight
Between crosses	19	153.21**	287.75**	495.23**	0.2313**
Between seasons	2	2467.71***	2367.47***	14084.02***	2.2335***
Between replications	2	18.48NS	23.88NS	108.12*	0.0114NS
Interaction between season x cross	38	96.28**	107.06**	430.06**	0.0251**
Residual	118	18.77	43.28	57.17	0.0056

18.2.1. Mortality due to Nuclear polyhedrosis(NP).

On an overall basis, the hybrids C_2 , C_3 , C_8 and C_{14} were least susceptible to NP, while the hybrids C_7 , C_9 , C_{17} and C_{19} were most susceptible. But when the seasons were individually considered, the hybrids C_2 , C_3 , C_6 , C_{10} , C_{14} and C_{16} were found least susceptible to NP (3.3% to 5.5%) for S_1 as could be seen in Fig 34. The NP mortality was relatively highest in S_2 in most of the hybrids with certain exception in C_4 , C_{13} , C_{15} and C_{18} (14.5% to 16.7%). For S_3 season, the hybrids C_2 , C_3 , C_4 , C_8 , C_{10} , C_{11} and C_{14} showed minimum incidence (2.8% to 8%). However, none of the hybrids were completely resistant to nuclear polyhedrosis.

~~These are the same as the different crosses due to~~
~~these are the same as the nuclear polyhedrosis under~~
~~these are the same as the nuclear polyhedrosis under~~

Fig.37. Percent cocoon harvest of different crosses due to artificial induction of Nuclear polyhedrosis under different seasons.



18.2.2. Total mortality.

The hybrids were highly variable with respect to total mortality. The lowest total mortality was exhibited by the hybrids C_2 , C_8 and C_{14} , while the highest by C_1 , C_7 , C_9 and C_{19} as could be seen in table 11. A wide fluctuating range in total mortality was observed among the hybrids reared under different seasons (Fig 35). Of the seasons, S_2 showed the highest mortality and it was alarmingly high for certain hybrids (C_1 , C_7 , C_9 , C_{11} and C_{19}). The total mortality of all the hybrids were found to be invariably depended and positively correlated with the mortality due to NP (Fig 36).

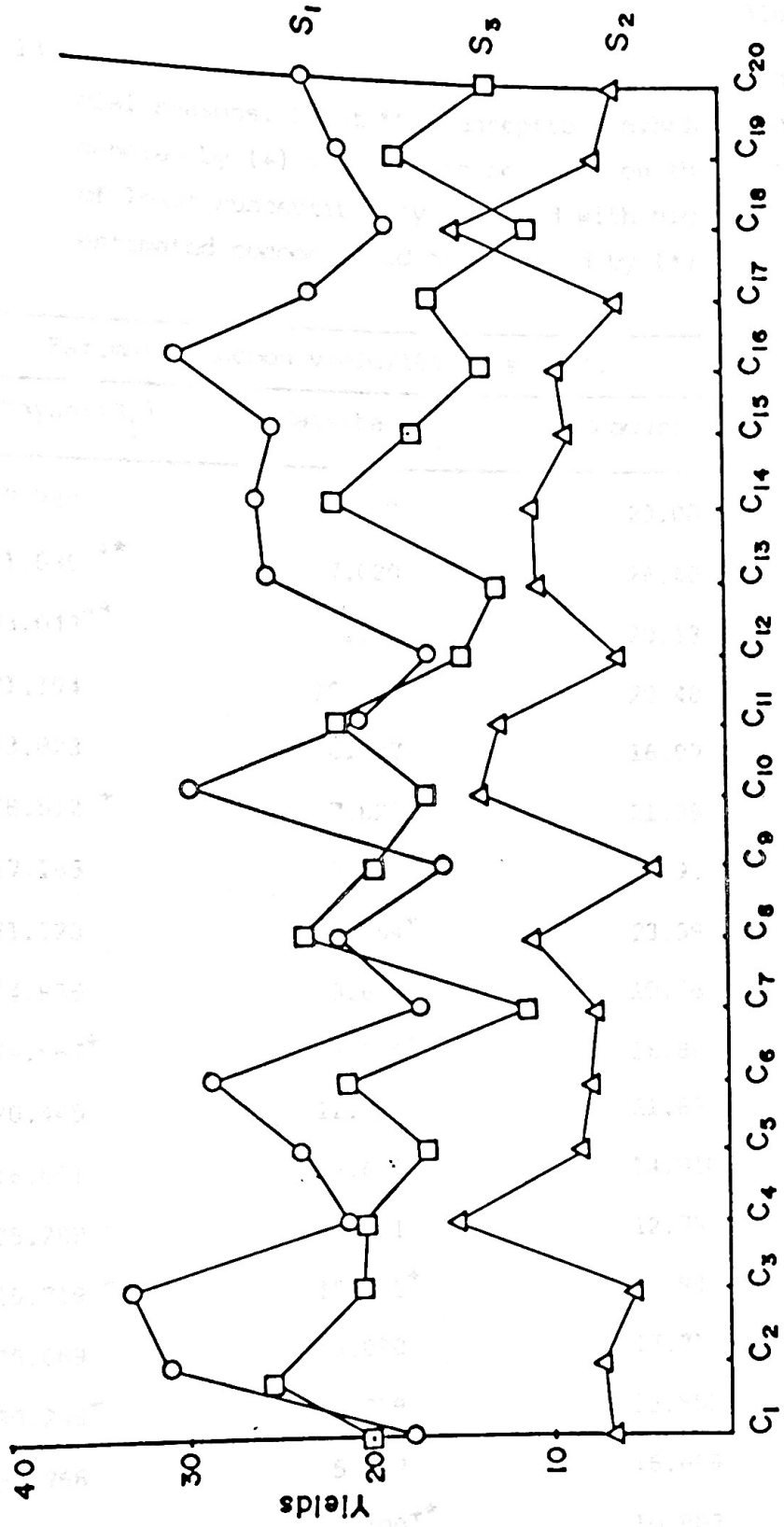
18.2.3. Percent cocoon harvest.

Induction of NP disease caused a significant difference in percent cocoon harvest among the races as well as seasons (table 11), which showed the highest cocoon harvest in C_{14} , C_2 , C_6 , C_3 , C_4 , C_8 and C_{11} (53% to 57%). S_1 season was the most favourable and S_2 was adversely unfavourable for all the hybrids (Fig 37). However, the hybrids C_2 , C_3 , C_6 , C_{10} and C_{16} were the highest yielder during this season (78-80%).

18.2.4. Average cocoon weight.

Single cocoon weight also significantly varied. The hybrids C_2 , C_{10} , C_{13} , C_{16} showed highest cocoon weight (table 11) as a general. In this case also S_1 produced best cocoons of highest weight (1.127-1.412 g), but S_2 affected badly (0.788- 1.021 g). However, the hybrids

Fig.38. Estimated cocoon yield of different crosses due to artificial induction of Nuclear polyhedrosis under different seasons.



Crosses

Table 13. Selection of suitable hybrids for different commercial seasons. Least NPV susceptible hybrids are denoted by (+) while those selected on the basis of least susceptibility combined with highest estimated cocoon yield are denoted by (*).

Hybrid code No.	Estimated cocoon yield/100 dfls (kg).		
	Agrahayani (S_1)	Jaistha (S_2)	Bhaduri (S_3)
C ₁	17.780	6.512	20.085
C ₂	31.035 ⁺⁺	7.020	26.404 ⁺⁺
C ₃	33.043 ⁺⁺	5.079	20.136 ⁺
C ₄	21.194	15.565 ⁺⁺	20.486 ⁺
C ₅	23.823	8.027	16.973
C ₆	28.512 ⁺	7.823	21.397
C ₇	17.185	7.365	10.953
C ₈	21.523	10.854 ⁺	23.587 ⁺⁺
C ₉	14.835	3.853	19.782
C ₁₀	29.667 ⁺	13.796 ⁺	16.884 ⁺
C ₁₁	20.445	12.732	21.837 ⁺
C ₁₂	16.601	5.663	14.810
C ₁₃	25.288	10.251	12.737
C ₁₄	25.719 ⁺	10.575 ⁺	21.944 ⁺
C ₁₅	25.069	8.890	17.713
C ₁₆	30.248 ⁺	9.218	13.553
C ₁₇	22.766	5.807	16.609
C ₁₈	18.745	15.100 ⁺⁺	10.867
C ₁₉	21.174	7.096	18.867
C ₂₀	23.159	5.965	13.141

C_2 , C_3 , C_4 , C_{13} and C_{16} (1.320-1.412 g) produced highest weight in S_1 ; C_7 , C_{10} , C_{18} , C_{19} (0.982-1.077 g) in S_2 and the hybrids C_5 , C_7 , C_{10} , C_{13} and C_{16} (1.304 - 1.521 g) showed the highest during S_3 .

18.2.5. Selection of suitable hybrids.

Mean cocoon yield per 100 dfls. (disease free layings) of the twenty hybrids studied for three seasons were estimated on the basis of maximum number of cocoons harvested and presented in table 13 & Fig 38. The hybrids showing relatively less susceptibility to NP are marked by (+) and those showing relatively highest estimated cocoon yield by (*). Thus, on the basis of NP susceptibility, the hybrids C_2 , C_3 , C_6 , C_{10} , C_{14} and C_{16} were identified as suitable for S_1 ; hybrids C_4 , C_8 , C_{13} , C_{14} , C_{15} and C_{18} for S_2 and finally C_2 , C_3 , C_4 , C_8 , C_{10} , C_{11} and C_{12} for S_3 season. On the other hand, based on the highest estimated cocoon yield per 100 dfls, the hybrids C_2 , C_3 and C_{16} were identified suitable for S_1 , C_4 and C_{18} for S_2 and C_2 and C_8 for S_3 seasons. However, both the selection criteria were considered together, then the hybrids C_2 , C_3 and C_{16} stand suitable for S_1 ; hybrids C_4 and C_{18} for S_2 and hybrids C_2 and C_8 for S_3 seasons.

18.3. Feeding of antiviral chemicals for controlling viral diseases of silkworm.

Feeding of antiviral chemicals, specially phenol significantly reduced the viral mortality ($p < 0.01$) in silkworm larvae (Appendix table XXXIII). In both the seasons under consideration phenol was proved highly

Table 14. Effect of feeding antiviral chemicals on the viral disease of silkworm.

Chemicals used	Dose %	Larval mortality due to viral disease (%)		
		June	August	Mean
Potassium permanganate	0.01	26.0	15.5	20.75
	0.03	26.0	16.2	21.08
	0.05	33.0	18.5	25.75
	0.08	34.0	16.5	25.25
	0.1	31.3	12.5	21.90
Phenol	1.0	16.3	18.8	17.58
	1.5	19.3	12.7	16.00
	2.0	21.3	16.5	18.92
	2.5	25.3	10.7	18.00
	3.0	23.3	13.3	18.33
Formalin	0.5	25.0	23.5	24.25
	0.8	33.0	24.2	28.58
	1.0	30.0	43.0	36.50
	1.5	34.3	58.0	46.17
	2.0	38.3	59.0	48.67
Normal feeding		32.3	25.5	28.92
Lsd ($p < 0.05$)		7.1	9.9	8.55
($p < 0.01$)		10.3	14.4	12.16

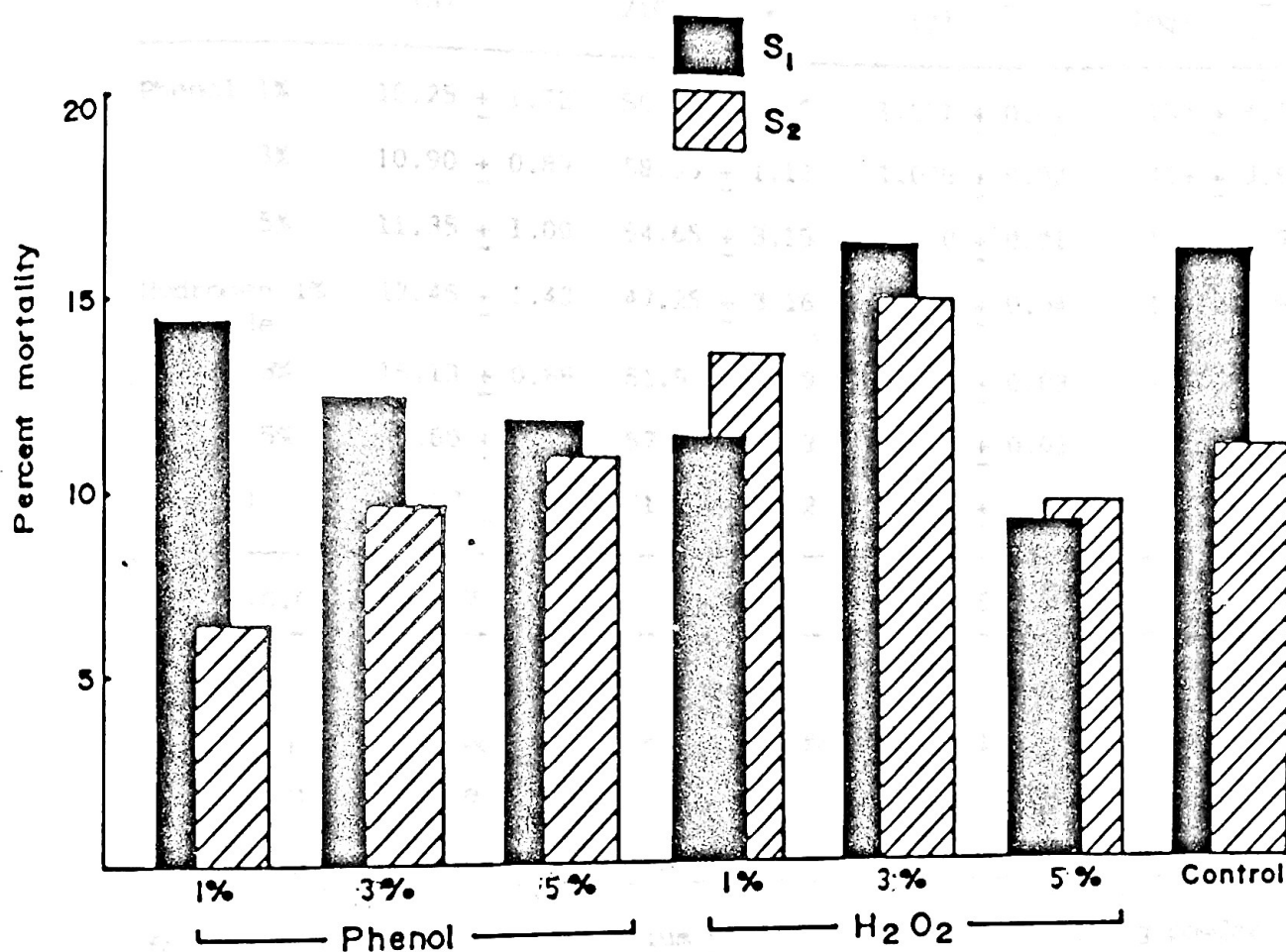
effective in controlling the disease compared to other chemicals as well as control group, the lower doses of 1.0% and 1.5% were more effective (Table 14). Feeding of potassium permanganate solution also caused lower mortality than the control, but the difference is insignificant. Feeding of formalin even at lower concentration produced adverse effect of increased viral mortality in both the seasons. The resultant mean viral mortality ranged from 16.0% in phenol (1.5%) solution to as much as 48.67% in formalin (2.0%) solution. The non-treated control group showed a moderate range upto 28.92%.

18.4. Spraying of antiviral chemicals for controlling viral disease in silkworm.

Spraying of the aqueous solutions of phenol and hydrogen peroxide directly over silkworms significantly ($p < 0.01$) lowered viral incidence in silkworm (Appendix table XXXIV). Treatment of 1% and 3% phenol and 5% hydrogen peroxide alongwith reduction in viral mortality, improved the effective rate of rearing, compared to nontreated control batch (Table 15).

The spraying of these chemicals did not affect the yield and quality of cocoons. Seasonwise effect of spraying these chemicals on the control of viral disease is shown in Fig. 39. From the figure it is clearly visible that there is a marked seasonal impact over treatments showing different results. But the high dose (5%) of both the chemicals were found more stable and showed similar performance in both the seasons.

Fig. 39. Effect of chemical spray on the control of viral diseases
of silkworm.



1% and live that on the larval mortality due to different diseases and exposed characters are shown in table 16. As per the data in the table that some of the disinfectants effectively reduced the larval mortality at significant level (Appendix table 16) and as a result of the interaction between treatments and disease was highly significant (Table 17).

Table 15. Effect of spraying some antiviral chemicals on different economic characters.

Treatments	Characters considered			
	Viral $\pm$ SE (%)	ERR $\pm$ SE /100 worms	Cocoon wt $\pm$ SE (g)	Shell wt $\pm$ SE (mg)
Phenol 1%	10.25 $\pm$ 1.72	56.13 $\pm$ 2.05	1.071 $\pm$ 0.01	139 $\pm$ 4.24
3%	10.90 $\pm$ 0.89	58.33 $\pm$ 1.13	1.038 $\pm$ 0.02	139 $\pm$ 3.67
5%	11.35 $\pm$ 1.00	54.65 $\pm$ 3.15	1.050 $\pm$ 0.01	144 $\pm$ 1.70
Hydrogen 1% peroxide	12.45 $\pm$ 1.43	47.25 $\pm$ 3.16	1.173 $\pm$ 0.04	151 $\pm$ 1.89
3%	16.13 $\pm$ 0.88	51.55 $\pm$ 2.59	1.072 $\pm$ 0.03	151 $\pm$ 2.13
5%	9.60 $\pm$ 0.27	57.62 $\pm$ 3.53	1.055 $\pm$ 0.02	148 $\pm$ 1.89
Control	13.67 $\pm$ 1.31	51.17 $\pm$ 2.22	1.079 $\pm$ 0.01	144 $\pm$ 2.49
Lsd ($p < 0.05$)	2.77	5.99	0.053	

18.5. Use of selected bed disinfectants for controlling viral and bacterial diseases.

The effect of using five selected bed disinfectants, namely formalin 2%, phenol 3%, potassium permanganate 0.1%, bleaching powder 5% and lime dust on the larval mortality due to different diseases and cocoon characters are shown in table 16. As could be seen in the table that none of the disinfectants effectively reduced the larval mortality at significant level (Appendix table XXXVIII) as a general. But the interaction between treatment and disease was highly significant ($p < 0.01$).

Among the tested disinfectants, phenol and bleaching powder reduced both the viral incidence and total loss at reasonable extent. Application of these two chemicals also resulted increased percentage of cocoon harvest, average cocoon and shell weights compared to others and non-treated control batches. Analysis of variance on these characters, however, showed an insignificant difference (Appendix tables XL, XLI) except shell weight which significantly differed ($p < 0.001$) among the treatments (Appendix table XLII).

Table 16. Effect of some bed disinfectants on different commercial traits of silkworm.

Disinfectants used	Observed mean value of different characters						
	Viral disease %	Bacterial disease %	Deficiency disease %	Total loss %	Cocoon harvest %	Cocoon wt(g)	Shell wt(mg)
Formalin 2%	13.33	1.66	1.00	16.00	80.17	1.121	149
Phenol 3%	2.50	2.33	3.66	8.50	89.83	1.168	190
Potassium permanga- nate 0.1%	11.16	2.50	1.16	14.83	80.83	1.078	172
Bleaching powder 5%	3.16	1.16	3.33	7.67	89.83	1.130	184
Lime dust	9.00	4.33	3.00	16.33	79.16	1.115	173
Water sprayed control	6.83	1.50	2.00	15.67	79.50	1.158	169
Non-treated control	12.00	2.66	1.66	10.17	84.66	1.158	178

18.6. In vitro control of bacteria pathogenic to silkworm.

Bactericidal effectiveness of the six chemicals used in the experiment is shown in table 17. Of the six chemicals used against bacterial infection, bleaching powder was highly effective in inhibiting the growth of all the four bacterial species used. Killing efficiency was noticed in all the dosages of bleaching powder except for 1% active chlorine where only two colonies were noted in case of Streptococcus sp. The most effective chemical for killing all the bacteria was 1% silver nitrate. Formalin was effective against three bacteria. Potassium hydroxide and phenol possessed both bactericidal^{cid} as well as bacteriostatic properties for different types of bacteria considered in this experiment. Ammonium chloride, however, was found to be completely ineffective.

All the three dosages of phenol were effective against Micrococcus sp., but 2% and 3% were effective against Bacillus sotto and Serratia marcescens. The later was comparatively more sensitive and Streptococcus was comparatively resistant to the chemicals used in this experiment.

From the observations, the minimum effective dose of the tested chemicals for killing specific pathogenic bacteria may be summarized as follows:

Streptococcus sp.: Silver nitrate (1%), Bleaching powder (2% a.c.),
Formalin (1%).

Table 17. Efficacy of certain chemicals in inhibiting the growth of bacteria pathogenic to silkworm in vitro.

Chemicals used	Concentration %	Bacterial species			
		<u>Streptococcus</u>	<u>Micrococcus</u>	<u>B.sotto</u>	<u>S.marcescens</u>
Phenol	1.0	+	-	+	++
	2.0	+	-	-	-
	3.0	+	-	-	-
Silver nitrate	0.1	-	-	+	+
	0.5	+	-	-	-
	1.0	-	-	-	-
Bleaching powder	1.0 a.c.*	+	-	-	-
	2.0 a.c.	-	-	-	-
	3.0 a.c.	-	-	-	-
Formalin	0.5	+	-	-	+++
	1.0	-	-	-	+++
	1.5	-	-	-	+++
Potassium hydroxide	2.0	+	++	-	-
	4.0	+	++	-	-
	6.0	+	-	-	-
Ammonium chloride	1.0	+++	+++	++	+++
	2.0	+++	+++	++	+++
	3.0	+++	+++	++	+++
Control		+++	+++	+++	+++

* Active chlorine.

Micrococcus sp. : Phenol (1%), silver nitrate (1%), Bleaching powder (1% a.c.), Formalin(0.5%),potassium hydroxide (6%).

Bacillus thuringiensis var sotto : Phenol (2%), silver nitrate (0.5%), Bleaching powder (1% a.c.) Formalin (0.5%) and potassium hydroxide (2%).

Serratia marcescens : Phenol (2%), silver nitrate (0.5%), Bleaching powder (1% a.c.) and potassium hydroxide (2%).

18.7. Application of antibiotics for controlling viral and bacterial diseases of silkworm.

Effect of using some antibiotics as a feed additive along with mulberry leaves for controlling viral and bacterial diseases is summarized in table 18. Mean results showed that all the tested five antibiotics fed for twelve hours have certain effect in reducing viral incidence in silkworm, although the difference between treatments was insignificant (table 19). Similar effect was observed in case of bacterial disease also. From the results the inference may be drawn that application of antibiotics (as tested) as feed additive have no effect on the control of these two diseases. The interactions between treatment x disease and also treatment x season were found insignificant in F-test analysis (table 19).

Table 18. Effect of feeding antibiotics on viral and bacterial diseases of silkworm.

Antibiotics	Dose PPM	Viral mortality %			Mean $\pm$ SE			Bacterial mortality%			Mean $\pm$ SE	
		May	July	September	May	July	September	May	July	September		
Terramycine	1000	2.0	22.3	5.7	10.0 $\pm$ 6.24	1.0	2.7	2.3	2.0 $\pm$ 0.51			
	2000	2.3	20.0	3.0	8.4 $\pm$ 5.79	1.3	3.0	2.0	2.1 $\pm$ 0.49			
	3000	1.0	22.3	4.8	9.3 $\pm$ 6.56	0.3	1.8	1.5	1.2 $\pm$ 0.45			
Chloram-phenicol	1000	0.3	16.5	5.7	7.7 $\pm$ 4.59	1.7	1.2	1.2	1.3 $\pm$ 0.16			
	2000	1.0	17.5	5.3	7.7 $\pm$ 5.11	2.0	0	2.0	1.3 $\pm$ 0.66			
	3000	2.0	14.3	5.5	7.2 $\pm$ 3.66	4.7	0.8	2.2	2.5 $\pm$ 1.14			
Strepto-mycine	1000	2.0	19.3	2.8	8.0 $\pm$ 5.64	0.7	3.2	4.7	2.8 $\pm$ 1.16			
	2000	0.7	19.2	4.5	8.1 $\pm$ 5.64	0.7	2.3	1.0	1.3 $\pm$ 0.49			
	3000	3.0	16.2	4.7	7.9 $\pm$ 4.15	2.0	2.3	1.7	2.0 $\pm$ 0.17			
Tetracycline	1000	1.7	18.7	7.3	9.2 $\pm$ 5.00	0.3	4.8	2.0	2.3 $\pm$ 1.31			
	2000	1.7	18.5	8.3	9.5 $\pm$ 4.89	0.3	3.0	5.2	3.4 $\pm$ 1.57			
	3000	1.3	19.3	8.8	9.8 $\pm$ 5.22	1.0	4.8	1.5	1.8 $\pm$ 0.60			
Sulfa-thiazole	1000	2.0	20.0	7.2	9.7 $\pm$ 5.35	0	6.2	1.8	2.6 $\pm$ 1.84			
	2000	0.7	12.5	7.0	6.7 $\pm$ 3.41	0.3	2.5	2.0	1.6 $\pm$ 0.66			
	3000	1.3	18.2	9.8	9.7 $\pm$ 4.88	0.7	4.2	2.2	2.3 $\pm$ 1.01			
Control	-	0	27.7	10.0	12.5 $\pm$ 8.10	3.7	0.8	3.7	2.7 $\pm$ 0.96			

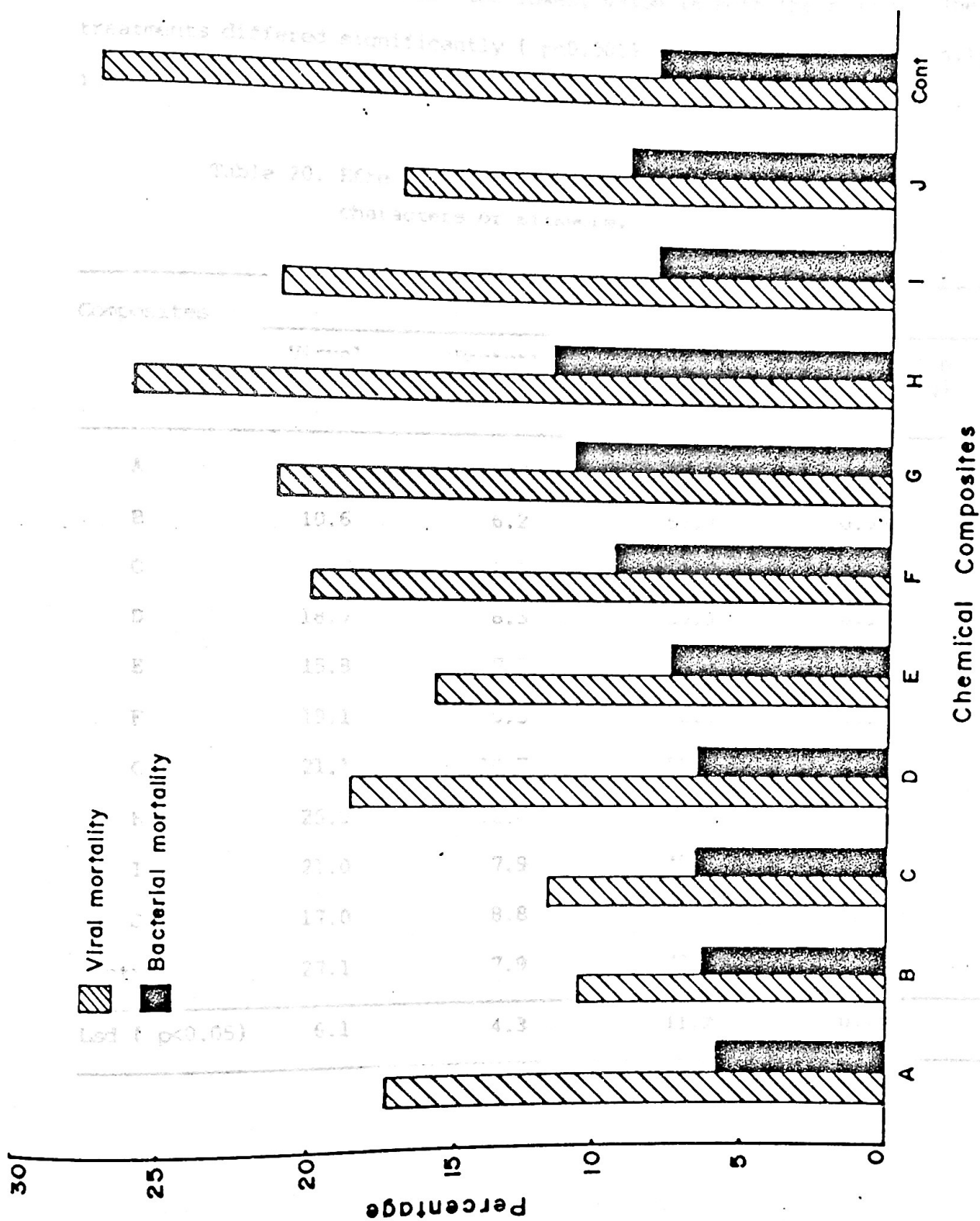
Table 19. Analysis of variance on the effect of antibiotics on viral and bacterial diseases of silkworm.

Source of variance	Degrees of freedom	Sum of squares	Mean squares	variance ratio
Between treatments	15	211.75	14.12	0.66 NS
Between diseases	1	3334.72	3334.72	155.37***
Between seasons	2	4477.14	2238.57	104.29***
Between replications	2	15.20	7.60	0.35NS
Interaction between treatment x disease	15	116.11	7.74	0.36NS
Interaction between treatment x season	30	315.74	10.52	0.49NS
Residual	223	4786.31	21.46	
Total	287	13256.97	46.19	

18.8. Dusting of some formulated chemical composites for controlling viral and bacterial diseases of silkworm.

Effect of dusting ten formulated composites on the viral and bacterial mortality have been shown in Fig 40. which clearly indicated that the composites B and C noticeably reduced the larval mortality due to viral disease to only 10.6% and 11.7% respectively as against 27.1% in the non-treated control. Other composites except composite - H also lowered viral incidence than the control batch. F-test analysis indicated

Fig. 40. Effect of dusting chemical composites for controlling
viral and bacterial diseases of silkworm.



that the disease was influenced by the seasonal climates and differed significantly ($p < 0.001$) from each other (Appendix table XLV). However, the composites B and C showed the lowest value in both the seasons. The treatments differed significantly ($p < 0.001$) among themselves, specially in controlling viral disease and effective rate of rearing (table 21).

Table 20. Effect of some chemical composites on different characters of silkworm.

Composites	Characters studied			
	Virval disease %	Bacterial disease %	ERR %	Av. cocoon weight (g)
A	17.4	5.8	47.7	1.090
B	10.6	6.2	57.7	0.997
C	11.7	6.6	54.5	1.012
D	18.7	6.5	56.3	0.977
E	15.8	7.5	55.2	1.013
F	19.1	9.3	50.4	0.981
G	21.1	10.7	58.8	1.031
H	25.9	11.6	38.0	1.066
I	21.0	7.9	45.0	1.012
J	17.0	8.8	46.7	1.069
Control	27.1	7.9	40.9	1.079
Lsd ($p < 0.05$)	6.1	4.3	11.2	0.104

In case of bacterial disease control, however, the formulated composites showed limited progress. On the other hand, certain composites like F, G, and H enhanced the disease incidence. Although the difference between treatments showed significant value (table 21), it was insignificant when compared with the control batch.

Table 21. ANOVA table showing mean squares of different characters in the analysis of variance.

Source of variance	Degrees of freedom	Mean squares			
		Viral	Bacterial	ERR	Av.cocoon wt.
Between composites	10	156.90 ^{***}	21.04 ^{**}	251.23 ^{***}	0.009 [*]
Between seasons	1	3566.95 ^{***}	284.38 ^{***}	21792.73 ^{***}	1.604 ^{***}
Between replications	2	1.79NS	1.58NS	22.20NS	0.00006NS
Interaction between composite x season	10	27.38NS	30.79 ^{**}	131.54 ^{**}	0.006NS
Residual	42	13.96	7.12	46.22	0.004

A wide variation is also observed among the populations in respect of effective rate of rearing (ERR) when dusted with different composites (table 20). Mean value of two seasons showed the highest ERR in the batches dusted with composite B (57.7%) followed by composites - D (56.3%), E(55.2%) and C (54.5%); while the lowest value was obtained

with composite H (38.0%) and the control batch (40.9%). The ERR differed significantly ($p < 0.001$) among the treatments and also between interaction with season ($p < 0.01$).

Average cocoon weight is not largely affected by dusting of different composites. The non-treated control batch showed the highest weight, while composite D resulted the lowest weight (table 21). Seasonal influence was more prominent and highly significant ($p < 0.001$) in this case as analysed in Appendix table XLVII.

CHAPTER 19.

DISCUSSION

The experimental results presented in the previous chapter exhibited several promising guidelines for suppressing viral/bacterial diseases of silkworm. However, a complete suppression was not achieved. In discussing the results obtained under different experiments one thing is clearly understood that in controlling these two diseases, optimization of the rearing environment should be the major factor of consideration. But in reality and practice, seasonal influence can not be avoided by any means. Therefore, control of either bacterial or viral pathogens must be accompanied by the prevailing situation of weather conditions, especially temperature, humidity and their fluctuations.

Thus the results presented in chapter 18.1. clearly indicated that there exists an interrelation between the silkworm strains and seasons for manifestation of nuclear polyhedrosis. Observations through five different environmental conditions reasonably proved that either high temperature ($< 28^{\circ}\text{C}$) or high relative humidity ($< 80\%$) with wide range of fluctuations or a combination of both is essential for the development of nuclear polyhedrosis irrespective of the NPV germ feeding which is in close agreement with the observations of Aruga (1961). This finding also corroborate the observations of Aratake et al (1973) who pointed out that high temperature of 30°C as well as the low temperature below 15°C , ill nutrition, overcrowding and certain chemicals act as the stressor of nuclear polyhedrosis. Their investigation supports the development of this disease by feeding NPV germs for 10-15

minutes only. This is contrary to the findings of the present study, where feeding for even six hours did not necessarily develop the disease in all the larvae. However, it was not possible to ascertain that all the larvae ingested same amount of NPV germs. Aruga and Watanabe (1964) urged that the difference in the amount of viral ingestion reflect the difference in resistance which was thought to be controlled by the Mendelian polygenes (Sidhu and Singh 1968).

Aruga (1961) pointed out that polyhedral virus pre-existent in a non-infective state and inducing stress disturbs the physiological condition of the larvae, culminate in the development of polyhedrosis. From the present findings, it is therefore, obvious that NPV germs alone can not develop the disease unless high temperature or high humidity exist as stressor. Aruga (1961) detected that pure lines are more resistant to polyhedral disease. This view was supported by Sidhu and Sing (1968) who found oily mutant strain as more resistant to polyhedral disease than the hybrids. In general, heritability scores were higher in Chinese strains compared to Japanese (Huang et al 1979).

From the foregoing discussions it may plausibly be concluded that the resistance to polyhedral disease in silkworm is an inherent character of the particular race and is different in different strains and breeds which is largely affected by the prevailing high temperature and high humidity during the rearing period.

The relatively resistant strains to NPV as screened out (SRI-oval and BSR-3) for different seasons are expected to serve the commercial purpose of cocoon production.

The twenty hybrids tested in chapter 18.2. exhibited wide range of tolerance against nuclear polyhedrosis under variant environmental conditions. Aruga (1958) pointed out some environmental factors such as, temperature, relative humidity and quality of mulberry leaves markedly influence the appearance of nuclear polyhedrosis in silkworm. However, relatively higher resistivity to NPV in the resulting hybrids must have been inherited from the conjugant parents. Of the parents, used in this experiment, SRI(o), BSR-3 and BR/84 were proved as less susceptible strains to NP disease (chapter 18.1). In the present screening, some of the hybrids showing significantly remarkable heterosis for NPV resistance did not have corresponding higher cocoon yield. Moreover, the resistivity to NPV and also higher cocoon yielding potentiality of different hybrids varied in different seasons. Aruga and Watanabe (1960) also found no universal correlation of polyhedral frequencies in induced inbred lines and untreated groups.

F_1 hybrids are reported to show extended heterosis to both NPV and CPV in the early larval stages (Aruga and Watanabe 1960, Aizawa and Furuta 1962, Aruga and Watanabe 1964), but when 5th instar larvae just after ecdysis are exposed to 5°C for 24 hours, the frequency of incidence would be higher in the hybrids than in inbred lines. On the

other hand, none of the hybrids was more resistant than parental lines when stressed by elevated temperature (Watanabe 1964), suggesting that the manifestation of heterosis on the resistance to virus infection might be partly controlled by temperature conditions. In the present experiment, elevated temperature was not used for inducing the disease, but frequently, the low temperature induced silkworms were exposed to high temperature and humidity conditions of the rearing seasons. Both these conditions were found to accelerate the disease.

Higher cocoon harvest percentage and single cocoon weight are important factors from the commercial point of view, but in the present study many hybrids showing higher values for these two attributes could not be selected due to their relatively higher susceptibility to NPV, total mortality and corresponding lower cocoon yield. However, on the basis of the performance of the hybrids tested in the present study, the hybrids C_2 (BSRI-83/2 x Nistari-m) may be recommended for S_1 (October-November) and S_3 (July-August) seasons, C_3 (BSRI-83/2 x BR/84) for S_1 , C_4 (BSRI-83/2 x SRI-o) and C_{18} (BSRI-802 x Nistari-m) for S_2 and finally C_8 (BSR-3 x SRI-o) for S_3 seasons. It is noteworthy to mention that the hybrids used in the present study were screened under the induced conditions and therefore, are expected to exhibit a better performance under natural conditions.

Feeding of antiviral chemicals especially phenol at 1% and 1.5% soaked with mulberry leaves, significantly lowered the incidence of viral

diseases in the treated larvae. Physically, the antiviral nature of phenol was first demonstrated by Watanabe (1951) who found to inactivate the silkworm virus by treating them in 2% phenol for one hour. However, its use as a feed additive for controlling the viral disease is not reported earlier. Earlier it was advocated that addition of cobalt or zinc salts with the mulberry leaves controls the spontaneous jaundice in silkworms (Gershenson 1958) although it was not supported by the sericulturists because of harmful effects of cobalt or zinc salts on larval skin. The recent workers suggest the use of less toxic chemicals like imanine 0.1% (Baburashvilli et al 1984), mayuran 0.4 mg/ml (Liu and Hou 1985), guanidine hydrochloride 0.01% (Miyajima et al 1986) etc. as a therapeutic against viral diseases. The result of the present investigation may solve an acute problem of silkworm rearing on its systematic use during unfavourable seasons. None of the tested doses of phenol showed any side effect on prolonged use.

Phenol 3% is also proved highly effective antiviral chemical as a spray on larval body as well as bed disinfectant. The next effective chemical spray is the hydrogen peroxide 5%, although Golanski (1961) found no reasonable result with this chemical. On the other hand, the author advocated the use of 0.5%-5% formalin on the larvae and mulberry leaves for controlling jaundice disease in commercial scale. In this experiment, however, formalin always showed adverse effect and enhanced the disease. This finding corresponds to the observations made by Hosny et al (1986). The other effective bed disinfectant observed in this experiment is the bleaching powder, controlling viral incidence, widely

used as general disinfectant in China. But use of only lime dust had no effect on the disease control.

A number of chemicals and doses were detected bactericidal when tested on culture media (chapter 18.6). Of the tested chemicals, bleaching powder and formalin are widely used as general disinfectant against all sorts of micropathogens of the silkworm in most of the silk producing countries. In China, bleaching powder having 0.3% active chlorine is used as a feed additive for the control of sotto disease caused by Bacillus thuringiensis var sotto. Rahman (1986) pointed out the economic advantage of using 1% chlorine containing bleaching powder over formalin as a general disinfectant. The results obtained herein, may be tried on living silkworms for control of bacterial disease. However, a test on their residual effect and also the economics needs to be evaluated before commercial applications.

None of the antibiotics used in the experiment (chapter 18.7) could produce any effect on disease control, although Afrikian (1960) found that feeding of larvae once a day with leaves soaked in solutions of streptomycine, aureomycine and tetracycline (500 units/ml) was enough for the maintenance of bacteriostatic concentrations of antibiotic in the intestine of larvae for 16-24 hours. Mladen et al (1984) suggested the use of chloramphenicol (0.25-0.50% conc.) and tylosine (500-1500 IU/cm³) once per day for the control of spontaneous nuclear polyhedrosis and flacherie in silkworm. Chinese workers also

are in favour of using chloramphenicol (500-1000 IU) as a feed additive for the control of cytoplasmic polyhedrosis, infectious flacherie as well as bacterial diseases. The ineffectiveness of the antibiotics as a therapeutic drug, however, may be questionable for its purity and products of local companies. The experiment requires further investigations with the products of several foreign companies.

Besides disease control, a number of workers found that antibiotic feeding influences the nitrogen metabolism and carbohydrate utilization (Murthy and Sreenivasaya 1953, Murthy et al 1954, Verma and Atwa 1963) and thus increases larval body weight with the subsequent increase in other economic cocoon characters (Majumdar and Medda 1975, Barman and Pasha 1985, Shidhar and Radha 1986 and Tayade et al 1986). But certain antibiotics like bruneomycine and bleomycine are reported to induce synthesis of nuclear polyhedrosis viral DNA in the pupae of silkworms (Biruykova et al 1985). In this study, however, the residual effect was not considered.

Dusting of different chemical composites posed different impacts on various economic characters. Certain composites especially A, B, C, D, E, F and J significantly decreased larval mortality due to viral disease when compared to non-treated control batch. The composite B (Lime powder, paraformaldehyde and sodium benzoat at the ratio of 94:3:3) and composite C (Lime powder, paraformaldehyde and benzoic acid at the ratio of 95:3:2) had the highest efficiency resulting in an overall decrease of 16.5% and 15.4% viral mortality compared to control.

While in India, it is claimed to decrease the disease only by 7.22% over control by the application of the most effective 'Reshamkeet oushad' (Samson et al 1987). Composites B, C, D and E also significantly improve the effective rate of rearing (54.5-57.7%) over the control (40.9%). Chinnaswamy et al (1986) reported that combination of paraformaldehyde and benzoic acid along with lime (2:2:96) on regular use reduced silkworm mortality and improved other economic characters, which is in agreement with the present findings. For bacterial disease control all the composites were proved to be ⁱⁿ⁻effective. However, the result of this finding may be utilized for commercial rearing for controlling the viral disease of silkworm.

Conclusion

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Section-F

Summary

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Appendices

CHAPTER 20.

SUMMARY

Under Bangladeshi environments, especially during hot and humid months, silkworm rearing is very much apt to the attack of bacterial and viral diseases; thereby causing a substantial loss to the cocoon growers of the country and also the silk industry. Through careful observation in different rearing seasons, four types of bacterial and three types of viral diseases affecting silkworms were detected. These are :-

1. Bacterial diseases:

1.1. Black thorax septicaemia - caused by Bacillus sp. and prevalent during high humid months of June to September.

1.2. Red-rose Septicaemia - caused by Serratia marcescens, which also occurred during high humid months.

1.3. Bacterial intoxication - caused by Bacillus thuringiensis var sotto and occurred during the period from May to August.

1.4. Bacterial gastro-enteric disease - caused by a number of bacterial species; two Bacilli sp. , Micrococcus sp. Diplococcus sp. and Streptococcus sp. prevalent during April - May.

2. Viral diseases.

2.1. Nuclear polyhedrosis or jaundice - caused by Borrelinavirus bombycis, is a threat to the sericulture industry of the country which occurs almost in three commercial rearing seasons except in chaitra.

2.2. Cytoplasmic polyhedrosis - comparatively less severe, caused by Smithiavirus bombycis. This is frequent only during autumn rearing or Bhaduri.

2.3. Gattine or infectious flacherie - found in all the commercial rearing seasons, but more frequent during Bhaduri crop, caused by a non-occluded free virus, Moratorvirus.

Incidence of both bacterial and viral diseases varied largely under the environment of different months as well as commercial seasons. The highest bacterial incidence of 19.8% was recorded in the month of October (Av. temperature 27.75°C , av. humidity 94% encountered with unsuitable immature and overmature leaves) and Jaistha crop (11.5%). But maximum viral incidence (NPV + Gattine) was observed during June (19.0%) and Bhaduri crop (23.5%). For both the diseases chaitra crop showed the lowest incidence. The incidence of bacterial, viral as well as deficiency diseases varied significantly among different silkworm races which constituted 25.60%, 38.48% and 25.20% respectively of the total mortality incurred annually.

In this study several factors affecting the susceptibility of the silkworms to flacherie and grasserie were investigated. With high temperature ($29.66^{\circ}\text{C} \pm 2.22$) and high humidity ($90.56\% \pm 6.68$) an average incidence of 11.67% bacterial and 25.33% viral diseases were recorded. The rate, however, varied significantly in ten different multi-voltine silkworm races.

The nutrition is important in the susceptibility of silkworm to both bacterial and viral diseases. Feeding of mature and oneday pre-

served leaves from 3rd to 5th instar largely affected bacterial incidence, while that of mature and water soaked leaves affected viral incidence with high frequency compared to the control groups. The quality of mulberry leaves significantly interacted with the environment of different seasons.

A study with the silkworm larvae held under stress condition of crowding, showed that more acute bacterial and viral diseases occurred in the crowded than in the uncrowded larvae. The spacing of $1250\text{cm}^2/100$ larvae caused the lowest incidence, while $625\text{cm}^2/100$ larvae caused highest incidence for both the diseases.

The feeding frequencies of three times/24 hours (8 hour's interval), four times/24 hours (6 hour's interval) and five times /24 hours (Approximately 5 hour's interval) did not affect the incidence of these diseases. But under different seasons, the treatments showed significant difference, specially three times feeding which caused highest viral incidence during May-June, had a favourable effect during June-July causing lowest incidence. It is assumed that environmental humidity has a close relationship with the feeding frequency for the outbreak of viral disease in silkworms.

Refrigeration of silkworm larvae at 5°C for 24 hours during the 5th instar caused highest bacterial disease, which was comparatively resistant to viral disease. But repeated refrigeration in the successive instars from 3rd to 5th resulted higher mortality due to nuclear polyhedrosis.

Rearing of silkworm larvae inoculated with NPV germs during six different environments resulted in an insignificant difference in mortality due to this disease. However, a comparatively higher incidence was recorded in the early three seasons (generations) which decreased in the later generations indicating certain degrees of resistance to NPV through per os feeding. On the other hand, feeding of a mixture of Streptococcus sp., Micrococcus sp., Bacillus sp. and Serratia marcescens for six gens. did not influence larval mortality due to bacterial disease; moreover the bacteria-fed line showed certain improvement in cocoon character.

Resistance to nuclear polyhedrosis disease was studied in four mutant strains and six acclimatized breeds under five different seasons of commercial importance. Variable resistance was observed among different races, but mutant races were found more resistant. From the three topmost NPV resistant races selection was given for only one race based on the combined effect on productivity due to resistance and higher percent cocoon harvest. The top ranking resistant mutant strains were SRI(Oval) and BR/84. Among breeds, BSR-3 and BSRI-83/1 also showed good resistance to NPV. Seasonal effect was found highly significant and played decisive role in the activation of the pathogens. High temperature ($< 28^{\circ}\text{C}$) and high humidity ($< 80\%$) with wide range of fluctuations or a combination of both were found as the major stressor or activator for the outbreak of nuclear polyhedrosis when NPV germs were fed to the larvae.

In selecting less susceptible multivoltine F_1 hybrids to NPV, twenty F_1 crosses were made among four local selected multivoltine races (males) and five improved acclimatized multi x bivoltine breeds (females). All the crosses were artificially induced with the NPV through refrigeration and reared under three different environmental conditions. Statistical analysis indicated highly significant differences due to NPV mortality, total loss, percent cocoon harvest and single cocoon weight among different crosses and under different environments. The Jaistha season accompanied by NPV induction showed most adverse effect for all the crosses. However, certain hybrids showing promising resistance were selected for different seasons on the basis of least NPV susceptibility and highest estimated cocoon yield per 100 disease free layings. Thus BSRI-83/2 x Nistari(m) was selected both for Agrahayani and Bhaduri, BSRI-83/2 x BR/84 for Agrahayani, BSRI-83/2 x SRI (oval) and BSRI-802 x Nistari(m) for Jaistha and finally BSR-3 x SRI (oval) were selected for Bhaduri.

An addition of phenol and potassium permanganate to the food of silkworm larvae decreased the frequency of viral mortality, especially favourable was the addition of phenol 1% and 1.5%. But feeding of formalin even at low concentration enhanced the frequency of viral mortality.

Phenol at 1% and 3% as a direct spray was also found significantly effective in reducing the viral diseases when compared to non-

treated control group. Similar effect was also noticed by spraying with hydrogen peroxide at 5% concentration. Treatment of both these chemicals also improved the effective rate of rearing without affecting the economic characters of cocoon.

Selected dose of five disinfectants were uniformly smeared on the rearing trays as prophylactic agents. They were formalin 2%, phenol 3%, potassium permanganate 0.1%, bleaching powder 5% and lime powder as dust. Of them, phenol and bleaching powder were found highly effective in reducing the viral population came from the external sources with corresponding reduction in larval mortality due to viral disease. These two disinfectants also improved percent cocoon harvest, average cocoon and shell weights of the treated batches. For bacterial disease, however, none of the disinfectants could have significant role compared to control batch.

Bactericidal effectiveness of certain chemicals, namely phenol (1%, 2%, 3%), silver nitrate (0.1%, 0.5%, 1%), bleaching powder (1%, 2%, 3% active chlorine), formalin (0.5%, 1%, 1.5%), potassium hydroxide (2%, 4%, 6%) and ammonium chloride (1%, 2%, 3%) were tested in vitro on the four pathogenic bacterial species to silkworm. The bacteria were Streptococcus sp., Micrococcus sp. Serratia marcescens and Bacillus sotto. Bleaching powder containing 2% and 3% active chlorine and 1% silver nitrate were highly effective against all the tested bacteria, while the others exhibited both bactericidal as well as bacteriostatic properties. Ammonium chloride was found completely ineffective. Minimum effective doses of the tested chemicals for killing specific bacteria were also discussed.

Enrichment of silkworm food with certain antibiotics like terramycin, chloramphenicol, streptomycin, tetracycline and sulfathiazole each at 1000, 2000 and 3000 ppm did not show any effect on disease control in the present study.

Attempts were also carried out to combat the malady of bacterial and viral diseases of silkworms through systemic application of some formulated composites on the larval body and beds. Among ten such tested composites, combinations of lime + paraformaldehyde + sodium benzoate (94:3:3) and lime powder + paraformaldehyde + benzoic acid (95:3:2) resulted in an overall decrease of 16.5% and 15.4% viral mortality compared to non-treated control batch. These two composites also significantly improved the effective rate of rearing. For bacterial disease control, however, the tested composites showed insignificant impact.

Since the etiological factors are the same for all the silkworm diseases and the etiological factors are the same for all the silkworm diseases before taking any curative measure, even in outbreaks, these two notorious diseases a large number of predisposing factors are related. Determination of actual problem becomes difficult. The outcome of the present investigation may solve the problem to a great extent. At the same time this would clarify many of the confusions which are very often raised by the traditional sericulturists as regards causal factors accentuating these diseases, especially the cases of leaf quality, spotting, frequency of feeding and germ inoculation.

As discussed earlier that onday preserved leaves although numerous bacterial diseases, it reduced viral mortality in many cases.

CHAPTER 21.

CONCLUSION

The cluster of works described in different chapters were designed in the light of actual problems which the sericulturists frequently attend in raising the silkworms under different seasons of the country. In practical sense, all the investigations carried out, were of applied in nature and any output comes through, would provide an immense impact in the field of sericulture.

The investigation clearly demonstrated the kinds of bacterial and viral diseases of silkworms under the climatic conditions of different months and seasons, their nature and extent of damages with the interactions of different ecological factors, which the cocoon growers very often face in raising the silkworms. Determination or diagnosis of the disease and the etiological factors are the prime need for all the sericulturists before taking any curative measure. When in outbreaking these two notorious diseases a large number of predisposing factors are related, determination of actual problem becomes difficult. The outcome of the present investigation may solve the problem to a great extent. At the same time this would clarify many of the confusions which are very often raised by the traditional sericulturists as regards causes or factors accentuating these diseases, especially the cases of leaf quality, spacing, frequency of feeding and germ inoculation.

As discussed earlier that oneday preserved leaves although enhances bacterial disease, it reduced viral mortality in rainy seasons.

Watersoaked leaves induces viral disease while mature leaves were always harmful for these two attributes. Spacing is very important factor for causing these diseases as was observed in this study, which confirmed that spacings below $937 \text{ cm}^2/100$ grown larvae is harmful for both the diseases. Deviation of one feeding from the normal i.e.; 3 times/24 hours or 5 times/24 hours has no relation with the outbreak of either flacherie or grasserie. Similarly, introduction of bacterial or viral pathogens through per os from the external sources can never induce the disease unless physiological status of the silkworms are disturbed by other stressors.

The investigation clearly established the relationship among host, pathogen and environment. That there is constitutional difference in the resistance or susceptibility to either flacherie or grasserie in different multivoltine races of silkworm. Therefore, selection of comparatively resistant races is one of the major factor for achieving success against these diseases. Anyway, keeping host as the constant, pathogen has nothing to do under balanced condition of environments, which include not only the temperature and humidity of the rearing period, but also the related managements which are responsible to restore or interfere with the microclimates of rearing room or rearing beds i.e.; leaf quality, spacing, feeding, bed cleaning etc. But under disturbed environmental conditions especially temperature and humidity, which the rearers have almost nothing to do with and which favours the growth of microbial population, then the application

of antibiological agents is a must to restore the microbial population at a limited extent, thereby relieving the chances of diseases.

In sericulture, absolute control of a disease by a single process has never been possible. 'Disease control' in this discipline does not mean relieve of diseased silkworm as in case of higher groups of animals and human beings. The short life span and a different biological processes in the silkworm could provide very little scope to the scientists to do something fruitful at the economic level. As a matter of fact 'silkworm disease control' may be referred to the killing of pathogenic microbes coming from external resources and also the latent microbes inhabiting the silkworms which induce disease under certain conditions. But inactivation of the microbial population inside insect body under stress condition is very limited.

In the present investigation, most of the control efforts have been carried out under stress conditions of temperature and humidity, as these two factors are supposed to be primarily responsible for bacterial and viral diseases. The experimental results discussed under the chapters 18.1, 18.2, 18.3, 18.4 and 18.8 are most important in the sense that these could reduce the larval mortality at economic level.

As discussed earlier that complete suppression of these diseases has never been possible. Therefore, the findings of these experiments should focus on this possibility in near future. As most of the experiments were designed on the basis of field condition, the results deserve chances of wide applicability under the Bangladeshi socioeconomic conditions of silkworm rearing.

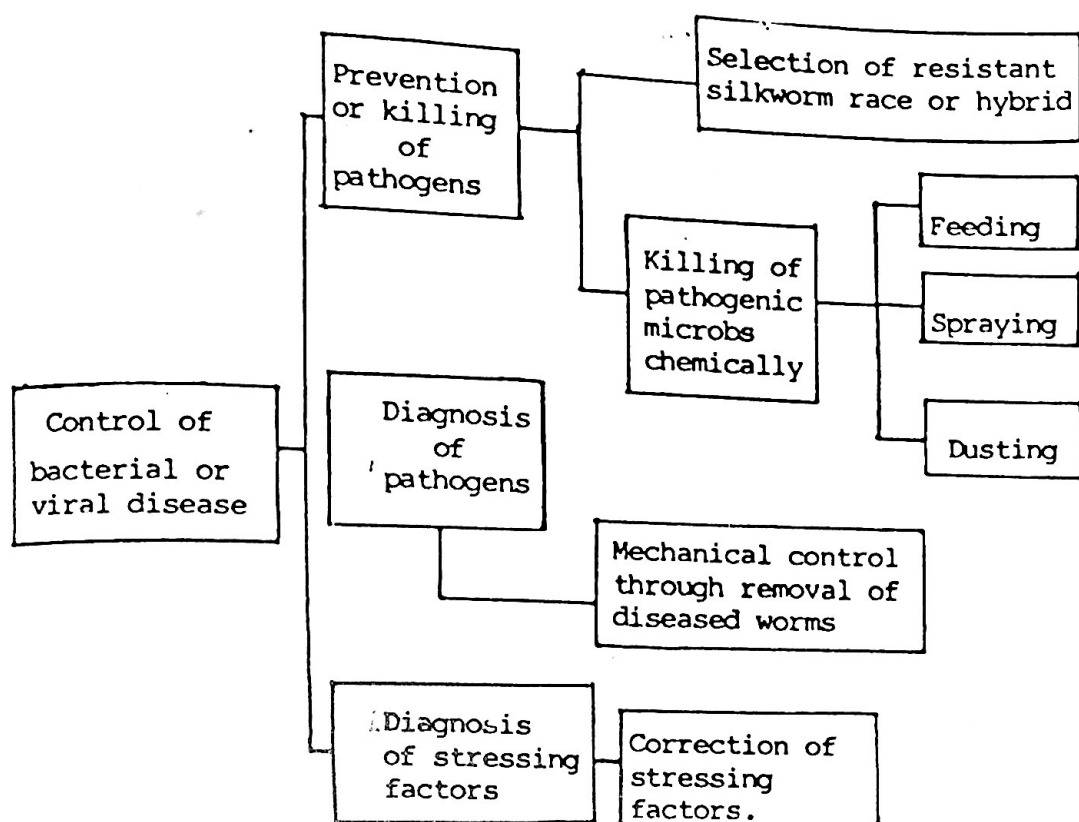


Fig. 41. Diagrammatic scheme of the method for the control of bacterial and viral diseases of silkworm.

From the facts, figures and discussions as already been accomplished in previous chapters, the thing becomes clear that in controlling or suppressing silkworm diseases of either bacterial or viral origin an "integrated method" has to be adopted rather going through a single process. The integrated method will include:- 1) selection of relatively resistant races or hybrids to these diseases for different seasons, especially summer and monsoon months, 2) correction of related ecological factors stressing disease and finally 3) application of antibacterial or antiviral agents in convenient forms. A diagrammatic scheme in this respect is suggested in Fig.41.

CHAPTER 22

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CHAPTER 2

APPENDICES

Appendix table - I

Analysis of variance showing the incidence of viral and bacterial diseases of silkworm under the environment of different months of 1986.

Months	Viral disease%			Mean $\pm$ SE	Bacterial disease%			Mean $\pm$ SE
	1	2	3		1	2	3	
January	0.5	1.0	1.5	1.0 $\pm$ 0.15	1.0	1.5	1.5	1.3 $\pm$ 0.09
February	2.3	3.0	0.3	1.9 $\pm$ 0.44	2.0	1.0	2.6	1.9 $\pm$ 0.25
March	3.0	2.0	3.0	2.7 $\pm$ 0.18	6.5	11.0	6.5	8.0 $\pm$ 0.82
May	1.5	2.0	3.0	2.2 $\pm$ 0.24	6.0	4.0	7.5	5.8 $\pm$ 0.55
June	13.5	18.5	25.0	19.0 $\pm$ 1.82	6.5	3.5	4.0	4.7 $\pm$ 0.50
July	10.5	13.0	15.5	13.0 $\pm$ 0.79	3.5	2.5	5.6	3.9 $\pm$ 0.50
August	15.5	12.0	18.0	15.2 $\pm$ 0.95	6.0	11.6	10.0	9.2 $\pm$ 0.91
September	11.5	11.5	10.0	11.0 $\pm$ 0.27	8.0	4.0	7.5	6.5 $\pm$ 0.68
October	20.5	12.0	14.5	15.7 $\pm$ 1.38	23.0	17.0	19.5	19.8 $\pm$ 0.95
November	1.0	1.0	2.0	1.3 $\pm$ 0.18	1.5	2.0	3.5	2.3 $\pm$ 0.32

Appendix table-I(continued)

Source of variance	Degrees of freedom	Sum of squares	Mean squares	variance ratio
Between months	9	1612.59	179.17	33.54***
Between diseases	1	56.65	56.65	10.60**
Between Replications	2	18.56	9.28	1.73NS
Interaction between Month x disease	9	550.97	61.22	11.46***
Residual	38	202.99	5.34	
Total	59	2441.76	41.38	

, * denote significant at 1% and 0.1% level respectively.

Calculation of multiple regression of bacterial mortality (Y) on Temperature (X_1) and relative humidity (X_2).

Y	X_1	X_2	$(Y - \bar{Y})$	$(X_1 - \bar{X}_1)$	$(X_2 - \bar{X}_2)$	$(Y - \bar{Y})^2$	$(X_1 - \bar{X}_1)^2$	$(X_2 - \bar{X}_2)^2$	$(X_1 - \bar{X}_1)(Y - \bar{Y})$	$(X_2 - \bar{X}_2)(Y - \bar{Y})$	$(X_1 - \bar{X}_1)(X_2 - \bar{X}_2)$
			(y)	x_1	x_2	y^2	x_1^2	x_2^2	$y x_1$	$y x_2$	$x_1 x_2$
1.33	20.0	84.0	-5.01	-7.35	-1.90	25.10	54.02	3.61	+38.82	+9.51	13.96
1.87	23.0	81.0	-4.47	-4.35	-4.90	19.98	18.92	24.01	+19.44	+21.90	21.31
8.00	26.5	76.5	+1.66	-0.85	-9.40	2.75	0.72	88.36	- 1.41	-15.60	7.99
5.83	28.0	80.0	-0.51	+0.5	-5.90	0.26	0.42	34.81	- 0.33	+ 3.00	-3.83
4.67	31.0	80.5	-1.67	+3.65	-5.40	2.78	13.32	29.16	- 6.09	+ 9.01	-19.71
3.87	30.0	90.5	-2.47	+2.65	+4.60	6.10	7.02	21.16	- 6.54	-11.36	12.19
4.20	31.2	90.5	-2.14	+3.90	+4.60	4.57	15.21	21.16	- 8.34	- 9.84	17.94
6.50	29.5	92.20	+0.16	+2.15	+6.10	0.02	4.62	37.21	+0.34	+ 0.97	13.11
19.80	27.7	94.0	+13.46	+0.40	+8.10	181.17	0.16	65.61	+5.38	+109.02	3.24
2.33	26.5	90.0	- 4.01	-0.85	+4.10	16.08	0.72	16.81	+3.40	- 16.44	3.48
$\bar{Y}$	$\bar{X}_1$	$\bar{X}_2$									
6.34	27.3	85.9	-	-	-	258.81	115.13	341.9	44.67	100.17	62.72
$\beta_1 = 0.2537$ $\beta_2 = 0.2464$ $\alpha = 21,764$ $R^2 = 0.139NS$ $Y = 0.2537x_1 + 0.2464x_2 - 21.764$											

Appendix table - III

Calculation of multiple regression of viral mortality (Y) on Temperature (X_1) and relative humidity (X_2).

Y	X_1	X_2	$(Y - \bar{Y})$	$(X_1 - \bar{X}_1)$	$(X_2 - \bar{X}_2)$	$(Y - \bar{Y})^2$	$(X_1 - \bar{X}_1)^2$	$(X_2 - \bar{X}_2)^2$	$(X_1 - \bar{X}_1)(Y - \bar{Y})$	$(X_2 - \bar{X}_2)(Y - \bar{Y})$	$(X_1 - \bar{X}_1)(X_2 - \bar{X}_2)$
			(Y)	x_1	x_2	y^2	x_1^2	x_2^2	yx_1	yx_2	x_1x_2
1.0	20.0	84.0	-7.28	-7.35	-1.00	52.99	54.02	3.61	53.50	13.83	13.96
1.87	23.0	81.0	-6.41	-4.35	-4.90	41.08	18.92	24.01	27.88	31.40	21.31
2.67	26.5	76.5	-5.61	-0.85	-9.40	31.47	0.17	88.36	4.76	52.73	7.99
2.17	28.0	80.0	-6.11	+0.65	-5.90	37.33	0.42	34.81	-3.97	36.04	-3.83
19.00	31.0	80.5	+10.72	+3.65	-5.40	114.91	13.32	29.16	39.12	-57.88	-19.71
13.00	30.0	90.5	+4.72	+2.65	+4.60	22.27	7.02	21.16	12.50	21.71	12.19
15.17	31.2	90.5	+6.89	+3.90	+4.60	47.47	15.21	21.16	26.87	31.69	17.94
11.00	29.5	92.0	+2.72	+2.15	+6.10	7.39	4.62	37.21	5.84	16.59	13.11
15.67	27.7	94.0	+7.39	+0.40	+8.10	54.61	0.16	65.61	2.95	59.85	3.24
1.33	26.5	90.0	-6.95	-0.85	+4.10	48.30	0.72	16.81	5.90	28.49	-3.48
$\bar{Y}$	$\bar{X}_1$	$\bar{X}_2$									
8.28	27.3	85.9	-	-	-	457.82	115.13	34.9	175.35	234.45	62.72

$$\beta_1 = 1.277 \quad \beta_2 = 0.5414 \quad \alpha = -65.41 \quad R^2 = 0.72^{***} \quad Y = 1.277x_1 + 0.4514x_2 - 65.41$$

Analysis of variance showing incidence of bacterial disease under different commercial rearing seasons (%).

Silkworm races	Jaistha			Bhaduri			Agrahayani			Chaitra		
	1	2	3	1	2	3	1	2	3	1	2	3
BSR-3	12.5	13.5	11.5	3.5	1.5	9.5	2.0	0.6	2.6	2.5	2.0	3.0
M ₂ P ₂	10.0	12.5	12.0	2.0	6.0	7.5	4.0	2.0	1.1	3.5	2.5	2.0
Nistari(S)	10.5	12.0	11.0	8.0	8.5	4.5	1.1	3.8	3.0	3.0	5.0	2.5
Nistari(M)	12.5	10.5	12.5	14.5	9.0	17.0	4.6	5.1	5.0	1.5	2.0	3.0
BSRI-801	10.0	10.0	11.0	4.0	1.5	8.5	8.1	11.1	9.0	2.5	4.0	5.0

Source of variance	Degrees of freedom	Sum of squares	Mean squares	variance ratio
Between Races	4	64.75	16.19	4.36**
Between Seasons	3	643.50	214.50	57.82***
Between Replications	2	12.87	6.43	1.73NS
Interaction between Races x seasons	12	234.44	19.53	5.26**
Residual	38	140.87	3.71	
Total	59	1096.43	18.58	

,* denote significant at 1% and 0.1% levels respectively.

Analysis of variance showing incidence of MPV₁₄ disease under different commercial rearing seasons(%).

Silkworm races	Jaistha			Bhaduri			Agrahayani			Chaitra		
	1	2	3	1	2	3	1	2	3	1	2	3
BSR-3	4.0	6.0	8.0	12.5	16.0	13.5	0.6	1.6	0.6	0.0	0.1	0.3
M ₂ P ₂	5.5	10.5	5.5	13.0	19.5	13.5	0.6	0.6	0.3	0.2	0.5	1.0
Nistari(S)	9.0	6.5	5.5	14.0	16.5	15.5	1.6	2.0	1.0	0.0	0.2	0.6
Nistari(M)	13.5	5.5	10.0	16.0	15.5	17.5	2.8	8.1	7.1	0.6	1.0	1.6
BSRI-801	3.0	4.5	6.0	16.0	19.0	12.5	2.8	6.8	3.6	2.0	1.5	1.0

Source of variance	Degrees of freedom	Sum of squares	Mean squares	variance ratio
Between races	4	62.24	15.56	4.05**
Between seasons	3	1903.81	634.60	165.26***
Between Replications	2	8.52	4.26	1.11NS
Interaction between Race x season	12	62.93	5.24	1.36NS
Residual	38	146.09	3.84	
Total	59	2183.59	37.01	

, * denot significant at 1% and 0.1% levels respectively.

Analysis of variance showing incidence of gattine disease under different commercial rearing seasons(%)

Silkworm races	Jaistha			Bhaduri			Agrahayani			Chaitra		
	1	2	3	1	2	3	1	2	3	1	2	3
BSR-3	0.0	1.5	3.5	5.5	12.5	12.0	0.0	0.3	0.2	1.0	1.5	0.0
M ₂ P ₂	1.0	3.5	0.0	5.0	9.5	9.0	0.0	0.5	0.5	3.0	2.0	1.0
Nistari(S)	4.0	5.5	2.0	8.0	10.0	2.5	0.8	1.0	0.0	2.0	1.0	1.5
Nistari(M)	5.0	4.5	6.0	12.0	5.5	13.0	0.8	0.5	0.3	3.5	1.5	1.5
BSRI -801	2.5	0.0	2.5	2.5	6.0	7.5	0.0	0.2	0.5	3.5	2.5	1.0
Source of variance	Degrees of freedom			Sum of squares			Mean squares			variance ratio		
Between races	4			29.28			7.32			1.83NS		
Between seasons	3			503.83			167.94			42.09***		
Between Replications	2			2.21			1.12			0.28NS		
Interaction between race x season	12			59.91			4.99			1.25NS		
Residual	38			151.65			3.99					
Total	59			746.88			12.66					

*** denotes significant at 0.1% level.

Analysis of variance showing incidence of deficiency disease under different commercial rearing seasons(%).

Silkworm races	Jaistha			Ithaduri			Agrahayani			Chaitra		
	1	2	3	1	2	3	1	2	3	1	2	3
BSR-3	6.0	9.0	12.0	3.0	4.5	6.0	9.1	0.0	10.6	1.5	3.5	8.5
M ₂ P ₂	3.5	8.5	12.5	2.5	3.5	7.0	0.0	1.1	0.0	7.5	1.5	3.5
Nistari(S)	6.5	11.0	4.0	7.5	8.0	4.5	1.1	1.0	1.0	4.5	1.5	5.0
Nistari(M)	11.5	9.5	19.0	7.5	9.0	11.0	10.0	13.6	11.6	7.0	4.0	2.5
BSRI-801	7.0	5.5	15.5	3.0	8.0	9.0	10.1	6.0	5.0	3.0	1.0	9.0

Source of variance	Degrees of freedom	Sum of squares	Mean squares	variance ratio
Between races	4	224.53	56.13	6.02 ^{**}
Between seasons	3	221.86	73.95	7.93 ^{**}
Between Replication	2	72.03	36.01	3.86 [*]
Interaction between race x season	12	155.86	12.97	1.39NS
Residual	38	354.36	9.32	
Total	59	1028.64	17.43	

*,** denote significant at 5% and 1% levels respectively.

Analysis of variance showing effect of high temperature and high humidity on the incidence of bacterial and viral disease (%).

Silkworm races	Bacterial mortality			Viral mortality			Total loss		
	1	2	3	1	2	3	1	2	3
BSRI-801	3.3	5.7	6.0	30.3	34.7	35.0	43.7	48.0	45.3
BSRI-802	3.7	9.3	6.7	28.0	27.3	29.3	39.0	47.3	43.3
BSRI-83/1	4.0	14.0	16.0	22.3	18.0	20.3	31.0	40.0	35.3
BSRI-83/2	21.3	14.3	10.0	47.3	28.5	32.3	81.0	48.3	63.0
BSRI-83/3	14.3	8.7	12.0	20.0	39.0	28.3	41.3	67.3	55.0
BSR-3	11.3	10.7	9.3	11.3	17.0	14.3	29.6	44.4	36.3
SRI(oval)	8.0	11.0	10.3	31.0	15.7	21.3	48.0	39.3	42.3
Nistari(S)	6.0	10.3	8.0	20.3	18.7	18.0	33.0	40.7	34.3
BR/84	7.7	9.3	8.0	23.7	28.7	26.3	39.0	44.0	41.3
NanNong	33.0	30.0	28.0	14.7	32.1	26.3	57.0	80.1	72.1

Analysis of variance showing effect of different quality mulberry leaves on the incidence of bacterial disease (IX).

Appendix table-VIII (continued)

Source of variance	Degrees of freedom	Sum of squares	Mean squares	Variance ratio
Between races	9	4148.25	460.92	11.85***
Between diseases	2	19048.80	9524.40	244.91***
Between replications	2	103.56	51.78	1.33NS
Interaction between race x disease	18	2290.71	127.26	3.27**
Residual	58	2256.02	38.89	
Total	89	27847.34	312.89	

, * denote significant at 1% and 0.1% level respectively.

Appendix table - IX

Analysis of variance showing effect of different quality mulberry leaves on the incidence of bacterial diseases(%).

Treatments	May - June			June - July			Total	Mean $\pm$ S
	1	2	3	1	2	3		
Tender	8.3	6.3	9.0	29.5	28.0	28.5	109.60	18.26 $\pm$ 4.
Mature	16.7	10.3	14.7	35.0	36.5	36.5	149.70	24.95 $\pm$ 5.
Water soaked	12.7	7.3	8.3	25.0	25.0	23.0	101.30	16.88 $\pm$ 3.
One day preserved	17.3	18.7	16.3	31.5	42.0	33.0	158.80	26.46 $\pm$ 4.
Normal	10.3	8.0	7.3	24.5	24.5	20.0	94.60	15.76 $\pm$ 3.

Source of variance	Degrees of freedom	Sum of squares	Mean squares	variance ratio
Between treatments	4	575.22	143.80	21.14***
Between seasons	1	2448.03	2448.03	359.98***
Between Replications	2	10.64	5.32	0.78NS
Interaction between treatment: x season	4	70.09	17.52	2.57NS
Residual	18	122.40	6.80	
Total	29	3226.38	111.25	Lsd(P<0.05) = 4.46

*** denotes significant at 0.1% level.

*, ** denote significant at 1% and 0.1% levels respectively.

Analysis of variance showing effect of different quality mulberry leaves on the incidence of viral diseases(%).

Treatment	May - June			June - July			Total	Mean $\pm$ SE
	1	2	3	1	2	3		
Tender	49.0	47.0	57.0	29.0	31.5	21.0	234.50	39.08 $\pm$ 5.70
Mature	74.3	52.0	60.3	31.0	34.0	22.0	273.60	45.60 $\pm$ 8.16
Water soaked	66.3	55.0	62.7	45.0	54.5	53.5	337.00	56.16 $\pm$ 3.07
One day Preserved	42.0	45.0	52.7	14.5	11.0	17.5	182.70	30.45 $\pm$ 7.42
Normal	47.0	49.5	46.5	25.5	28.0	26.5	223.00	37.16 $\pm$ 4.74

Source of variance	Degrees of freedom	Sum of squares	Mean squares	variance ratio
Between treatments	4	2270.73	567.68	15.82***
Between seasons	1	4363.30	4363.30	121.63***
Between Replications	2	14.10	7.05	0.19NS
Interaction between treatment x season	4	522.24	130.56	3.64*
Residual	18	645.71	35.87	
Total	29	7816.08	269.52	Lsd(P<0.05) =10.24

*, *** denote significant at 5% and 0.1% levels respectively.

Appendix table - XI
 Analysis of variance showing effect of different spacing on the outbreak of bacterial diseases.

Treatment Cm ² /100 worms	May-June			June-July			Total	Mean $\pm$ SE.
	1	2	3	1	2	3		
1250	5.7	5.3	4.7	7.0	6.7	6.0	35.40	5.90 $\pm$ 0.35
937	7.5	5.0	8.7	6.2	6.2	8.0	41.60	6.93 $\pm$ 0.56
750	6.8	6.2	6.8	10.2	13.0	12.2	55.20	9.20 $\pm$ 1.22
625	8.7	7.2	7.0	18.0	19.7	17.7	78.30	13.05 $\pm$ 2.45

Source of variance	Degrees of freedom	sum of squares	Mean squares	variance ratio
Between treatments	3	180.68	60.22	45.40 ***
Between seasons	1	109.65	109.65	82.66 ***
Between Replications	2	0.20	0.10	0.07NS
Interaction between treatment x season	3	109.72	36.57	27.57 ***
Residual	14	18.57	1.32	
Total	23	418.83	18.21	Lsd(P<0.05) = 1.33

***, denotes significant at 0.1% level.

Appendix table-XII
 Analysis of variance showing effect of different spacings
 on the outbreak of Viral diseases.

Treatment Cm ² /100 worms	May-June			June-July			Total	Mean $\pm$ SE
	1	2	3	1	2	3		
1250	22.5	23.0	24.4	25.7	26.7	26.0	148.3	24.7 $\pm$ 0.69
937	25.7	33.2	24.0	25.2	28.7	27.2	164.0	27.3 $\pm$ 1.35
750	38.4	36.2	35.8	33.0	28.6	32.6	204.6	34.1 $\pm$ 1.41
625	40.8	36.0	38.8	33.6	38.5	34.8	222.5	37.1 $\pm$ 1.11
750	107.5	107.25	31.10	41.29	10.52	44.74		
Source of variance	Degrees of freedom		sum of squares	Mean squares		Variance ratio		
Between treatments	3		596.36	198.78		29.85***		
Between seasons	1		13.80	13.80		2.07NS		
Between Replications	2		3.79	1.89		0.28NS		
Interaction between treatment x season	3		55.14	18.38		2.76NS		
Residual	14		93.20	6.65				
Total	23		762.29	33.14		Lsd(P<0.05) = 4.53		

***, denotes significant at 0.1% level.

Appendix table-XIII

Coefficient correlation and regression equation between spacing(X)
and Viral disease(Y).

Spacing X	(X - $\bar{X}$) $\bar{X}=890.5$ x	(X - $\bar{X}$) ² x ²	Viral disease Y	(Y - $\bar{Y}$) $\bar{Y}=30.81$ y	(Y - $\bar{Y}$) ² y ²	(X - $\bar{X}$)(Y - $\bar{Y}$) xy
1250	+359.5	129240.25	24.72	-6.08	37.08	-2189.35
937	+ 46.5	2162.25	27.33	-3.48	12.11	-161 .82
750	-140.5	19742.25	34.10	+3.29	10.82	- 462.24
625	-265.5	70490.25	37.08	+6.27	39.31	-1664.68
$\Sigma X=$ 3562	$\Sigma x=0$	$\Sigma x^2=$ 221633	$\Sigma Y=$ 123.23	$\Sigma y=0$	$\Sigma y^2=$ 99.32	$\Sigma xy=$ -4478.09

Regression equation of Y on X

$$Y - \bar{Y} = \frac{\Sigma xy}{\Sigma x^2} (X - \bar{X})$$

$$Y - 30.81 = \frac{-4478.09}{221633} (X - 890.5)$$

$$Y = 48.8 - 0.020X$$

$$\text{and } r = -0.954^{***}$$

Analysis of variance showing effect of different feeding treatments on the outbreak of bacterial disease.

Appendix table -XIV

Coefficient correlation and regression equation between spacing(X) and bacterial disease(Y).

Spacing X	(X - $\bar{X}$) $\bar{X}=890.5$	(X - $\bar{X}$) ² x ²	Bacterial disease Y	(Y - $\bar{Y}$) $\bar{Y}=877$	(Y - $\bar{Y}$) ² y ²	(X - $\bar{X}$)(Y - $\bar{Y}$) xy
1250	+359.5	129240.25	5.90	-2.87	8.23	-1031.76
937	+ 46.5	2162.25	6.93	-1.84	3.38	- 85.56
750	-140.5	19742.25	9.20	+0.43	0.18	- 60.41
625	-265.5	70490.25	13.05	+4.28	18.31	-1136.34
$\Sigma X = 3562$	$\Sigma x = 0$	$\Sigma x^2 = 221633$	$\Sigma Y = 35.08$	$\Sigma y = 0$	$\Sigma y^2 = 30.10$	$\Sigma xy = -2314.07$
Regression equation of Y on X						
$Y - \bar{Y} = \frac{\Sigma xy}{\Sigma x^2} (X - \bar{X})$						
$Y - 8.77 = \frac{-2314.07}{221533} (X - 890.5)$						
Total						

***, denotes significant at $Y = 17.67 - 0.010X$

and $r = -0.895^{***}$

Appendix table xv

Analysis of variance showing effect of different feeding frequencies on the outbreak of bacterial diseases(%).

No. of feeding/ 24 hours	May - June			June- July			Total	Mean $\pm$ SE.
	1	2	3	1	2	3		
3	6.7	8.3	4.0	8.5	11.5	14.5	53.50	8.91 $\pm$ 1.50
4	3.3	3.3	5.3	10.0	16.0	17.0	54.90	9.15 $\pm$ 2.54
5	9.3	6.7	9.0	10.0	14.5	18.0	67.50	11.25 $\pm$ 1.71

Source of variance	Degrees of freedom	Sum of squares	Mean squares	variance ratio
Between treatments	2	19.81	9.90	1.62NS
Between seasons	1	228.26	228.26	37.43***
Between Replications	2	34.02	17.01	2.78NS
Interaction between treatment x season	2	24.02	12.01	1.96NS
Residual	10	60.98	6.09	
Total	17	367.09	21.59	

***, denotes significant at 0.1% level.

**, denotes significant at 1% level.

Appendix table XVI

Analysis of variance showing effect of different feeding frequencies on the outbreak of Viral diseases (%).

No. of feeding/ 24 hours	May - June			June - July			Total	Mean $\pm$ SE
	1	2	3	1	2	3		
3	40.3	48.0	41.0	37.0	27.5	30.0	223.80	37.30 $\pm$ 3.10
4	41.0	36.3	38.3	36.0	37.0	34.5	223.10	37.18 $\pm$ 0.92
5	27.3	31.3	39.0	41.5	45.5	46.5	231.10	38.51 $\pm$ 3.17

Source of variance	Degrees of freedom	sum of squares	Mean squares	variance ratio
Between Treatments	2	6.54	3.27	0.18NS
Between seasons	1	2.72	2.72	0.15NS
Between Replications	2	3.24	1.62	0.08NS
Interaction between treatment x season	2	424.86	212.43	11.76**
Residual	10	180.54	18.05	
Total	17	617.90	36.34	

** , denotes significant at 1% level.

, * denote significant at 1% and 0.1% levels, respectively.

Appendix table - XVII

Analysis of variance between refrigeration and outbreak of bacterial disease.

Age of silkworm	April '85			June '85			Total	Mean $\pm$ SE.
	1	2	3	1	2	3		
3rd	0	0	6	12	10	9	36	6.17 $\pm$ 1.66
4th	9	0	0	14	9	2	25	4.17 $\pm$ 1.58
5th	9	12	3	33	34	22	113	18.83 $\pm$ 4.91
3rd-5th	0	6	3	0	3	0	12	2.00 $\pm$ 0.92

Source of variance	Degrees of freedom	sum of squares	Mean squares	variance ratio
Between Treatment	3	1027.46	342.49	23.57***
Between Replication	2	58.58	29.29	2.01NS
Between season	1	345.04	345.04	23.77***
Interaction between treatment x season	3	477.46	159.15	10.95**
Residual	14	203.39	14.53	
Total	23	2111.93	91.82	

, * denote significant at 1% and 0.1% levels, respectively.

Appendix table - XVIII

Analysis of variance between effect of refrigeration and outbreak of Viral disease.

Age of silkworm	April '85			June '85			Total	Mean $\pm$ SE
	1	2	3	1	2	3		
3rd	117	135	126	95	80	95	648	108 $\pm$ 7.94
4th	60	51	57	147	162	178	655	109 $\pm$ 22.12
5th	33	33	54	115	65	81	381	63 $\pm$ 11.72
3rd-5th	219	171	177	173	114	174	1028	171 $\pm$ 12.52

300 silkworms were taken in each replication.

Source of variance	Degrees of freedom	Sum of squares	Mean squares	variance ratio
Between Treatment	3	35481.46	11827.15	35.21***
Between Replication	2	1429.33	714.66	2.13NS
between season	1	2301.04	2301.04	6.85*
Interaction between treatment x season	3	22205.46	7401.82	22.03***
Residual	14	4702.67	335.90	
Total	23	66119.96	2874.79	

*, *** denote significant at 5% and 0.1% levels respectively.

Appendix table - XIX

't' test showing difference in NPV mortality between NPV germ fed and non fed control groups.

Generations	NPV fed			Control		
	1	2	3	1	2	3
G ₁	24.3	23.6	22.3	13.5	18.5	25.0
G ₂	25.3	31.0	27.0	19.7	26.0	30.7
G ₃	24.0	29.5	15.0	20.5	12.0	14.5
G ₄	0	0	0	0.5	1.0	1.5
G ₅	0.5	1.0	2.0	3.0	2.0	3.0
G ₆	1.0	0.6	6.0	1.5	2.0	3.5

	<u>NPV fed</u>	<u>Control</u>
$\bar{X}$	12.95	11.02
SD	12.53	10.25
SE	2.95	2.41
SEd	$= \sqrt{2.95^2 + 2.41^2}$	
	$= 2.31$	
't'	$= \frac{D}{SEd} = \frac{1.93}{2.31} = 0.83 \text{ NS. (D= difference between two means)}$	

Appendix table - XX

't' test showing difference in total mortality between NPV germ fed and non fed control groups.

Generations	NPV			Control		
	1	2	3	1	2	3
G ₁	36.0	34.0	33.0	23.0	20.0	29.0
G ₂	47.0	47.3	44.0	57.3	61.0	70.7
G ₃	29.5	35.0	21.0	23.0	17.0	19.0
G ₄	0.5	2.0	1.0	3.5	5.0	3.0
G ₅	3.0	5.5	2.5	6.5	11.0	6.5
G ₆	7.7	8.7	10.7	11.5	9.5	16.0

	<u>NPV fed</u>	<u>Control</u>
$\bar{X}$	20.46	21.83
SD	17.52	20.46
SE	4.13	4.82

$$SEd = \sqrt{4.13 + 4.82}$$

$$= 2.99$$

$$'t' = \frac{D}{SEd} = \frac{1.37}{2.99} = 0.458 \text{ NS (D = difference between two means)}$$

Appendix table - XXI

't' test showing difference in average cocoon weight(mg)
between NPV germ fed and non fed control.

Generations	NPV fed			Control		
	1	2	3	1	2	3
G ₁	750	806	774	945	906	926
G ₂	822	896	829	949	943	856
G ₃	1054	893	958	1038	1078	1044
G ₄	1111	1123	1143	1098	1104	1086
G ₅	975	1038	1052	852	1039	916
G ₆	973	1049	945	834	940	751

	<u>NPV fed</u>	<u>Control</u>
$\bar{X}$	955.05	955.83
SD	124.03	106.03
SE	29.25	25.00

$$SEd = \sqrt{29.25 + 25.0}$$

$$= 7.36$$

$$'t' = \frac{D}{SEd} = \frac{0.78}{7.36} = 0.105 \text{ NS}$$

Appendix table - XXII

't' test showing difference in average shell weight(mg)
between NPV germ fed and non fed control.

Generations	NPV fed			Control		
	1	2	3	1	2	3
G ₁	104	100	98	120	125	125
G ₂	116	120	112	125	123	125
G ₃	154	138	150	153	158	151
G ₄	152	154	152	151	150	145
G ₅	162	170	168	140	148	136
G ₆	130	138	132	118	122	109

	<u>NPV fed</u>	<u>Control</u>
$\bar{X}$	136.11	134.66
SD	23.34	14.92
SE	5.50	3.51

$$SED = \sqrt{5.50 + 3.51}$$

$$= 3.00$$

$$'t' = \frac{D}{Sed} = \frac{1.45}{3.00} = 0.483 \text{ NS}$$

Appendix table - XXIII

't' test showing difference in bacterial mortality(%)
between bacteria-fed and non-fed control groups.

Generations	Bacteria-fed			Control		
	1	2	3	1	2	3
G ₁ (May)	7.5	11.0	9.3	8.5	7.5	7.0
G ₂ (July)	11.0	10.0	10.2	8.0	14.0	9.0
G ₃ (August)	9.5	9.5	10.0	12.5	14.0	12.0
G ₄ (October)	49.0	47.5	45.0	45.0	47.0	43.0

	<u>Bacteria fed</u>	<u>Control</u>
$\bar{X}$	19.12	18.95
SD	16.95	15.91
SE	4.90	4.59
SEd	$= \sqrt{4.90^2 + 4.59^2}$	
	$= 3.08$	

$$'t' = \frac{D}{SEd} = \frac{0.17}{3.08} = 0.05 \text{ NS.}$$

Appendix table - XXIV

't' test showing difference in total mortality(%)
between bacteria-fed and non-fed control groups.

Generations	Bacteria-fed			control		
	1	2	3	1	2	3
G ₁ (May)	34.0	35.5	34.5	28.0	24.0	25.5
G ₂ (July)	17.0	15.0	15.5	16.0	19.5	20.0
G ₃ (August)	40.0	30.5	32.3	35.0	31.5	30.0
G ₄ (October)	54.0	54.0	52.2	52.0	49.0	50.3

	<u>Bacteria-fed</u>	<u>control</u>
$\bar{X}$	34.54	31.73
SD	14.05	12.48
SE	4.06	3.60
SEd	$= \sqrt{4.06 + 3.60}$ $= 2.76$	
't'	$= \frac{D}{SEd} = \frac{2.81}{2.76} = 1.01 \text{ NS.}$	

Appendix table - XXV

't' test showing difference in average cocoon weight(mg)
between bacteria-fed and non-fed control groups.

Generations	Bacteria-fed			control		
	1	2	3	1	2	3
G ₁ (May)	820	790	805	820	750	815
G ₂ (July)	820	791	812	825	750	758
G ₃ (August)	1102	1037	1109	1040	1034	1030
G ₃ (October)	775	1054	840	689	705	1000

	<u>Bacteria-fed</u>			<u>control</u>		
$\bar{X}$	896.25			851.33		
SD	134.69			135.84		
SE	38.92			39.26		

$$SEd = \sqrt{38.92 + 39.26}$$

$$= 8.84$$

$$'t' = \frac{D}{SEd} = \frac{44.92}{8.84} = 5.08^*$$

Between varieties	9	211.35	24.59	1.14
Between seasons	4	13462.74	4065.85	1.91**
Between field trials	7	10.87	15.47	1.01
Interaction between variety & season	36	4419.15	122.52	4.75**
Residual	12	2401.27	200.11	
Total	149	21137.34	1777.06	

*, ** denote significant at 1% and 0.1% levels respectively.

Appendix table - XXVI

Analysis of variance showing viral mortality in different silkworm varieties under five different seasons.

Varieties	Mean viral mortality(%)					Total	Mean $\pm$ SE
	S ₁	S ₂	S ₃	S ₄	S ₅		
BSRI-801	33.3	0.2	5.2	34.5	31.1	104.3	20.8 $\pm$ 6.68
BSRI-802	39.5	0.3	5.0	32.8	27.2	104.8	20.9 $\pm$ 6.95
BSR-3	22.8	0.3	6.7	26.4	16.9	73.1	14.6 $\pm$ 4.34
Nan Nong	27.8	0.7	7.2	34.7	16.9	87.3	17.4 $\pm$ 5.60
SRI(oval)	13.2	0.2	4.2	27.4	22.7	67.7	13.5 $\pm$ 4.66
BR/84	31.8	2.2	1.7	21.7	23.8	81.2	16.2 $\pm$ 5.42
Nistari(S)	31.0	2.3	4.5	35.3	19.7	92.8	18.5 $\pm$ 6.00
BSRI-83/1	12.5	2.0	12.0	30.8	19.5	76.8	15.3 $\pm$ 4.26
BSRI-83/2	8.7	0.7	6.0	34.0	35.6	85.0	17.0 $\pm$ 6.59
BSRI-83/3	12.7	0.7	12.7	29.3	29.1	84.5	16.9 $\pm$ 4.88
Lsd(p<0.05)	12.6	1.9	5.6	12.6	9.8		

Source of variance	Degrees of freedom	sum of squares	Mean squares	variance ratio
Between varieties	9	851.38	94.59	3.14 **
Between seasons	4	19462.43	4865.60	161.45 ***
Between Replications	2	30.87	15.43	0.51 NS
Interaction between variety x season	36	4419.85	122.52	4.06 **
Residual	98	2953.27	30.13	
Total	149	27708.79	185.96	

** , *** denote significant at 1% and 0.1% levels respectively.

Appendix table - XXVII

Analysis of variance showing total mortality in different silkworm varieties under five different seasons.

Varieties	Mean total mortality(%)					Total	Mean $\pm$ SE
	S ₁	S ₂	S ₃	S ₄	S ₅		
BSRI-801	40.0	0.2	13.5	46.1	45.8	145.6	29.1 $\pm$ 8.38
B SRI-802	44.0	7.2	14.5	40.2	43.1	149.0	29.8 $\pm$ 6.99
BSR-3	40.8	1.5	16.3	34.4	29.9	122.9	24.6 $\pm$ 6.27
Nan Nong	47.7	35.5	17.0	49.1	52.6	201.9	40.4 $\pm$ 5.82
SRI(oval)	14.5	2.2	7.8	32.6	41.9	99.0	19.8 $\pm$ 6.72
BR/84	34.2	5.8	6.0	28.8	41.5	116.3	23.3 $\pm$ 6.59
Nistari(S)	33.0	5.3	10.5	40.9	36.8	126.5	25.3 $\pm$ 6.50
BSRI-83/1	15.3	4.0	18.3	38.8	35.5	111.9	22.4 $\pm$ 5.82
BSRI-83/2	16.0	1.3	16.0	38.8	64.3	136.4	27.3 $\pm$ 9.86
BSRI-83/3	24.7	1.7	20.8	38.0	48.6	133.8	26.8 $\pm$ 7.13
Lsd(p<0.05)	10.4	3.0	8.8	15.7	9.3		

Source of variance	Degrees of freedom	sum of squares	Mean squares	variance ratio
Between varieties	9	4299.03	477.67	12.98***
Between seasons	4	30939.20	7734.80	210.18***
Between Replications	2	178.25	89.13	2.42 NS
Interaction between variety x season	36	7011.70	1940.76	5.29***
Residual	96	3607.37	36.80	
Total	149	46035.55	308.96	

*** denotes saignificant at 0.1% level.

Analysis of variance showing mortality of different crosses due to
NPV disease under different seasons.

Crosses	November			April-May			July-August			Total	Mean $\pm$ SE
	1	2	3	1	2	3	1	2	3		
C ₁	10.0	14.0	10.2	18.0	16.6	19.4	16.5	15.0	14.4	134.1	14.9 $\pm$ 1.00
C ₂	8.5	2.5	5.5	17.5	17.0	22.5	1.5	1.5	5.4	81.9	9.1 $\pm$ 2.48
C ₃	6.0	8.0	2.5	25.5	18.0	11.4	5.5	4.0	6.1	87.0	9.7 $\pm$ 2.36
C ₄	13.5	24.0	20.4	15.0	12.5	22.6	6.0	6.0	12.0	132.0	14.7 $\pm$ 2.06
C ₅	6.3	6.3	7.2	17.3	14.6	23.3	6.5	9.5	14.9	105.9	11.8 $\pm$ 1.90
C ₆	3.5	7.0	5.4	19.0	17.5	28.6	7.5	9.0	8.1	105.6	11.7 $\pm$ 2.58
C ₇	9.7	18.7	12.7	24.0	37.5	27.9	20.5	28.5	33.4	222.9	24.8 $\pm$ 3.26
C ₈	10.7	6.3	3.4	13.2	14.5	15.8	6.5	7.5	7.9	85.8	9.5 $\pm$ 1.32
C ₉	13.3	11.0	12.6	29.5	26.5	31.9	13.0	21.0	13.4	172.2	19.1 $\pm$ 2.58
C ₁₀	5.5	3.0	3.5	30.0	23.5	27.5	9.5	6.5	7.1	116.1	12.9 $\pm$ 3.41
C ₁₁	8.7	11.0	10.0	38.2	41.0	23.7	8.0	6.0	8.5	155.1	17.2 $\pm$ 4.30
C ₁₂	11.2	10.3	13.0	27.5	17.0	29.6	13.1	16.0	15.0	152.7	16.9 $\pm$ 2.17
C ₁₃	8.0	4.5	7.0	15.5	11.0	18.5	37.5	18.5	25.0	145.5	16.2 $\pm$ 3.25
C ₁₄	2.0	6.7	2.1	15.5	19.5	16.0	9.5	6.5	8.0	85.8	9.5 $\pm$ 1.95
C ₁₅	12.7	8.7	8.9	10.5	14.0	20.5	14.0	6.0	10.9	106.2	11.8 $\pm$ 1.31

Crosses	November			April-May			July-August			Total	Mean + SE
	1	2	3	1	2	3	1	2	3		
C ₁₆	3.0	4.0	2.9	15.5	23.5	19.5	24.0	30.0	24.0	146.4	16.3+3.28
C ₁₇	13.3	11.7	14.3	26.5	30.0	24.5	23.0	14.0	17.0	174.3	19.4+2.10
C ₁₈	9.7	11.0	11.1	11.0	25.5	10.6	26.0	15.0	28.6	148.5	16.5+2.46
C ₁₉	11.7	11.3	9.7	15.5	34.5	28.6	15.5	14.0	27.5	168.3	18.7+2.83
C ₂₀	7.7	8.3	5.0	26.0	20.5	29.4	17.0	17.5	21.6	153.0	17.0+2.66
Source of variance	Degrees of freedom			sum of squares			Mean squares			variance ratio	
	1	2	3	1	2	3	1	2	3		
Between crosses	19			2911.05			153.21			8.16	**
Between seasons		2		4935.42			2467.71			131.47	***
Between Replications		2		36.95			18.48			0.98NS	
Interaction between Cross x season		38		3658.51			96.28			5.13	**
Residual		118		2215.00			18.77				
Total		179		13756.93			76.85				

, * denote significant at 1% and 0.1% levels respectively

Appendix table - XXIX

Analysis of variance showing total larval mortality in different crosses due to artificial induction of NPV disease.

Crosses	November			April-May			July-August			Total	Mean±SE
	1	2	3	1	2	3	1	2	3		
C ₁	35.0	39.7	36.3	35.3	50.6	57.2	30.0	40.0	33.5	357.6	39.7±2.74
C ₂	30.0	16.5	23.1	35.5	38.0	41.1	14.0	12.5	8.6	219.3	24.4±3.81
C ₃	19.0	18.0	26.9	44.0	41.0	30.8	22.0	21.5	30.9	254.1	28.2±2.94
C ₄	43.5	39.0	25.5	30.5	35.0	46.1	20.5	16.5	32.5	289.1	32.1±3.15
C ₅	22.7	21.6	26.8	35.3	27.9	46.0	25.0	27.0	39.0	271.3	30.1±2.57
C ₆	19.0	24.5	29.7	36.5	46.0	38.7	29.0	30.0	29.5	282.9	31.4±2.50
C ₇	36.4	48.4	41.8	45.0	68.5	41.0	33.5	49.0	49.0	412.6	45.8±3.18
C ₈	31.7	14.7	15.4	32.5	36.5	27.9	21.5	31.0	17.1	228.3	25.4±2.60
C ₉	32.2	27.8	25.5	53.5	43.0	47.5	44.0	43.5	33.1	350.1	38.9±3.00
C ₁₀	18.5	21.0	15.4	39.5	41.0	41.0	25.5	28.5	25.6	256.0	28.4±3.10
C ₁₁	28.1	31.6	30.9	61.2	50.2	47.0	21.0	27.5	18.4	315.9	35.1±4.53
C ₁₂	29.0	32.3	43.4	42.0	26.0	43.0	26.0	40.0	33.9	315.6	35.1±2.26
C ₁₃	20.0	19.5	19.6	28.5	23.0	28.6	48.0	32.5	35.6	255.3	28.4±2.95
C ₁₄	16.3	26.0	12.6	25.5	29.0	22.3	26.0	17.5	20.1	195.3	21.7±1.71
C ₁₅	30.0	27.0	30.3	36.5	38.0	44.0	37.0	21.5	26.7	291.0	32.3±2.20

Appendix table - XXIX (continued)

Crosses	November			April-May			July-August			Total	Mean+SE
	1	2	3	1	2	3	1	2	3		
C ₁₆	15.0	19.0	16.1	23.5	36.5	36.0	40.0	36.0	35.0	257.1	28.6+3.14
C ₁₇	26.0	18.1	35.1	39.0	40.0	32.6	37.5	32.0	30.5	290.8	32.3+2.17
C ₁₈	20.0	23.0	25.7	25.0	42.0	23.0	39.5	26.0	42.5	266.7	29.6+2.82
C ₁₉	35.7	32.7	27.3	33.5	51.5	47.0	27.0	25.5	47.7	327.9	36.4+3.10
C ₂₀	20.4	22.6	19.1	38.5	27.0	44.9	31.5	33.0	41.1	278.1	30.9+2.93

Source of variance	Degrees of freedom	sum of squares	Mean squares	variance ratio
Between crosses	19	5467.29	287.75	6.65**
Between seasons	2	4734.95	2367.47	54.70***
Between Replications	2	47.77	23.88	0.55NS
Interaction between Cross x season	38	4068.48	107.06	2.47**
Residual	118	5407.46	43.28	
Total	179	19425.95	108.52	

, * denote significant at 1% and 0.1% levels respectively.

Appendix table - xxx

Coefficient correlation and regression equation between NPV(X)
and total mortality(Y) due to artificial induction of NPV disease.

Cross	NPV X	$(X - \bar{X})$ $\bar{X}=14.90$ x	$(X - \bar{X})^2$ x ²	Total mortality Y	$(Y - \bar{Y})$ $\bar{Y}=31.75$ y	$(Y - \bar{Y})^2$ y ²	$(X-\bar{X})(Y-\bar{Y})$ xy
C ₁	14.90	0	0	39.73	7.98	63.68	0
C ₂	9.10	-5.80	33.64	24.37	-7.38	54.46	42.80
C ₃	9.67	-5.23	27.35	28.23	-3.52	12.39	18.40
C ₄	14.67	-0.23	0.05	32.12	-0.63	0.39	0.14
C ₅	11.77	-3.13	9.79	30.14	-1.61	2.59	5.04
C ₆	11.73	-3.17	10.04	31.43	-0.34	0.11	1.07
C ₇	24.77	9.87	97.41	45.84	14.09	198.53	139.06
C ₈	9.53	-5.37	28.83	25.37	-6.38	40.70	34.26
C ₉	19.13	4.23	17.89	38.90	7.15	51.12	30.24
C ₁₀	12.90	-2.00	4.00	28.44	-3.31	10.95	6.62
C ₁₁	17.23	2.33	5.43	35.10	3.35	11.22	7.80
C ₁₂	17.30	2.40	5.76	35.07	3.32	11.02	7.96
C ₁₃	16.17	1.27	1.61	28.37	-3.38	11.42	4.29
C ₁₄	9.53	-5.37	28.83	21.70	-10.05	101.00	53.96
C ₁₅	11.80	-3.10	9.61	32.33	0.58	0.33	1.79
C ₁₆	16.27	-1.37	1.87	28.57	-3.18	10.11	4.35
C ₁₇	19.37	4.47	19.98	32.31	0.56	0.31	2.50
C ₁₈	16.50	1.60	2.56	29.63	-2.12	4.49	3.39
C ₁₉	18.70	3.80	14.44	36.43	4.68	21.90	17.78
C ₂₀	17.00	2.10	4.41	30.90	-0.85	0.72	1.78
$\sum X =$ 298.04		$\sum x =$ 0.04	$\sum x^2 =$ 323.5	$\sum Y =$ 634.98	$\sum y =$ 0.57	$\sum y^2 =$ 607.44	$\sum xy =$ 383.23

$$Y = 16.85 + 1.18X \text{ and } r = + 0.864^{***}$$

Analysis of variance showing percentage of cocoon harvest
in different crosses on artificial induction of NPV disease.

Crosses	November			April-May			July-August			Total	Mean $\pm$ SE
	1	2	3	1	2	3	1	2	3		
C ₁	45.7	40.3	48.7	20.7	37.3	17.9	55.5	52.0	55.4	373.5	41.5 $\pm$ 4.41
C ₂	68.0	80.0	74.0	19.5	34.5	18.6	83.0	59.5	68.4	501.9	55.8 $\pm$ 7.90
C ₃	81.0	84.1	79.5	24.5	18.5	19.4	65.5	61.5	55.4	483.4	53.7 $\pm$ 8.26
C ₄	41.5	56.0	54.9	67.0	53.5	43.0	60.5	54.0	49.0	478.5	53.2 $\pm$ 2.51
C ₅	64.0	68.3	72.9	37.3	24.0	29.3	49.5	47.5	31.1	423.9	47.1 $\pm$ 5.67
C ₆	80.0	65.0	74.1	35.0	26.5	26.4	70.5	56.0	64.0	497.5	55.3 $\pm$ 6.51
C ₇	46.0	42.7	53.8	24.0	26.5	24.5	25.0	22.0	37.0	301.5	33.5 $\pm$ 3.66
C ₈	71.0	49.3	53.7	45.0	36.5	37.0	58.0	53.0	76.0	479.5	53.3 $\pm$ 4.27
C ₉	40.0	44.3	44.7	17.0	16.0	15.9	54.0	57.5	58.6	348.0	38.7 $\pm$ 5.62
C ₁₀	70.5	79.0	65.4	41.0	46.0	41.1	42.5	25.0	52.5	463.0	51.4 $\pm$ 5.37
C ₁₁	63.7	54.7	55.9	46.0	43.0	40.0	63.5	49.5	61.0	477.3	53.0 $\pm$ 2.78
C ₁₂	48.2	42.4	47.4	14.5	26.0	24.0	44.5	32.5	43.9	202.5	33.7 $\pm$ 4.29
C ₁₃	61.5	64.5	53.1	38.5	32.5	42.4	36.5	22.0	33.6	384.6	42.7 $\pm$ 4.45
C ₁₄	76.9	70.0	73.5	33.0	43.5	36.0	49.5	77.5	59.5	519.2	57.7 $\pm$ 5.56
C ₁₅	72.0	60.0	70.5	37.5	31.5	33.9	61.0	42.5	45.6	454.5	50.5 $\pm$ 4.91

Appendix table - XXXI (continued)

Crosses	November			April-May			July-August			Total	Mean±SE.
	1	2	3	1	2	3	1	2	3		
C ₁₆	83.0	64.0	78.5	40.5	31.0	34.1	25.0	41.0	24.9	422.0	46.9±7.07
C ₁₇	63.3	50.7	69.6	18.5	26.0	22.1	38.5	46.0	53.8	388.5	43.2±5.72
C ₁₈	48.3	53.2	52.4	53.5	44.5	49.9	28.5	31.5	24.9	386.7	42.9±3.60
C ₁₉	47.3	60.7	61.7	20.5	25.0	22.6	70.5	41.0	52.0	405.3	45.0±5.97
C ₂₀	75.3	67.7	62.5	18.5	24.0	21.1	31.5	27.5	27.4	355.5	39.5±7.00

Source of variance	Degrees of freedom	sum of squares	Mean squares	Variance ratio
Between crosses	19	9409.42	495.23	8.66 ^{**}
Between seasons	2	28168.05	14084.02	246.35 ^{***}
Between Replications	2	416.24	208.12	3.64 [*]
Interaction between cross x season	38	16377.82	430.99	7.54 ^{**}
Residual	118	6746.68	57.17	
Total	179	61118.21	341.44	

*, **, *** denote significant at 5%, 1% and 0.1% levels respectively.

Analysis of variance showing average cocoon weight of different crosses due to artificial induction of NPV disease (g).

Crosses	November			April-May			July-August			Total	Mean $\pm$ SE
	1	2	3	1	2	3	1	2	3		
C ₁	1.380	1.280	1.300	0.870	0.889	0.815	1.225	1.276	1.198	10.223	1.137 $\pm$ 0.067
C ₂	1.414	1.383	1.397	1.176	0.867	0.858	1.145	1.226	1.385	10.851	1.205 $\pm$ 0.068
C ₃	1.408	1.433	1.305	0.846	0.788	0.808	1.147	1.039	1.126	9.900	1.100 $\pm$ 0.078
C ₄	1.455	1.336	1.382	0.972	0.947	0.937	1.351	1.201	1.207	10.788	1.198 $\pm$ 0.063
C ₅	1.209	1.135	1.139	0.921	0.906	0.831	1.276	1.250	1.449	10.116	1.124 $\pm$ 0.063
C ₆	1.017	1.318	1.229	0.840	0.976	0.854	1.000	1.223	1.110	9.567	1.063 $\pm$ 0.052
C ₇	1.194	1.234	1.190	1.052	1.009	0.885	1.276	1.470	1.166	10.476	1.164 $\pm$ 0.052
C ₈	1.140	1.303	1.268	0.959	0.894	0.895	1.308	1.223	1.243	10.233	1.137 $\pm$ 0.054
C ₉	1.216	1.092	1.142	0.824	0.806	0.734	1.142	1.200	1.147	9.303	1.033 $\pm$ 0.059
C ₁₀	1.230	1.238	1.321	1.076	1.177	0.978	1.360	1.428	1.433	11.241	1.249 $\pm$ 0.048
C ₁₁	1.257	1.227	1.035	1.013	0.950	0.998	1.315	1.217	1.233	10.245	1.138 $\pm$ 0.043
C ₁₂	1.260	1.106	1.243	0.924	0.846	0.864	1.234	1.143	1.298	9.918	1.102 $\pm$ 0.056
C ₁₃	1.452	1.374	1.410	0.883	0.896	0.933	1.449	1.428	1.272	11.097	1.233 $\pm$ 0.079
C ₁₄	1.183	1.171	1.150	0.909	1.012	0.899	1.156	1.034	1.338	9.852	1.094 $\pm$ 0.044
C ₁₅	1.215	1.253	1.246	0.891	0.892	0.809	1.147	1.148	1.269	9.870	1.096 $\pm$ 0.056

Crosses	November			April-May			July-August			Total	Mean±SE.
	1	2	3	1	2	3	1	2	3		
C ₁₆	1.321	1.354	1.279	0.967	0.843	0.809	1.555	1.492	1.426	11.046	1.227±0.088
C ₁₇	1.234	1.299	1.187	0.864	0.867	0.885	1.304	1.190	1.109	9.939	1.104±0.057
C ₁₈	1.303	1.164	1.187	1.079	0.952	1.032	1.276	1.250	1.314	10.557	1.173±0.040
C ₁₉	1.227	1.222	1.208	1.030	1.153	0.943	1.094	1.148	1.109	10.134	1.126±0.029
C ₂₀	1.139	1.133	1.109	0.988	0.939	0.887	1.440	1.451	1.672	10.758	1.195±0.083

Source of variance	Degrees of freedom	sum of squares	Mean squares	variance ratio
Between crosses	19	0.5946	0.0313	5.59**
Between seasons	2	4.4670	2.2335	398.84***
Between Replications	2	0.0228	0.0114	2.03NS
Interaction between cross x season	38	0.9542	0.0251	4.48**
Residual	118	0.6634	0.0056	
Total	179	6.072	0.0374	

, * denote significant at 1% and 0.1% levels respectively.

Analysis of variance between feeding antiviral chemicals and NPV disease(%).

Treatments	Dose (%)	June			August			Total	Mean $\pm$ SE
		1	2	3	1	2	3		
Potassium permanganate	0.01	19.0	27.0	32.0	12.0	19.5	15.0	124.5	20.7 $\pm$ 2.78
	0.03	32.0	21.0	25.0	16.5	12.0	20.0	126.5	21.1 $\pm$ 2.58
	0.05	31.1	33.0	35.0	21.0	12.0	22.5	154.5	25.7 $\pm$ 3.27
	0.08	34.0	36.0	32.0	14.5	22.0	13.0	151.5	25.2 $\pm$ 3.77
	0.1	29.0	36.0	29.0	10.0	16.0	11.5	131.5	21.9 $\pm$ 4.01
Phenol	1.0	13.0	15.0	21.0	19.0	20.0	17.5	105.5	17.6 $\pm$ 1.14
	1.5	19.0	19.0	20.0	12.0	8.0	18.0	96.0	16.0 $\pm$ 1.80
	2.0	18.0	17.0	29.0	14.5	20.5	14.5	113.5	18.9 $\pm$ 2.00
	2.5	21.0	28.0	27.0	11.0	9.0	12.0	108.0	18.0 $\pm$ 3.15
	3.0	25.0	23.0	22.0	10.0	20.5	9.5	110.0	18.3 $\pm$ 2.54
Formalin	0.5	33.0	19.0	23.0	25.0	24.5	21.0	145.5	24.2 $\pm$ 1.80
	0.8	35.0	31.0	33.0	27.5	23.0	22.0	171.5	28.6 $\pm$ 1.96
	1.0	30.0	33.0	27.0	57.0	36.0	36.0	219.0	36.5 $\pm$ 3.97
	1.5	33.0	36.0	34.0	54.5	58.0	61.5	277.0	46.2 $\pm$ 4.91
	2.0	23.0	45.0	47.0	66.0	38.0	73.0	292.0	48.7 $\pm$ 6.84
Normal	-	29.0	32.0	36.0	38.0	20.0	18.5	173.5	28.9 $\pm$ 3.03

Source of variance	Degrees of freedom	sum of squares	Mean squares	variance ratio
Between treatment	15	5770.58	584.70	7.28**
Between seasons	1	392.04	392.04	3.95*
Between Replications	2	35.29	17.64	0.22NS
Residual	77	6183.92	80.31	
Total	95	15381.83	161.91	

*,** denote significant at 5% and 1% levels respectively.

Appendix table - XXXIV

Analysis of variance between chemical spray and viral disease(%)

Treatment	July-August			September-October			Total	Mean+SE
	1	2	3	1	2	3		
T ₁	15.0	14.0	14.0	4.9	7.6	6.0	61.5	10.25+1.86
T ₂	11.5	12.0	13.5	7.3	12.3	8.8	65.4	10.90+0.96
T ₃	16.0	9.5	10.0	13.0	9.3	10.3	68.1	11.35+1.08
T ₄	14.5	8.5	11.5	19.0	9.6	11.6	74.7	12.45+1.55
T ₅	15.5	19.5	15.5	13.2	18.3	14.8	96.8	16.13+0.95
T ₆	9.5	8.5	10.0	9.0	10.3	10.3	57.6	9.60+0.29
T ₀	13.5	17.5	19.0	11.5	11.8	10.5	83.8	13.96+1.42

Source of variance	Degrees of freedom	sum of squares	Mean squares	variance ratio
Between treatments	6	189.30	31.55	3.93**
Between seasons	1	57.40	57.40	7.16*
Between Replications	2	15.99	7.99	0.99NS
Residual	32	256.78	8.02	
Total	41	519.47	12.67	

*, ** denote significant at 5% and 1% levels respectively.

Appendix table-XXXV

Analysis of variance between spraying antiviral chemicals and percent cocoon harvest.

Treatment	July-August			September-October			Total	Mean $\pm$ SE
	1	2	3	1	2	3		
T ₁	50.5	52.5	53.0	55.3	64.3	61.2	350.8	56.13 $\pm$ 2.22
T ₂	54.5	58.5	58.0	63.7	57.3	58.0	350.0	58.33 $\pm$ 1.22
T ₃	48.0	67.5	61.5	45.7	53.7	51.5	327.9	54.65 $\pm$ 3.41
T ₄	32.0	56.5	45.0	51.7	49.0	49.3	283.5	47.25 $\pm$ 3.42
T ₅	46.5	44.0	45.5	58.0	57.0	58.3	309.3	51.55 $\pm$ 2.81
T ₆	48.0	65.5	51.5	62.7	57.7	60.3	345.7	57.62 $\pm$ 2.74
T ₀	53.0	57.5	52.0	46.5	56.0	42.0	307.0	51.17 $\pm$ 2.40

Source of variance	Degrees of freedom	sum of squares	Mean squares	variance ratio
Between Treatments	6	577.09	96.18	2.55*
Between seasons	1	80.65	80.65	2.14NS
Between Replications	2	237.99	118.99	3.16NS
Residual	32	1205.84	37.68	
Total	41	2101.57	51.26	

* denotes significant at 5% level.

Appendix table - XXXVI

Analysis of variance showing effect of spraying antiviral chemicals on the average cocoon weight (g).

Treatment	July-August			September-October			Total	Mean $\pm$ SE
	1	2	3	1	2	3		
T ₁	1.099	1.104	1.100	1.050	1.035	1.038	6.426	1.071 $\pm$ 0.013
T ₂	1.091	1.109	1.104	0.986	0.952	0.989	6.231	1.038 $\pm$ 0.028
T ₃	1.062	1.096	1.085	1.042	1.025	0.990	6.300	1.050 $\pm$ 0.015
T ₄	1.430	1.084	1.090	1.14	1.148	1.140	7.039	1.173 $\pm$ 0.052
T ₅	1.161	1.147	1.132	0.987	1.008	0.995	6.430	1.072 $\pm$ 0.033
T ₆	1.104	1.122	1.108	0.984	1.024	0.988	6.330	1.055 $\pm$ 0.025
T ₀	1.113	1.078	1.100	1.139	1.044	1.000	6.474	1.079 $\pm$ 0.020

Source of variance	Degrees of freedom	sum of squares	Mean squares	variance ratio
Between Treatments	6	0.072	0.012	4.00**
Between seasons	1	0.078	0.078	26.00**
Between Replications	2	0.007	0.003	1.00NS
Residual	32	0.1036	0.003	
Total	41	0.2606	0.006	

** denotes significant at 1% level.

Appendix table - XXXVII

Analysis of variance showing effect of spraying antiviral chemicals on the average shell weight (mg).

Treatments	July-August			September-October			Total	Mean+SE
	1	2	3	1	2	3		
T ₁	145	150	152	129	128	130	834	139+4.59
T ₂	145	147	150	132	130	128	832	139+3.97
T ₃	140	143	152	145	140	142	862	144+1.84
T ₄	148	145	150	159	152	155	909	151+2.04
T ₅	145	146	148	158	155	156	908	151+2.31
T ₆	155	150	153	142	146	145	891	148+2.04
T ₀	150	148	152	137	140	138	865	144+2.69

Source of variance	Degrees of freedom	Sum of squares	Mean squares	variance ratio
Between Treatments	6	1036.28	172.71	3.92**
Between seasons	1	384.02	384.02	8.73**
Between Replications	2	35.76	17.88	0.41NS
Residual	32	1408.06	44.00	
Total	41	2864.12	69.86	

**denotes significant at 1% level.

Appendix table - XXXVIII

Analysis of variance showing the effect of some disinfectants on silkworm diseases(%).

Treatments	Viral disease			Bacterial disease			Deficiency disease			Total	Mean $\pm$ SE
	1	2	3	1	2	3	1	2	3		
T ₁	19.5	11.0	9.5	1.5	2.0	1.5	0.5	1.5	1.0	48.0	5.3 $\pm$ 2.07
T ₂	3.0	1.5	3.0	1.0	3.5	2.5	4.0	4.5	2.5	25.5	2.8 $\pm$ 0.35
T ₃	12.5	7.0	14.0	6.0	1.5	0.0	0.5	1.0	2.0	44.5	4.94 $\pm$ 1.66
T ₄	3.0	2.5	4.0	1.0	2.0	0.5	1.5	3.5	5.0	23.0	2.5 $\pm$ 0.46
T ₅	9.5	14.0	3.5	2.5	5.5	5.0	2.5	2.5	4.0	49.0	5.4 $\pm$ 1.22
T ₆	11.0	4.0	5.5	1.0	0.0	3.5	0.0	3.5	2.5	31.0	3.44 $\pm$ 1.06
T ₀	18.5	3.0	14.5	2.0	2.5	3.5	2.5	2.0	0.5	49.0	5.4 $\pm$ 2.01

Source of variance	Degrees of freedom	Sum of squares	Mean squares	variance ratio
Between Treatments	6	90.25	15.04	1.76NS
Between diseases	2	504.02	252.01	29.47***
Between Replications	2	15.17	7.58	0.88NS
Interaction between treatment x disease	12	279.92	23.33	2.73**
Residual	40	342.00	8.55	
Total	62	1231.36	19.86	

** , *** denote significant at 1% and 0,.1% levels respectively.

Appendix table - XXXIX

Analysis of variance showing effect of bed disinfectants
on total mortality (%).

Treatments	Replications			Total	Mean+SE
	1	2	3		
T ₁	21.5	14.5	12.0	48.0	16.0+1.32
T ₂	8.5	9.0	8.0	25.5	8.5+0.23
T ₃	19.0	9.5	16.0	44.5	14.8+2.28
T ₄	5.5	8.0	9.5	23.0	7.7+0.95
T ₅	14.5	22.0	12.5	49.0	16.3+2.35
T ₆	7.0	23.0	17.0	47.0	15.7+3.80
T ₀	11.5	7.5	11.5	30.5	10.2+1.08

Source of variance	Degrees of freedom	Sum of squares	Mean squares	variance ratio
Between treatments	6	260.48	43.41	1.78NS
Between Replications	2	4.09	2.05	0.08NS
Residual	12	291.74	24.31	
Total	20	556.31	27.81	

Appendix table - XL

Analysis of variance showing effect of bed disinfectants on percentage of cocoon harvest.

Treatments	Replications				Total	Mean $\pm$ SE
	1	2	3	4		
T ₁	74.0	82.5	84.0	140.5	240.5	80.2 $\pm$ 2.54
T ₂	89.5	90.0	90.0	170.0	269.5	89.8 $\pm$ 0.13
T ₃	76.0	87.0	79.5	142.5	242.5	80.8 $\pm$ 2.64
T ₄	91.0	90.5	88.0	172.0	259.5	89.8 $\pm$ 0.75
T ₅	81.5	71.0	85.0	137.5	237.5	79.2 $\pm$ 3.43
T ₆	91.5	70.5	76.5	138.5	238.5	79.5 $\pm$ 5.10
T ₀	85.0	89.0	80.5	154.5	254.5	84.7 $\pm$ 2.12

Source of variance	Degrees of freedom	Sum of squares	Mean squares	variance ratio
Between treatments	6	404.95	67.49	1.62NS
Between Replications	2	4.67	2.33	0.05NS
Residual	12	498.33	41.52	
Total	20	907.95	45.39	

Appendix table - XLII

Analysis of variance showing the effect of bed disinfectants on average shell weight(mg).

Treatments	Replications			Total	Mean+SD
	1	2	3		
T ₀	178	181	175	534	178+2.45
T ₁	151	148	150	449	149+1.25
T ₂	190	192	188	570	190+1.63
T ₃	170	175	172	517	172+2.05
T ₄	183	187	182	552	184+2.16
T ₅	178	170	172	520	173+3.39
T ₆	165	170	174	509	169+3.68

Source of variance	Degrees of freedom	Sum of squares	Mean squares	variance ratio
Between treatments	6	2957.90	492.98	47.45 ***
Between Replications	2	8.00	4.00	0.38NS
Residual	12	124.67	10.38	***
Total	20	3090.57	154.53	0.32NS

*** denotes significant at 0.1% level.

Residual	42	238.68	5.68	7.12
Total	65	1104.67	16.99	16.99

*, ** denote significant at 1% and 0.1% levels, respectively.

Appendix table - XLIII

Analysis of variance showing effect of dusting chemical composites on bacterial disease(%).

Composites	March			June			Total	Mean $\pm$ SE
	1	2	3	1	2	3		
CA	4.3	2.0	4.3	13.0	4.3	6.7	34.6	5.8 $\pm$ 1.43
CB	3.0	2.7	8.3	6.0	10.0	7.3	36.3	6.2 $\pm$ 1.06
CC	6.7	11.7	8.0	3.3	5.3	4.7	39.7	6.6 $\pm$ 1.10
CD	9.3	8.0	5.0	4.0	6.7	6.0	39.0	6.5 $\pm$ 1.10
CE	5.0	3.7	3.3	9.0	13.0	11.3	45.3	7.5 $\pm$ 1.51
CF	7.0	7.3	8.0	15.7	9.0	9.0	56.0	9.3 $\pm$ 1.18
CG	7.7	3.0	3.3	13.7	17.3	19.3	64.3	10.7 $\pm$ 2.62
CH	2.7	6.0	14.3	19.0	13.7	13.7	69.4	11.6 $\pm$ 2.21
CI	2.7	5.3	6.0	11.7	11.3	10.7	47.7	7.9 $\pm$ 1.39
CJ	6.7	6.0	6.7	6.7	14.3	12.7	53.1	8.8 $\pm$ 1.35
CO	6.7	7.0	6.7	8.3	10.0	8.7	47.4	7.9 $\pm$ 0.49

Source of variance	Degrees of freedom	Sum of squares	Mean squares	variance ratio
Between treatments	10	210.38	21.04	2.95**
Between seasons	1	284.38	284.38	39.94***
Between Replications	2	3.17	1.58	0.22NS
Interaction between treatment x season	10	307.96	30.79	4.32**
Residual	42	298.98	7.12	
Total	65	1104.87	16.99	Lsd(P<0.05) =4.39

, * denote significant at 1% and 0.1% levels, respectively.

*** denotes significant at 0.01 level.

Appendix table - XLIV

Analysis of variance showing the effect of dusting chemical composites on viral disease(%).

Composites	March			June			Total	Mean+SE
	1	2	3	1	2	3		
CA	12.3	12.0	5.7	21.3	24.3	28.7	104.3	17.4+3.23
CB	6.7	4.0	6.3	13.0	17.7	15.7	63.4	10.6+2.09
CC	5.7	6.3	5.0	16.7	16.7	20.0	70.4	11.7+2.50
CD	13.0	9.0	12.3	27.0	25.7	25.3	112.3	18.7+3.03
CE	10.7	6.3	5.7	21.0	24.0	27.0	94.7	15.8+3.48
CF	8.0	18.0	13.0	30.0	20.7	25.0	114.7	19.1+2.95
CG	12.7	12.7	12.3	24.0	27.0	38.0	126.7	21.1+3.89
CH	14.7	15.3	15.0	34.0	43.7	32.7	155.4	25.9+4.67
CI	12.3	14.3	8.3	39.7	26.3	25.3	126.2	21.0+4.34
CJ	12.7	15.3	13.0	19.3	19.3	22.7	102.3	17.0+1.47
CO	14.0	20.3	21.0	35.0	35.0	37.3	162.6	27.1+3.64

Source of variance	Degrees of freedom	Sum of squares	Mean squares	variance ratio
Between treatments	10	1569.02	156.90	11.23***
Between seasons	1	3566.95	3566.95	255.42***
Between Replications	2	3.58	1.79	0.13NS
Interaction between treatment x season	10	273.85	27.38	1.96NS
Residual	42	586.52	13.96	
Total	65	5999.92	92.31	Lsd(P<0.05) =6.14

*** denotes significant at 0.1% level.

Analysis of variance showing effect of dusting chemical composites on effective rate of rearing/100 worms.

Composites	March			June			Total	Mean $\pm$ SE
	1	2	3	1	2	3		
CA	64.0	67.0	63.0	31.0	31.3	29.7	286.0	47.7 $\pm$ 6.98
CB	68.6	77.0	81.3	39.7	40.0	39.7	346.3	57.7 $\pm$ 7.50
CC	78.0	88.0	65.3	29.7	22.3	33.7	327.0	54.5 $\pm$ 10.80
CD	70.0	79.0	76.3	35.7	41.0	36.0	338.0	56.3 $\pm$ 7.78
CE	79.3	72.0	63.3	43.7	37.7	35.0	331.0	55.2 $\pm$ 7.04
CF	67.3	76.6	76.3	21.0	37.0	24.0	302.2	50.4 $\pm$ 9.71
CG	77.6	60.3	69.0	22.0	23.0	23.0	274.0	45.8 $\pm$ 9.67
CH	75.0	49.7	46.6	13.0	17.0	27.7	228.3	38.0 $\pm$ 8.72
CI	60.3	69.7	53.3	25.0	23.0	38.7	270.0	45.0 $\pm$ 7.13
CJ	51.0	70.3	59.3	30.7	37.0	31.7	280.0	46.7 $\pm$ 6.02
CO	53.6	47.3	49.6	29.3	36.3	29.3	245.4	40.9 $\pm$ 3.97

Source of variance	Degrees of freedom	Sum of squares	Mean squares	variance ratio
Between treatments	10	2512.26	251.23	5.43***
Between seasons	1	21792.73	21792.73	471.47***
Between Replications	2	44.40	22.20	0.48NS
Interaction between treatment x season	10	1315.42	131.54	2.84**
Residual	42	1941.37	46.22	
Total	65	27606.18	424.71	Lsd(P<0.05) =11.18

, * denote significant at 1% and 0.1% levels respectively.

Appendix table - XLVI

Analysis of variance showing effect of chemical composites on average cocoon weight (g).

Composites	March			June			Total	Mean $\pm$ SE
	1	2	3	1	2	3		
CA	1.226	1.285	1.279	0.948	0.943	0.864	6.545	1.090 $\pm$ 0.071
CB	1.200	1.189	1.194	0.830	0.859	0.714	5.986	0.997 $\pm$ 0.082
CC	1.177	1.121	1.164	0.842	0.892	0.876	6.072	1.012 $\pm$ 0.058
CD	1.153	1.040	1.076	0.823	0.869	0.904	5.865	0.977 $\pm$ 0.048
CE	1.172	1.141	1.259	0.900	0.793	0.813	6.078	1.013 $\pm$ 0.075
CF	1.135	1.096	1.120	0.875	0.807	0.855	5.888	0.981 $\pm$ 0.056
CG	1.229	1.232	1.171	0.875	0.823	0.859	6.189	1.031 $\pm$ 0.073
CH	1.195	1.142	1.177	0.842	1.140	0.904	6.400	1.066 $\pm$ 0.056
CI	1.188	1.211	1.240	0.808	0.787	0.842	6.076	1.012 $\pm$ 0.082
CJ	1.228	1.191	1.202	0.901	1.073	0.819	6.414	1.069 $\pm$ 0.064
CO	1.226	1.253	1.229	0.863	0.820	1.088	6.479	1.079 $\pm$ 0.072

Source of variance	Degrees of freedom	Sum of squares	Mean squares	variance ratio
Between Treatments	10	0.096	0.009	2.07*
Between seasons	1	1.604	1.604	370.25***
Between Replications	2	0.0001	0.00006	0.01NS
Interaction between treatment x season	10	0.063	0.006	1.38NS
Residual	42	0.181	0.004	
Total	65	1.945	0.029	Lsd(P<0.05) = 0.104

*, *** denote significant at 5% and 0.1% levels respectively.

Appendix table - XLVII

Maximum and minimum room temperatures of the three experimented years.

Months	Av. Maximum temperatures °C			Av. Minimum temperature °C		
	1985	1986	1987	1985	1986	1987
February	26.54	26.01	28.65	22.66	23.53	22.17
March	25.36	28.22	31.18	25.33	25.82	28.06
April	32.75	29.81	30.58	28.62	27.19	25.74
May-June	31.43	30.18	32.96	26.59	27.68	27.36
June-July	30.75	29.52	30.69	28.29	27.46	29.41
July-August	33.72	29.97	28.63	23.28	27.11	26.39
September	33.35	30.83	30.52	24.39	27.59	28.08
October	29.14	29.96	28.53	25.64	25.96	23.93
November	25.68	27.80	27.01	23.52	25.58	21.69
December	22.55	24.64	23.89	22.05	24.08	21.95

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Appendix table - XLVIII

Maximum and Minimum room relative humidity of the three experimented years.

Months	Av. Maximum humidity %			Av. Minimum humidity %		
	1985	1986	1987	1985	1986	1987
February	83.06	87.94	88.41	77.06	78.64	79.81
March	73.82	77.39	73.22	69.78	70.99	70.44
April	69.89	88.41	93.63	61.89	79.29	73.49
May-June	91.51	88.00	87.10	86.73	78.74	82.34
June-July	93.06	92.83	99.80	88.92	86.77	70.43
July-August	94.63	93.25	96.42	89.49	88.75	88.44
September	94.02	95.29	97.62	89.10	85.83	89.10
October	91.77	93.13	85.55	86.17	87.31	75.99
November	90.72	93.58	93.59	80.18	85.22	77.79
December	88.52	91.93	87.13	76.48	82.93	79.83

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