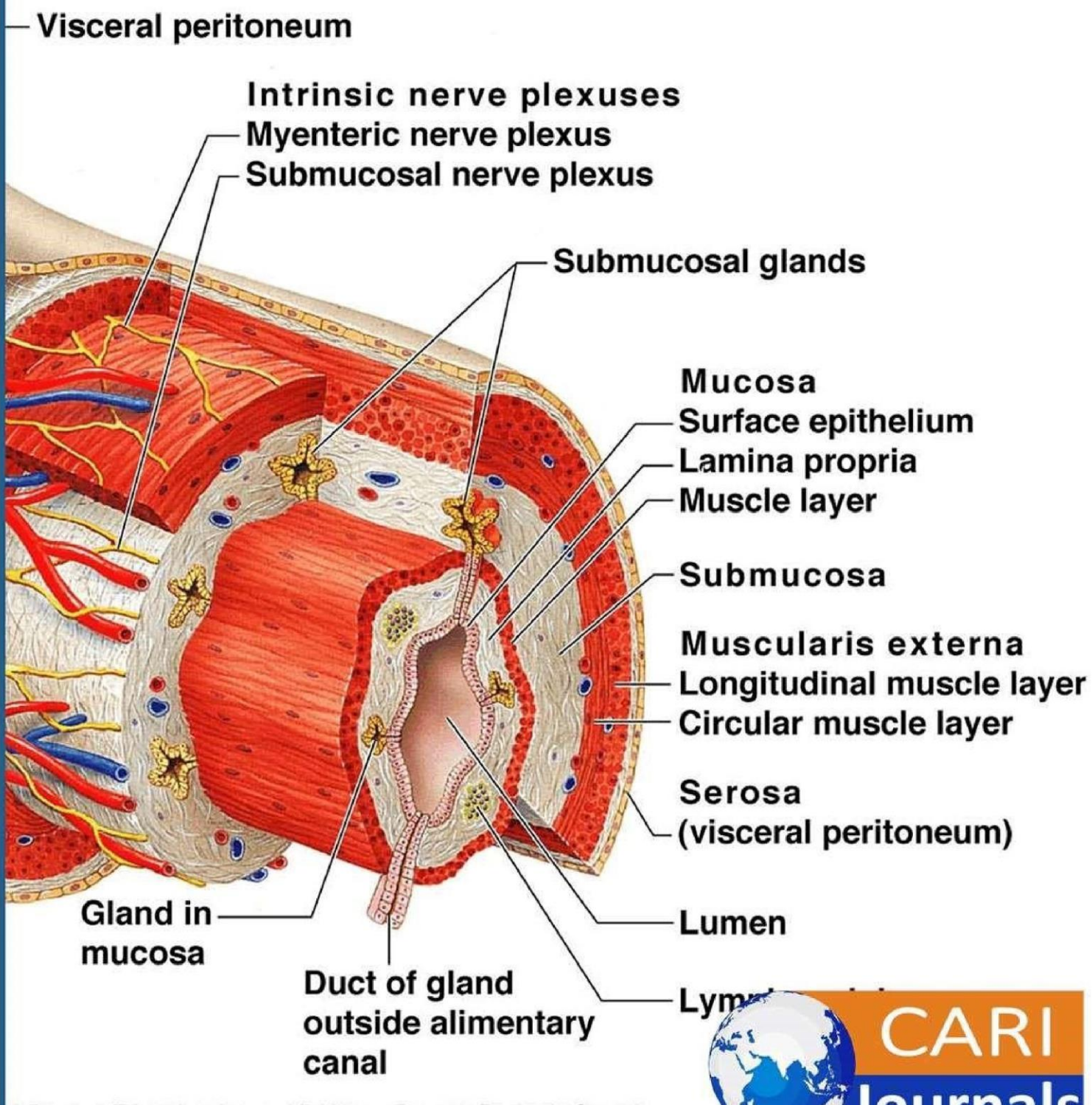
International Journal of
Biological Studies
(IJBS)

Studies on Synergistic Effects of Different Bio-Active Chemicals on Nuclearpolyherosis (Grasserie Disease) of Silkworm, *Bombyx mori* Linnaeus



CARI Journals

International Journal of Biological Studies
ISSN: 2957-7764 (Online)
Vol. 2, Issue No.2, pp 1- 20, 2022
www.carijournals.org

Studies on Synergistic Effects of Different Bio-Active Chemicals on Nuclearpolyherosis (Grasserie Disease) of Silkworm, *Bombyx mori* Linnaeus

Suman Deb[1]*, Ajit Kumar Sinha[2], Atul Kr Saha[3] and Niladri Hazra[4]

[1&2] Ranchi University, Ranchi, Jharkhand, India 834008

sinhaajitkumar58@gmail.com

[3]Central Sericultural Research and Training Institute, Berhampore, West Bengal, India 742101

sahaatul@rediffmail.com

[4]Department of Zoology, The University of Burdwan, West Bengal, India -713104,
hazra.niladri@gmail.com

*Author for correspondence: sumandev22@gmail.com

Abstract

Purpose: Larval hind gut of multivoltine breed silk worm *Bombyx mori* L. (Nistari) was studied in both favourable and unfavourable rearing seasons after Grasserie caused by *Bombyx mori* Nuclearpolyherosis Virus (*Bm*NPV) using Histological Analysis, Scanning Electron Microscopy and Transmission Electronic Microscopy.

Methodology: Chemical treatments using various bio active chemicals: Curcumin, Allicin and Azadirachtin were administered to minimize the extent of damage.

Findings: The present study clearly indicates that Azadirachtin is more effective in comparison to its other counterparts and it is the major finding of this extensive study.

Unique Contribution to Theory, Policy and Practice: The uniqueness of this type of bio chemical control of Grasserie disease which is not only a cost effective and eco-friendly technique, but also it is less hazardous and simple controlling procedure of Grasserie. Therefore, this extensive study may help the stakeholders to step ahead on the progression of sustainable agriculture while avoiding crop loss by means of cost-effective pathogen management technology.

Keywords: Silk worm: *Bombyx mori* L, Nuclearpolyherosis (Grasserie Disease), Synergistic Efffects**,** Bio-Active chemicals: Curcumin, Alicin and Azadirachtin, Hind Gut, Unfavourable Rearing Season.

INTRODUCTION

Silk and silk products have been associated with luxury and traditionally been expensive. Natural silk is produced in very few countries of world. China and India are the major silk producers among the 60 silk producing countries in the world. Silkworm, *Bombyx mori* L has long been reared as a beneficial insect. China and India contributed 104000 MT (77.89%) and 19690 MT (22.11%) respectively of the Global silk production in 2021 amongst 60 sericulural countries in the world (Anonymous, 2022) compare with last year and at the end of XI 5-year plan over X 5-year Plan, respectively. Mulberry silk industry in India is now passing through a critical phase with reducing area under mulberry and almost stagnating raw silk production in spite of continuous efforts. Nevertheless, the production is comparatively lower due to various reasons such as, changing economic scenario, conversion of large chunks of agricultural land for construction of housing and business establishments opportunities for more remunerative but easy to do jobs for farm workers, reducing water resources and unpredictable rains etc. could be some of the reasons attributable to the reduction of mulberry area can be attributed (Rath et. al., 2000). Besides, the cocoon production is comparatively low due to disease is noteworthy. All silkworms suffer from various diseases like Pebrine (Protozoa: Microsporida), Flacherie (Bacteria), Grasserie (Virus) and Muscardine (Fungus). Thus, these pathogens have a great role in deterioration of sericulture industry. Most of the commercially reared silkworm species exploited is highly susceptible to various diseases; therefore, it is very often attacked by the pathogens. Approximately, 40% crop loss is attributed due to diseases in India.

Viral disease in *Bombyx mori* L. comprises mainly inclusion of polyhedra caused by *Bombyx mori* Nuclear Polyhedrosis Virus (*Bm*NPV) (Family: Baculoviridae). A major disease problem next to pebrine in tropical countries like India is Grasserie. It is caused also due to physiological disorder. Grasserie has been causing great economic loss in all the sericultural countries and it is estimated around 70-80% of the total crop loss due to the disease. However, it is reported that maximum crop loss was found throughout the seasons mainly due to Grasserie. Usually high temperature (30°C - 45°C) and humidity (90%-100%) are prevalent in tropical regions in conductive to proliferation of polyhedrosis diseases. Moreover, few recent reports on the presence of anti-viral proteins against some viruses, strongly suggest the presence of functional anti-viral immune system in the silkworm. Information on haemolymph protein of the silkworm responsible for protection through innate immune system from diseases including viral infections is reported (Deb *et. al.*, 2014).

Sericulture in West Bengal and other North-Eastern states experiences low temperature (less than 25°C) and low relative humidity (less than 65%) during Nov-Mar, which is very congenial for fungal diseases. At the same time, Agrahayani (Oct-Nov) and Falgooni (Feb-Mar) are the most very good and important commercial crops in West Bengal and there is a huge demand for bed-disinfectant during this period to ensure crop. But the crop loss is also attributed during this favourable seasons due to fungal i.e., Muscardine disease. Some time out break was also noticed even application of popular bed disinfectant as efficacy of bed disinfectant against Muscardine is not promising. Gattine contributes about

10-30% crop loss during dry seasons (May-June) in particular (Subba Rao *et al.*, 2001). Gattine is said to be caused due to infection of an ultra-virus and a bacterium, *Streptococcus bombycis* as secondary invader (Zhou *et al*., 1998). A number of room disinfectant, 5% Bleaching powder, Sanitech, Decol, Serichlor, Formalin spray etc. and bed disinfectant like Labex, Vijetha, Sericillin, Rakshak etc. made up with chemical formulation and Amruth, Ankush etc eco-friendly plant-based formulation are widely used in the field for control of silkworm diseases.

MATERIALS AND METHODS

Rearing Schedule: For three consecutive favourable rearing seasons, month wise February–March (2016-21) and November–December (2016-21), all the experimental procedure was conducted. All the recommended package of practices of rearing was followed. The rearing was followed after Krishnaswamy (1978). The egg of *B.mori* L multivoltine breed of Nistari were taken from Silkworm Breeding and Genetics Laboratory of Central Sericultural Research and Training Institute, Berhampore, West Bengal,India and reared using traditional methods after feeding with tender Mulberry: *Morus alba* (S-1635 variety) leaves. At first the eggs were washed in 2% formalin solution for surface disinfection. Rearing house as well as rearing appliances was also disinfected by spraying with 5% normal bleaching powder solution. After proper room disinfection and taking proper measures for disinfection the rearing was commenced. Brushing of silkworm larvae is a process of separating the newly hatched larvae from the empty egg shells or egg sheets and transforming them to rearing bed. On the hatching day the egg sheets were exposed to light in the morning and at least one hour of exposure can result uniform hatching (Saha *et al.* 2008). Chopped tender mulberry leaves were provided (size: 0.5 cm^2) by sprinkling as thin layer over the hatched larvae. The silkworm larvae were kept for 10-20 minutes as such. The larvae were then transferred to rearing trays providing optimum spacing. Wet pad of foam and paper greased with paraffin was provided in the tray to maintain the optimum humidity. Larval stages of silkworm could be divided into five instars and during this period moulting occurs four times. The first three instars are called young age or *chawki* and the last two instars are called late age. The young age larvae were fed with tender leaves of mulberry plant and the fourth instar larvae were fed with mature leaves whereas the fifth instar larvae were given with cut twigs and mature leaves throughout the rearing and it was followed in all the rearing seasons.

Isolation and Purification of Virus: Grasserie infected pupae, obtained from silk growers of Birchandrapur village; Birbhum District of West Bengal, India was used in the present study. The polyhedra were isolated from the infected pupae and purified by centrifugation at 3000 rpm for 10 minutes using percoll cushions (PVP coated silica particles, Sigma chemicals Co., USA). After centrifugation, the polyhedra were stored at 4ºC. Third instar '0' hour larvae were perorally inoculated with *Bm*NPV (1×10^6 Obs/ml). Polyhedra were counted using a Neubauer haemocytometer (German Fine Optic) under light microscope (×600) and determined the inoculum concentration after Undeen (1997). *Bm*NPV isolates were propagated in *B. mori* silkworm larvae and purified from live moth using Percoll Cushions (PVP coated Silica particle, Sigma Chemicals Co., USA).

Inoculum Concentration and Experiments: Freshly ecdysed Vth instar 100 larvae were considered for the experiment.1.5×10^8 Obs/ml were inoculated using conventional diet contamination method at '0' hour (Bhattacharya *et al.*, 1993). Briefly the procedure involved dipping leaf dishes (28.27 sq. cm) in 1.0 ml of the polyhedral suspension, drying and then allowing the larvae to feed on the dishes for a period of 6 hours. Normal and *Bm*NPV infected samples were maintained. *Bm*NPV inoculated Vth instar larvae were isolated from the population for different investigation.

Histopathological Study: The method was followed after Baker (1958**).** In the laboratory animals were anaesthetized followed by sacrificed in anhydrous condition. Both normal and *Bm*NPV infected tissues were dissected out and then fixed for histological studies. Alcoholic Bouin's fluid was used as fixative. After fixation, the samples were processed according to the general paraffin processing protocol involving dehydration of the tissue by gradation of alcohol, like 30% ethanol, 50% ethanoland 70% ethanol. Storage at 70% ethanol + lithium carbonate treatment for picric acid, 90% ethanol and finally 100% ethanol treatment was done. Later, clearing was done by using cedar wood oil. One to 2 hours was used for clearing. Hind gut tissues can be left in cedar wood oil indefinitely. It does not harden the tissue. After clearing, the samples were transferred to a mixture of xylene saturated with paraffin. It was kept in the paraffin at about 35ºC for 15 minutes. Hot paraplast was infiltrated in a 58ºC paraffin oven in an open container. Three consecutive changes following time sequences: 30 minutes, 30 minutes and 1 hour respectively were done. A small amount melted paraplast was poured (fresh) into stainless steel container. The paraplast infiltrated tissues were transferred with forceps and oriented properly in the centre of the depression. The top surface of the paraplast was cooled by blowing gently on it. As soon as a scum of paraplast has formed on top, the cup was sunk gently into a cold-water bath. The cup was cooled thoroughly in cold running tap water. Embedding was done following all the precautions and the blocks were prepared for sectioning with rotary microtome after trimming and mounting on a metal block holder. The thickness of sections was set at 5µm using rotary microtome. Clean glass slides smeared with Myers' albumen and flooded with distilled water were used for placing the tissue sections, stretched in hot oven. Sections were dried over night before staining. The sections were covered carefully with a coverslip without drying. Photomicrographs were taken from tissue sections with oil immersion under light microscope (×1000) (Leitz Diaplan Phase Contrast Microscope).

Scanning Electron Microscopic (SEM) Study: 4g dry powder of Paraformaldehyde (EM grade) was dissolved in 100 ml of distilled water at 60ºC. Mouth of conical flask was covered with aluminium foil. A drop of 0.1N sodium hydroxide was used for dissolve properly. The solution was cooled and filtered. Equal volume of 0.2M phosphate buffer (pH 7.4) to the paraformaldehyde solution to make it a 2% solution in 0.1 M phosphate buffer was added. Required quantity of glutaraldehyde (from the concentrated 25% stock solution, EM grade) was added in paraformaldehyde solution. In this case, 10 ml of glutaraldehyde (from stock solution) was added to 90 ml of 2% paraformaldehyde solution. The final concentration of the fixative was 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1M phosphate buffer. Similar technique was followed after Inoue and Osatake (1988). Silkworms were perfused,

first with 0.9% phosphate buffer saline of pH 7.2 for 10 minutes and then with 2% paraformaldehyde for 20 minutes. Larval Hind gut tussue at different larval stages of both healthy and infection of Nistari were collected for scanning electron microscopic study. Larval integument was fixed in a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer for 8-12hr at 4ºC. 5-7 mm in length and breadth of the integument sample was taken for the study followed by wash in buffer for 3 times, each for one-hour duration at 4ºC. The samples were kept inside a thermos flask using cool pack for transportation. *Critical Point Drying for SEM* was conducted with Hitachi (HCP-2). Gold coating was done by IB2 Ion Coater (Eiko Engineering, Japan). Samples were observed under SEM (S-530–Hitachi, Japan.) and photomicrograph was taken (×300-1500). {conducted at: **University Science Instrumentation Centre (USIC), The University of Burdwan, West Bengal, India**}

Transmission Electron Microscopic (TEM) Study: Paraformaldehyde solution was made in 100ml of distilled water by dissolving 4g of dry power (EM Grade) at 60ºC. The mouth of the conical flask was covered with aluminium foil before the solution was cooled and filtered; later equal volume of 0.2M Phosphate buffer (pH 7.4) was added to the paraformaldehyde solution to make it a 2% solution in 0.1M Phosphate buffer. Then required quantity of glutaraldehyde (from the 25% concentrated stock solution, EM Grade) is added to the paraformaldehyde solution. To prepare 0.2M phosphate buffer (pH 7.4), 3.12 g of sodium dihydrogen orthophosphate (NaH_2PO_4, $2H_2O$: Molecular weight 156) was added to 100ml of distilled water and solution A was prepared. 2.84 g of disodium hydrogen orthophosphate anhydrous (NA_2HPO_4: MW 142) was added to 100ml of distilled water to make solution B. 19 ml of solution A was mixed with 81 ml of solution B and the final pH was adjusted with HCl or NaOH). Each sample was washed in buffer for three times each for one-hour duration at 4ºC. The samples were kept in thermos flask using cooling pack to the instrumentation centre. The technique was followed after Hall (1995). The samples were post fixed in 1% osmium tetroxide (OsO_4) for 1 hour at 4ºC. The samples were dehydrated in an ascending grade of acetone, infiltrated and embedded in araldite CY 212 (TAAB, UK). Thick Sections (1µm) were cut with an ultramicrotome, mounted on to glass slide, stained with aqueous toluidine blue and observed under light microscope for gross Observation of the area and quality of the tissue fixation. For electron microscope examination thin sections of gray silver colour interference (70-80nm) were cut and mounted in 300 mesh-copper grids. Sections were stained with alcoholic uranyl acetate and alkaline lead citrate, washed gently with distilled water and observed under a Morgagni 268D transmission electron microscope (FEI Company, The Netherlands) at an operating voltage 80 kV. Images were digitally acquired by using a CCD camera (Megaview III, Fei Company) attached to the microscope. *Bm*NPV inoculated Vth instar larvae were isolated from the population for the electron micrograph study. Each larva was perfused in 0.9% phosphate buffer saline (pH 7.2) for 10 minutes followed by 2% paraformaldehyde solution for 20 minutes. Larval hind gut was dissected out and cut into pieces measuring 2×2 mm size. The sample was fixed in a mixture of 2% paraformaldehyde solution and 2.5% glutaraldehyde in 0.1M phosphate buffer for 8 to 12 hours at 4ºC. Samples were observed under TEM (268 D (10kV) of FEI, Company, The

Netherlands and photomicrograph were taken (×1000 -8000) {conducted at: **All India Institute of Medical Sciences (AIIMS), New Delhi, India**}.

Application of Disinfectants:

Azadirachtintin: Molecular formula: $C_{35}H_{44}O_{16}$, Molecular weight: 720.7 g/mol**.** It is an active compound of Neem oil found in seeds of Neem tree (*Azadiracta indica*). Ranging from dark yellow to brown in colour, Azadirachtin is an active natural pesticide used for several years in Indian subcontinent. Azadirachtin is the most active component for repelling and killing pests and parasites from ancient time. It can be applied in wide range to crops and ornamental plants for controlling insects and pests. Azadirachtin is an amorphous dust soluble in water and alcohol or emulsifiable concentrates. Azadirachtin can repel and reduce the feeding of Nematodes. It has been observed that 90% of Azadirachtin dose is excreted out by the insects within seven hours, and remaining dose is enough to reach the target. In water, half-life of Azadirachtin ranges from 48 minutes to 4 days. It also breaks down rapidly in plant body and the half-life is 1-2.5 days. The remaining components are broken by microbes and environmental elements. Azadirachtin is nontoxic to most animal and plant except fish and other aquatic animals. It is commercially available and economically cost effective easy to use compound. It has been purchased from T. Stanes and Company Ltd. Coimbatore, Tamil Nadu, India, for all the experiments.

Allicin: Molecular formula: $C_6H_{10}OS_2$, Molecular mass: 162.28 g/mol**.** It is an active compound of Garlic **(***Allium sativum***).** It is an organo sulphur compound found in Alliaceae family plants. Anti-inflammatory property of Allicin has made it more accepted in bio medical research. Allicin quickly disintegrates into series of sulphur compounds which are able to control various activities. Allicin is an integral part of defence mechanism against attack by pests and pathogens in plant. Allicin in a defence molecule of Garlic with broad spectrum antimicrobial properties. Allicin can react with thiol groups and can inactivate microbial essential enzyme system. Besides bacteria, the effects of Allicin have been investigated in fungi and protozoa and even in viruses. Commercially available Allicin is water and alcohol soluble and easy to use for the stakeholders. It has been commercially purchased from Vihaan Foddertech, Siddhapur, Maharastra, India, for the present experimental purposes.

Curcumin: Molecular formula: $C_{12}H_{20}O_6$, Molecular mass: 368.38 g/mol**.** It is a yellow phytophenol pigment and active compound isolated from Turmeric (*Curcuma longa*), with a variety of pharmacological properties. It can block the formation of reactive Oxygen species resulting in inhibition of unnatural growth of cells. The desirable preventive or putative therapeutic properties of Curcumin have also been considered to be associated with its antioxidant and anti-inflammatory properties. Curcumin have antioxidant properties. It is therefore highly explored in cancer research arena. Laboratory research clearly indicates that it can slower down the progression and spread of cancerous growth. The major biochemical activity of Curcumin is mediated through its properties to inhibit cyclooxygenase -2 and lipoxygenase. It can induce Nitric Oxide synthesis and down regulation of protein kinase C. Improper upregulation of above stated components are associated with pathophysiology of

certain animal cells that results in improper growth and development. Curcumin is a beta diketone compound found in the root stuff of turmeric plant. It is a well-tested hetero protective flavouring bio pigment having intraceutical and antifungal properties. Factors that enhance the activities of Curcumin are absence of potent and selective target specific activity, limited tissue distribution, low bio availability and extensive metabolism specificity. It has been purchased from Sangam Agro-Chemicals Pvt. Ltd. Gurugram, Haryana, India, or all the present experimental purposes. Curcumin in excess dose has less lethal effect as it is eliminated in faeces 90% or above.

Bleaching powder: Bleaching powder is a white amorphous powder, with a pungent smell of chlorine. For effective disinfection, a high grade of bleaching powder with an active chlorine content of 30% must be used. It should be stored in sealed bags, away from moisture, failing which it will be rendered ineffective. The action of bleaching powder is optimal under wet and contact condition and therefore the surface of equipment and walls of the rearing room should be drenched with this solution. Bleaching powder solution (5%) can be prepared by dissolving 50g of bleaching powder in 1l of water. This solution contains 1.6% active chlorine. For room disinfection one litre solution is required to disinfect 2.5 sq. m areas. It is also called chlorinated lime powder; it is generally used for washing of rearing rooms and appliances but can also be effectively used as a spray. As it is hygroscopic, if left exposed moisture is absorbed from the atmosphere and it becomes ineffective. It decomposes into water and acids, liberating free chlorine with moisture of ordinary temperature. The disinfecting action of bleaching powder is caused by the release of nascent oxygen. The chlorine produced from bleaching powder has a bactericidal action. The available chlorine in commercial bleaching is around 25-30% and is not completely soluble in water and hence only the supernatent solution is used for spraying. As it is a strong oxidising agent it affects metallic goods and textiles and care should be taken to protect these things during use.

Paraformaldehyde: Paraformaldehyde (PFA) is the smallest polyoxymethylene, the polymerization product of formaldehyde with a typical degree of polymerization of 8–10 units. Paraformaldehyde commonly has a slight odour of formaldehyde due to decomposition. Paraformaldehyde is a polyacetal. Paraformaldehyde forms slowly in aqueous formaldehyde solutions as a white precipitate, especially if stored in the cold. Formalin actually contains very little monomeric formaldehyde; most of it forms short chains of polyformaldehyde. A small percent of methanol is often added as a stabilizer to limit the extent of polymerization. Paraformaldehyde can be depolymerized to formaldehyde gas by dry heating and to formaldehyde solution by water in the presence of a base or heat. The very pure formaldehyde solutions obtained in this way are used as a fixative for microscopy and histology. The resulting formaldehyde gas from dry heating paraformaldehyde is flammable. Once paraformaldehyde is depolymerized, the resulting formaldehyde may be used as a fumigant, disinfectant, fungicide and fixative. Longer chain-length (high molecular weight) polyoxymethylenes are used as thermoplastic and are known as polyoxymethylene plastic.

Experimental Layout: Three sets of treatment, each with five replications were kept in the rearing room and larvae were brushed normally on the paraffin sheet and fresh mulberry

chopped leaves were fed to the silkworms and allowed the silkworm for development. Hatching% were counted and recorded. Temperature and R.H% were also recorded. The IIIrd instar larvae were counted after undergoing their second moult and 100 larvae were kept for each treatment and replications kept in five trays were dusted with different bed disinfectants This work has been conducted at Silkworm Breeding and Genetics Research Unit, Central Sericultural Research and Training Institute, Berhampore, West Bengal, India for five consecutive years including both favourable and unfavourable rearing seasons (2016-21). The experiments were laid out in completely randomised design with below stated treatments replicated five times, with untreated control sets for each experiments along with Sericilin, Labex, Dusting 1 containing Bio active compounds with Bleaching powder and Paraformaldehyde mixture (30:20:950) and Dusting 2 containing mixtures of Bleaching powder and Paraformaldehyde (20:980), (Deb *et al*., 2021). Before the commencement of silkworm rearing the appliances were sundried and rearing room along with rearing appliances were thoroughly cleaned and dried up using 2% Bleaching Powder solution and Absolute Alcohol. The entire room was later disinfected following standard protocol (Dandin *et al*., 2003). The rearing room was kept air tight for 24 hours and after that the room was kept open and used for rearing. The temperature during the incubation of silkworm eggs was ranged from 23-25 ºC and the relative humidity was ranged between 85-90%. Complete hatching took place after few days of incubation. New leaves and tender shoots of Mulberry were put over the hatching larvae, which crawled up the leaves and twigs, and then these were removed with silkworms to the rearing trays. Rearing schedule (Krishnaswamy, 1978) was continued till the end of second instar larvae enters moulting.

The third instar larvae were provided with chopped tender Mulberry (*Morus alba*) leaves (S1 variety) of required quantity and quality. The Mulberry leaves for feeding of Silkworm larvae were cut freshly every morning, and covered with wet clean and clear muslin cloth to protect that from loss of water. The leaves were cleaned and given to the first and second instar larvae as strips or buds. Afterwards, the whole leaves were distributed in a usual manner four times a day, till the beginning of fourth instar. Regular cleaning of rearing beds was carried out by removing uneaten leaves and faeces, to avoid infection spreading. After thirty minutes of initial feeding, 90 larvae were transferred to each experimental tray in each experimental tray with the mulberry leaves. Bed disinfectants were dusted once 30 minutes after resume from each moult and an additional dusting was done on 5^{th} day of the fifth instar after bed cleaning. For dusting muslin cloth was used. During the course of rearing, disease wise larval mortality were recorded. Mortality was also recorded during harvesting of cocoons.

In this study, data pertaining to Effective Rearing Rate (ERR) %, Single Cocoon weight, Single Shell weight and Shell (%) were recorded. Chemicals and botanicals are screened as inducer was dusted with selective dose to the larvae after resuming from 4^{th} moult (1^{st} day '0' hr). Dusting was done 3-4g/ sq. ft. of bed area on silkworm body. Dusting was done on silkworm larvae after each moult 30-45 minutes before the resumption of feed. One additional dusting was done on the 4^{th} day of Vth instar after bed cleaning. On the appearance of disease symptom, dusting frequency increased every day.

Observations and Data collection: Observations like Larval mortality percentage (%) were recorded till the formation of cocoon. Mature larval weight (wt.), Cocoon wt., Shell wt., Effective Rearing Rate (ERR) % was observed and recorded. The data was analysed statistically to verify the result.

RESULTS AND DISCUSSIONS

Histology of Larval Hind gut: The histology of normal hind gut shows presence of columnar epithelial cells arranged in the densely packed coiled gut portions. The nucleus is prominent ant the compactness of the tissue is clearly visible (**F**ig.1a). The affected hind gut portion in histology shows presence of deformed columnar epithelial cells without predominant nucleus, the cells are quite spindle shaped in appearance. The occluded bodies are prominent and the budded virus is clearly present in the tissue sections. It is very clear that the appearance of occluded bodies within the cell gives an appearance of hollow chamber (**F**ig.1b).

Larval Hind gut under SEM: The overall appearance of the hind gut shows coiled branched alimentary canal with presence of nodule like structures at different positions. The sectional view of normal larval hind gut shows presence of digestive diverticula with secretory cell line along with specialized absorptive cells (**F**ig.2a). The grasserie infected larval hind gut shows deformed absorptive cells present in the sectional view along with empty secretory cells although digestive diverticula are observed (**F**ig.2b).

Larval Hind gut under TEM: The hind gut portion of the normal silkworm larvae under transmission electron microscopy clearly shows different soft tissues of the alimentary canal and the section appears like irregular canals spread all over (**F**ig. 3a). In the *Bm*NPV infected counterpart the presence of chain like occluded bodies are clearly observed. The chain may be discontinuous in some regions which may be due to when the carrying capacity is exceeded. The chain is distributed all over the soft tissue of the gut (**F**ig. 3b).

Following the methodology stated earlier, the chemical treatment was conducted to study the effect of different bio active chemicals to control grasserie.

Effective Rate of Rearing (ERR %): ERR was recorded during favourable rearing season compared to the control and dusting 1 was 38.000 ± 0.408 (Table No. 1b), 36.000 ± 0.418 (Table No. 2b) and 36.010 ± 0.528 (Table NO. 3 b). Silkworm larvae treated with Azadirachtin had highest ERR% under normal conditions while those treated with Allicine and Curcumin. SINGLE COCOON WEIGHT (SCW): Single Cocoon Weight is the weight (in grams) of a single cocoon i.e., the combined weight of one shell and the pupa inside. SCW in the Azadirachtin treatment showed the reading of 1.248 ± 0.001 (Table No. 1b) which was seen to be in the range of 1.218 ± 0.001 to 1.198 ± 0.001 (Table NO. 2 b and 3 b).

SINGLE SHELL WEIGHT (SSW): Single Shell Weight is the weight (in grams) of a single cocoon shell. SSW ranged from 0.209 ± 0.001 to 0.189 ± 0.007 (Table NO. 1 b, 2 b and 3 b). Azadirachtin performed significantly better in all the parameters in comparison to its two counterparts: Allicin and Curcumin after challenged with *Bm*NPV in unfavourable rearing season.

Although there is a huge utilization of image processing and application of technology in various fields of Agriculture based industries, there is a need of development of effective techniques. India being the seventh largest country in the world and 38% of its GDP depends on agriculture, we need to focus more on improving technology in this field by utilizing the modern solutions and techniques (Yashaswini *et al*.2020).

Conclusions

The healthy larval hind gut had layers of different tissues whereas infected hind guts were dilated with a series of Obs in soft tissue which gradually increased in number and later released polyhedra in gut juice. Deformed columnar epithelial tissues were filled with Obs, and BVs were exposed from hind gut epithelial layer in advanced stage of infection, whose hind gut wall appeared emerged. During *Bm*NPV infection process, two viral phenotypes were encountered: one form was Obs within polyhedra and is responsible for primary infection in hind gut cells. The second form of virus, BV, never became occluded and was released into haemolymph. BV spreads infection from cell to cell within insect body (Haas-Stapleton *et al*., 2005). The initiation of replication of BV is believed to occur in cells by endocytosis (Herniou *et al*., 2003). However, the silk glands and majority of insect tissues have a fibrous extracellular matrix, the basal lamina (BL). Therefore, BV needs to penetrate BL for systemic infection. Reports reveal that BL organization and thickness influences the passage of macromolecules into certain tissues where it acts as a selective filter (Rahman and Gopinathan, 2004). Infected hind gut showed deformed absorptive cells present in the sectional view along with empty secretory cells and digestive diverticula under SEM observations. The fully transformed lipid globule gradually discharged Obs in adjacent matrix and released polyhedral structures. Healthy hind gut possessed several layers of different tissues while infected hind guts were dilated due to the presence of Obs in soft tissue. The number of Obs gradually increased in chain with disease progression. The chain broke up once the number of Obs reached highest carrying capacity thereby releasing polyhedra in gut juice. Diseases in sericulture are the major hurdle to gain profit. Although there are many biological solutions to overcome these diseases, they are not so much effective. The effective utilization of modern technologies in early identification and detection of various diseases in silkworm leads to effective sustainable sericulture. There was positive correlation of ingestion of food with larval weight, cocoon weight and shell weight (Magadum *et al*., 1996). Diseased silkworms spun flimsy cocoons, which are unfit for reeling (Geetha Bai and Mahadevappa, 1995). In addition to retardation of growth, the economic parameters were also adversely affected by infection lending credence to the fact that inhibition of normal growth /development results in poor cocoon and silk quality (Krishnan *et al.*, 1998). There is a need of improvement of technology in effective detection of silkworm diseases at the earliest at lowest possible cost, because early detection of disease leads to early treatment, that in turn enhances recovery and productivity is increased. Increased productivity leads to upliftment of economy. A number of attempts have been taken by the different Sericulture Institute to control the silkworm disease, however, loss due to disease is still continue rather increasing due to prevalent of new strains of pathogens, which are becoming resistant in the present degraded scenario and silkworms are fail to combat the attack of pathogens (Deb *et*

al., 2021). Therefore, it is the demand of this era to develop the new technology, which is sustainable in the field of sericulture and asses the technology already available in the field but not adaptable by the farmers through refinement and make suitable for the use in the field with less input, easily adaptable, eco-friendly and of course low cost for accessible by the farmers.

REFERENCES

Anonymous (2022) Seri-info, *Annual report*, Directorate of Textiles (Sericulture Division), Govt. of West Bengal, pp 5-7.

Bhattacharya, J. (1992) Some biotic and abiotic factors influencing the outbreak of viral diseases in silkworm *B. mori* L. *Indian Silk*, 45(1): 45-46.

Baker, J. R. (1958) Principles of biological microtechnique. Methuen, London, pp 23-56.

Dandin, S. B., Jayaswal, J. and Giridhar, K. (2003) Handbook of Sericulture Technologies, Central Silk Board, Bangalore. pp. 226-228.

Deb, S., Sinha, A.K. and Saha, A. (2021) Studies on Synergistic Effects of Different Chemicals on Nuclear Polyhedrosis (Grasserie Disase) of Silkworm, *B. mori* L. *African J. Bio. Sciences*, 3(4):104-113, ISSN: 2663-2187.

Deb, S., Sinha, A. K. and Saha, A. (2021) Studies on Integumental Dystrophy of *B. mori* L, caused by Nuclearpolyherosis Virus (*Bm*NPV), Leading to Grasserie Disease and its Synergistic Management. *Int. J. A. R. E. S. M.*, 9(7):255 -261.

Deb, S., Chakrabarty, S., Saha, A. K., Hazra, N. (2014) Effect of grasserie on Gonadosomatic Index (GSI) of different commercial breeds and hybrid of *Bombyx mori* L. *J. Experimental Zoology*, 17(2):543-549.

Geethabai, M. and Mahadevappa, L. (1995) Comparative studies on the pathogenicity of *Nosema bombycis* Nageli and *Nosema* sp. (Microsporidia: Nosematidae) to *Bombyx mori* L. (Lepidoptera: Bombycidae) due to secondary contamination. *Sericologia*, 35(4): 681-685.

Hall, D. H. (1995) Electron microscopy and three-dimensional image reconstruction methods. *Cell Biology*, 48: 395–436.

Haas-Stapleton, E. J., Washburn, J. O. and Volkman, L. E. (2005) *Spodoptera frugiperda* resistance to oral infection by *Autographa californica* multiple nucleopolyhedro virus linked to aberrant occlusion-derived virus binding in the hind gut. *Journal of General Virology*, 86: 1349-1355.

Herniou, E. A., Olszewski, J. A., Cory, J. S. and O'Reilly, D. R. (2003) The genome sequence and evolution of Baculoviruses. *Annual Review of Entomology*, 48: 211-234.

Inoue, T. and Osatake, H. (1988) A new drying method of biological specimens for scanning electron microscopy: the t-butyl alcohol freeze drying method. *Archives of Histology Cytology*, 1: 53-59.

Krishnan, N., Mahanan, M. M., Mitra, P., Sen, S. K. and Saratchandra, B. (1998) Qualitative and Quantitative aspects of nuclear polyhedrosis virus infection in silkworm *Bombyx mori* (Lepidoptera: Bombycidae). *Annual report of the Entomological Society of America*, 16(2): 27-32.

Krishnaswami, S. (1978) New technology of silkworm rearing. Central Sericultural Research and Training Institute, Mysore, India, pp 1-10.

Magadum, S. B., Remadevi, O. K., Dutta, R. K. and Shivashankar, N. (1996) Correlation between various nutritional parameters of silkworm, *Bombyx mori* L. during fifth instars. *Indian Journal of Sericulture*, 35(2): 138-141.

Rath, S.S., Singh, B.M.K. and Sinha, B.R.R.P. (2000) Effect of Uzi parasitism on nutritional parameters and silk production in *Anthera mylitta* D. *Int. J. wild silk moth*. 5: 179-188.

Rahman, M. M. and Gopinathan, K. P. (2004) Systemic and *in vitro* infection process of *Bombyx mori* nucleopolyhedrovirus. *Virus Research*, 101: 109-118.

Saha, A. K., Dutta Biswas, T., Das. S. K. and Moorty, S. M. (2008) Bivoltine rearing during adverse season in West Bengal. *Indian Silk*, 47(1): 5-7.

Subba Rao, G., Chandra, A. K. and Bhattacharya, J. (2001) Incidence of crop loss from adopted rearer level in West Bengal (India) due to silkworm diseases. *Indian Journal of Seric*ulture, 30(2): 167.

Undeen, A. H. (1997) *Microsporidia* (*Protozoa*): A Handbook of Biology and Research Technique. Southern Association Agricultural Experiment Station, Directors, Southern *Cooperative Series*, Bulletin, pp 387.

Yashaswini,B., Madhusudhan, N. and Suresh,D.(2020) Automated smart sericulturebased on iot and image processing techniques. IEEEExplore, 10(6), 26201-26206.

Zhou, C. E., Ko, R. and Maeda, S. (1998) Polyhedron like inclusion body formation by a mutant nucleopolyhedrovirus expressing the granulin gene from a granulovirus. *Virology*, 240: 282-294.

ACKNOWLEDGEMENTS:

Authors are greatly thankful to **Mr. Sayan Deb**, M.E. (Jadavpur University, India) for his support to develop and prepare the photomicrographs. We are also thankful to Ms. **Shalini Das**, M. Stat. (Aliah University, India) for her support to prepare and analyse the data statistically. We acknowledge **Dr. Srikanta Bannerjee** University Science Instrumentation Centre, The University of Burdwan, for his help during Scanning Electron Microscopic investigations and **Dr. Tapas Chandra Nag** and Team Members, Sophisticated Analytical Instrumentation Facility, All India Institute of Medical Sciences, New Delhi for their cooperation during Transmission Electron Microscopic studies.

Table No. 1a. Economic parameters before challenge with *Bm*NPV during Unfavourable Rearing Season of 2016-21 (pool data) using Azadirachtin.

Treatment	Vth Instar Larval Weight (g)	Effective Rearing Rate (%)	Single Cocoon Weight (g)	Single Shell Weight (g)	Shell (%)
Dusting 1 (D1)	1.207 ± 0.019	44.000 ± 0.408	1.264 ± 0.081	0.219 ± 0.000	17.139 ± 0.008
Dusting 2 (D2)	1.140 ± 0.025	41.667 ± 0.624	1.227 ± 0.074	0.218 ± 0.001	16.637 ± 0.006
Sericillin (SER)	1.333 ± 0.015	51.667 ± 0.624	1.355 ± 0.092	0.258 ± 0.001	17.990 ± 0.012
Labex (LAB)	1.057 ± 0.010	49.000 ± 0.408	1.277 ± 0.076	0.214 ± 0.001	16.503 ± 0.006
Control	1.432 ± 0.014	53.067 ± 0.064	1.324 ± 0.091	0.322 ± 0.000	19.224 ± 0.016
CD (Critical Difference)	0.110**	2.481**	0.005**	0.002**	0.047**
CV% (Critical Variance)	3.627	2.035	0.159	0.361	0.104
SE. m ±	0.507	0.935	0.457	0.464	0.408

Table No. 1b. Economic parameters after challenge with *Bm*NPV during Unfavourable Rearing Season of 2016-21 (pool data) using Azadirachtin.

Treatment	Vth Instar Larval Weight (g)	Effective Rearing Rate (%)	Single Cocoon Weight (g)	Single Shell Weight (g)	Shell (%)
Dusting 1 (D1)	1.150 ± 0.004	38.000 ± 0.408	1.248 ± 0.001	0.209 ± 0.001	16.546 ± 0.013
Dusting 2 (D2)	1.100 ± 0.004	31.333 ± 0.624	1.244 ± 0.002	0.202 ± 0.001	16.023 ± 0.010
Sericillin(SER)	1.123 ± 0.012	35.000 ± 0.408	1.246 ± 0.002	0.204 ± 0.002	16.483 ± 0.014
Labex (LAB)	1.010 ± 0.011	31.000 ± 0.408	1.235 ± 0.002	0.206 ± 0.001	16.450 ± 0.008
Control	± 0.014	± 0.308	± 0.002	± 0.001	± 0.013
CD (Critical Difference)	0.069 **	0.964 **	0.007 **	0.006 **	0.057 **
CV% (Critical Variance)	2.470	7.921	0.223	1.017	0.136
SE. m ±	0.417	0.695	0.449	0.364	0.411

Table No. 2a. Economic parameters before challenge with *Bm*NPV during Unfavourable Rearing Season of 2016-21 (pool data) using Allicin.

Treatment	Vth Instar Larval Weight (g)	Effective Rearing Rate (%)	Single Cocoon Weight (g)	Single Shell Weight (g)	Shell (%)
Dusting 1 (D1)	1.207 ± 0.019	44.000 ± 0.408	1.264 ± 0.001	0.219 ± 0.000	17.139 ± 0.008
Dusting 2 (D2)	1.140 ± 0.025	41.667 ± 0.624	1.227 ± 0.001	0.218 ± 0.001	16.637 ± 0.006
Sericillin (SER)	1.333 ± 0.015	51.667 ± 0.624	1.355 ± 0.002	0.258 ± 0.001	17.990 ± 0.012
Labex (LAB)	1.057 ± 0.010	49.000 ± 0.408	1.277 ± 0.001	0.214 ± 0.001	16.503 ± 0.006
Control	1.432 ± 0.014	53.067 ± 0.064	1.324 ± 0.091	0.322 ± 0.000	19.224 ± 0.016
CD (Critical Difference)	0.110**	2.481**	0.005**	0.002**	0.047**
CV% (Critical Variance)	3.627	2.035	0.159	0.361	0.104
SE. m ±	0.507	0.935	0.457	0.464	0.408

Table No. 2b. Economic parameters after challenge with *Bm*NPV during Unfavourable Rearing Season of 2016-21 (pool data) using Allicin.

Treatment	Vth Instar Larval Weight (g)	Effective Rearing Rate (%)	Single Cocoon Weight (g)	Single Shell Weight (g)	Shell (%)
Dusting 1 (D1)	1.189 ± 0.006	36.000 ± 0.418	1.218 ± 0.001	0.199 ± 0.001	19.046 ± 0.014
Dusting 2 (D2)	1.120 ± 0.004	30.021 ± 0.612	1.184 ± 0.002	0.212 ± 0.001	18.018 ± 0.008
Sericillin(SER)	1.113 ± 0.012	29.000 ± 0.418	1.326 ± 0.00	0.276 ± 0.000	15.444 ± 0.017
Labex (LAB)	1.009 ± 0.001	30.010 ± 0.408	1.247 ± 0.001	0.219 ± 0.001	14.240 ± 0.008
Control	1.652 ± 0.004	50.061 ± 0.054	1.124 ± 0.071	0.322 ± 0.004	18.248 ± 0.016
CD (Critical Difference)	0.057 **	0.854 **	0.009 **	0.007 **	0.055 **
CV% (Critical Variance)	2.491	7.901	0.323	1.009	0.136
SE. m ±	0.551	0.786	0.348	0.585	0.419

Table No. 3a. Economic parameters before challenge with *Bm*NPV during Unfavourable Rearing Season of 2016-21 (pool data) using Curcumin.

Treatment	Vth Instar Larval Weight (g)	Effective Rearing Rate (%)	Single Cocoon Weight (g)	Single Shell Weight (g)	Shell (%)
Dusting 1 (D1)	1.207 ± 0.019	44.000 ± 0.408	1.264 ± 0.001	0.219 ± 0.000	17.139 ± 0.008
Dusting 2 (D2)	1.140 ± 0.025	41.667 ± 0.624	1.227 ± 0.001	0.218 ± 0.001	16.637 ± 0.006
Sericillin (SER)	1.333 ± 0.015	51.667 ± 0.624	1.355 ± 0.002	0.258 ± 0.001	17.990 ± 0.012
Labex (LAB)	1.057 ± 0.010	49.000 ± 0.408	1.277 ± 0.001	0.214 ± 0.001	16.503 ± 0.006
Control	1.432 ± 0.014	53.067 ± 0.064	1.324 ± 0.091	0.322 ± 0.000	19.224 ± 0.016
CD (Critical Difference)	0.110**	2.481**	0.005**	0.002**	0.047**
CV% (Critical Variance)	3.627	2.035	0.159	0.361	0.104
SE. m ±	0.507	0.935	0.457	0.464	0.408

Table No. 3c. Economic parameters after challenge with *Bm*NPV during Unfavourable Rearing Season of 2016-21 (pool data) using Curcumin.

Treatment	Vth Instar Larval Weight (g)	Effective Rearing Rate (%)	Single Cocoon Weight (g)	Single Shell Weight (g)	Shell (%)
Dusting 1 (D1)	1.165 ± 0.008	36.010 ± 0.528	1.198 ± 0.001	0.189 ± 0.007	16.546 ± 0.013
Dusting 2 (D2)	1.107 ± 0.010	29.252 ± 0.624	1.244 ± 0.002	0.207 ± 0.004	16.023 ± 0.010
Sericillin(SER)	1.139 ± 0.017	33.001 ± 0.501	1.241 ± 0.007	0.204 ± 0.005	16.483 ± 0.014
Labex (LAB)	1.004 ± 0.011	30.001 ± 0.407	1.235 ± 0.004	0.206 ± 0.001	16.450 ± 0.008
Control	1.552 ± 0.004	52.040 ± 0.051	1.321 ± 0.067	0.752 ± 0.007	19.658 ± 0.017
CD (Critical Difference)	0.087 **	0.844 **	0.007 **	0.006 **	0.059 **
CV% (Critical Variance)	2.850	8.674	0.223	1.017	0.187
SE. m ±	0.545	0.656	0.352	0.658	0.842

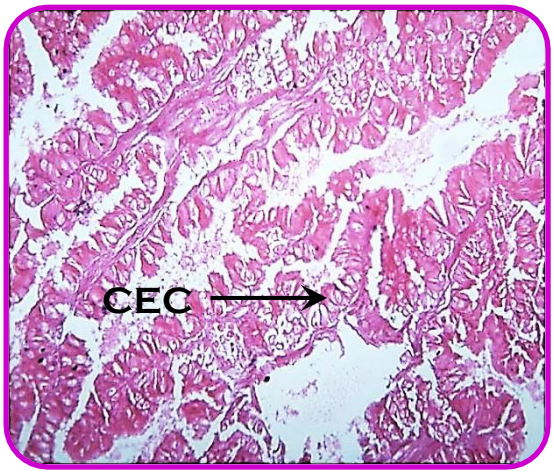


Fig: 1a. Histology of normal larval hindgut (×1000, CEC: Columnar Epithelial Cells).

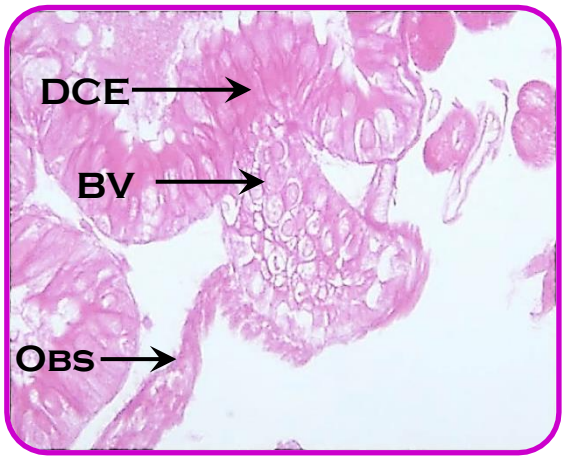


Fig: 1b. Histology of *Bm*NPV infected larval hindgut (×1000, DCE: Deformed Columnar Epithelial Cells, Obs: Occluded body, BV: Budded Virus).

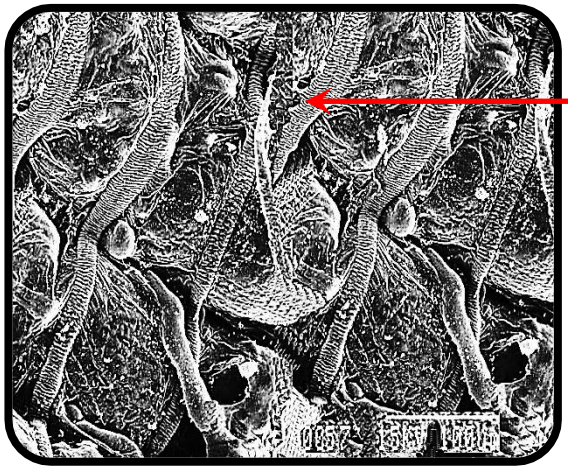


Fig: 2a. SEM of larval hindgut external view (×300, Hg: Hindgut).

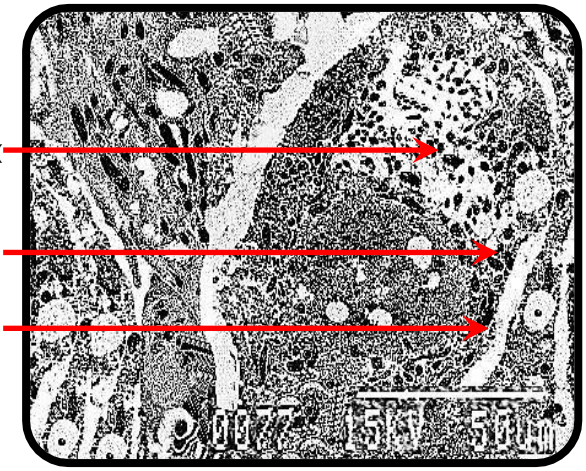


Fig: 2b. SEM of BmNPV infected larval hindgut cross sectional view (×1000, ESC: Empty Secretory Cell, DAC: Deformed Absorptive Cell, DD: Digestive Diverticula).

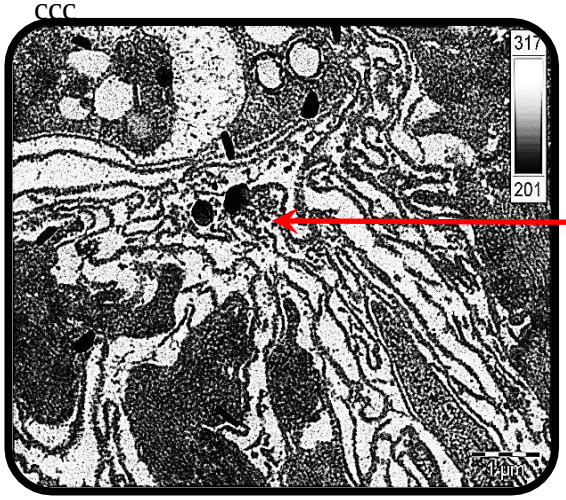


Fig: 3a. TEM of hindgut of normal Vth instar larva of B. mori (Gt: Gut tissue), Bar = 1 µm.

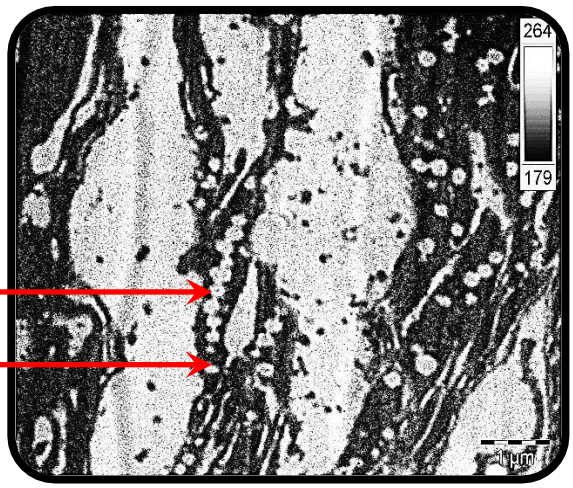


Fig: 3b: TEM of hindgut of BmNPV infected Vth instar larva of B. mori (Obs: Occluded bodies, Gt: Gut tissue), Bar = 1 µm.