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STUDIES OF DISEASE OUTBREAK IN SILKWORM REARING IN SUB-TROPICAL REGION OF POONCH DISTRICT

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Abstract

Due to prolong domestication, silkworm develops less immunity and less adaptability for which they are often attacked by different agents and is vulnerable to various pathogens resulting in disease that causes loss of almost 20% of the total cocoon production every year. Any abnormal condition of a part, organ or system of an organism from various causes such as infection inflammation environmental factors or genetic defect and characterized by an identifiable group of signs symptoms or both. Diseases of silkworm are big challenge to the rearers.. Grasserie, Muscardine, Pebrine, Flacherie, and Cytoplasmic polymerasic are the major diseases observed in cocoon culture. caused by Nucleopolyhedrosis Virus (BmNPV). Generally, bivoltine silkworms are more susceptible to diseases as compared to multivoltine silkworm breed. The factors responsible for disease incidence are higher pathogen load, wider fluctuation of temperature between day and night, higher humidity in the later stage of rearing and poor quality of mulberry leaves available for rearing in North West Indian condition. The most popular methods of disease control in silkworm are prophylactic. There is no race of *B. mori,* totally resistant or immune to diseases and pests. Hence, proper disinfection is required to kill all the pathogens present in the rearing environment and to eliminate risk of disease transmission as "Prevention is better than cure" is best in silkworm rearing. The present study deals with pathogen and pathogenesis of silkworm along with their prevention and control measures.

Keywords: Disease, Silkworm, Multivoltine, Immune, Pest, Cocoon, Pathogen, Grasserie, Environment.

Introduction

Sericulture-the production of raw silk by means of raising worms on mulberry leaves. UT of J&K have two distinct Sericultural zones viz, Temperate and sub-tropical based on agro climatic conditions. Jammu and Kashmir is a bivoltine sericulture state having only two rearing of silkworm larva in a year but its silk has outstanding quality on international level. As an important economic insect, silkworm *Bombyx mori* (L.) (Lepidoptera: Bombycidae) has numerous advantages in life science, such as low breeding cost, large progeny size, short generation time, and clear genetic background. The mulberry silkworm, B. mori produces silk, which are influenced by many pathogens includes fungi, bacteria, virus and protozoan which cause damages to cocoons reached to 75% .The frequency of

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infections at silkworm breeding extremely diminishes the production of silk. Protozoan Diseases: Pebrine- This is one of the most virulent protozoan disease caused by protozoa- Nosema bombycis.

Bacterial Diseases- Silkworm suffering from this disease exhibit diarrhoea and vomiting. It is caused by Bacillus bombycis, B. sotto, Streptococcus bombycids', Pseudomonas aeroginosa Viral diseases- Grasserie- Symptoms-This disease is characterized by the jaundice like symptoms which are collectively known as Grasserie- or Polyhedrosis. Other symptoms include- Loss of appetite, formation of loose and shiny white skin with swellings in the inter-segmental zones.

Fungal diseases known as Muscardine - In muscardine the body of the larvae get mummified due to deposition of calcium oxalate. Hence the disease is also called as 'calcino'. Symptoms includes- Sluggish movement of the larvae, loss of appetite, vomiting, shrinkage of body with loose cuticle are observed. These diseases not only affect the silkworm health but may also cause crop failure at field level.

Silkworm diseases are highly contagious and the causative agents get easily dispersed leading to outbreak of diseases.. Disease outbreak is one of the major constraints in attaining high productivity in sericulture. In India, the average silkworm cocoon crop loss due to various diseases is to the tune of 15 -47% (Tayal et al., 2017). The white muscardine (fungal) and Pebrine (protozoan) are the two major diseases of silkworm and has been the great scourge of sericulture (Krishnaswami, 1993).

Methodology adopted:

- Materials used:
- Diseased larva
- Autoclaved mortar pestle
- Distilled water
- Glass slide
- Microscope

Using whole larva method procedure

1. Take a live diseased larva and crush it in an autoclaved mortar and pestle by applying gentle force.
2. Crush the larval body to a fine homogenous paste of uniform thickness by addition of small amount double distilled water
3. Take a fresh glass slide and make a uniform smear of crushed larva on it with the help of another fresh slide. Cover the slide by gently placing a cover slip on it with the help of forcep or needle.
4. Observe the slide under light microscope.

Using haemolymph method procedure

1. Take a live diseased larva and give a sharp prick to its abdominal segment or appendages. Body fluid i.e., haemolymph starts oozing out.
2. Place 1 or d drops of haemolymph directly on the surface of a fresh glass slide.
3. Cover the slide by gently placing a cover slip on it with the help of forcep or needle.
4. Observe the slide under light microscope.

Disease to be observed:

The present study revealed that which type of disease occurs during rearing. In this study the viral disease occurs during rearing. Viruses are obligate intracellular pathogens which need host cells to replicate. Viral genetic material is made up of different forms of DNA or RNA and is enclosed in a protein coat and sometimes a lipoprotein envelope. The genetic material and surrounded protein are called Nucleocapsid and the entire structure is called Virion or virus particle. The

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Silkworm Bombyx mori *L.* is an important economic insect and also a tool to convert mulberry leaf protein into commercially valuable silk protein. Silk is called the "Queen of textiles" due to its glittering lusture, softness, elegance, durability and tensile properties. Silkworm disease is a phenomenon of Physiological disorders caused by invasion of pathogenic microorganism and parasites, or influence of physical and chemical factors and other adverse environmental factors, showing a variety of abnormal conditions or death of the Silkworm. Mulberry silkworm, *Bombyx mori L.* is affected by a number of diseases caused by Viruses, Bacteria, fungi and Protozoa. Viral diseases of Silkworm comprise of inclusion and non-inclusion types. The inclusion virus disease form typical inclusion bodies. They are nuclear Polyhedrosis and cytoplasmic Polyhedrosis which can be more easily identified through ordinary microscopy. The non- inclusion type consists of infectious flacherie and Densonucleosis which can be detected only through electron microscopy and Serological tests. These diseases are known to occur in almost all the Silkworm rearing areas of the world causing considerable damage to the silkworm cocoon crop. A number of measures have been suggested for the prevention and control of these diseases, care is also needed to be taken to see that they are not exposed to stress conditions like temperature, humidity, and mulberry leaf content. During the study it has been observed that the larvas were infected with Nuclear Polyhedrosis (Grasserie) disease caused by Virus (BmNPV).

Symptoms of Nuclear Polyhedrosis;

During early part of the disease no symptoms are noticed except the worms being slightly sluggish. Initially the skin shows oily and shiny appearance. The external symptoms of the BmNPV disease of the Silkworm in the fourth and fifth instar larva, which are visible about a week after infection, include the following:

- Swelling of inter segmental regions
- Larvae become lethargic and loose appetite
- Shining and yellowing body
- Hyperactivity
- Crawling around trays and hanging
- White ooze filled with polyhedra
- Death of worms
- Diseased larva loose the clasping power of abdominal legs, except the caudal legs by which it hangs with the head downwards
- The larva become restless and impatient cause fatigue

If infection occurs in the early instars, the worms fail to spin the cocoons and die, whereas infection occurs at later stages, the worms could spin the cocoon but subsequently die inside producing melted cocoons, and the affected cocoons become unfit for reeling.

RESULTS AND DISCUSSION

The result on the present investigation entitled "Studies of disease outbreak in silkworm rearing in sub-tropical region of Poonch district". The experimental result showed that there were significant changes in the growth, development and economical parameters of Silkworm due to outbreak of diseas

Causes of the disease

This disease is caused by Borrelina bombycis virus belonging to the sub-group A of the family Baculoviridae. The shape is usually octadecahedral or hexahedral and sometimes tetragon or trigon. Polyhedral Bodies under Light Microscope & their Shapes. Infection mostly takes place through feeding rarely wounds.

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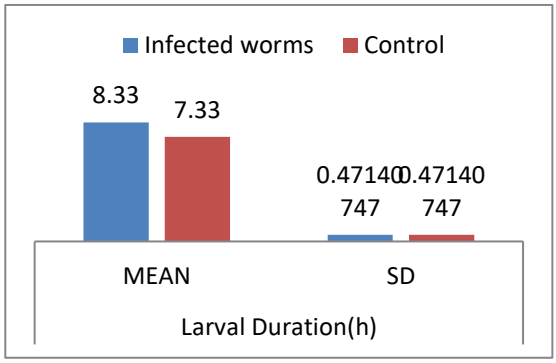
Larval duration

Breed(FC1XFC2)	Larval Duration(h)	
	MEAN	SD
Infected worms	8.33	0.47140747
Control	7.33	0.47140747

Table1: Larval duration of Infected and Control worms

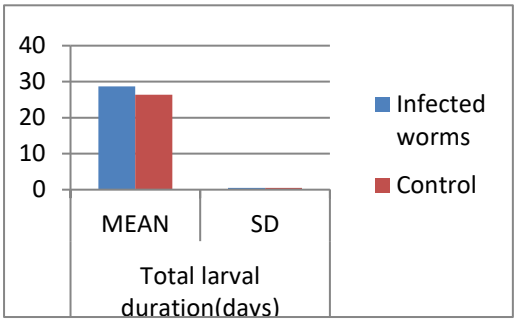


Graph 1: Larval duration of Infected and Control worms

Total larval duration

Breed(FC1XFC2)	Total larval duration(days)	
	MEAN	SD
Infected worms	28.66	0.47141631
Control	26.33	0.47140747

Table 2: Total larval duration of Infected and Control worms

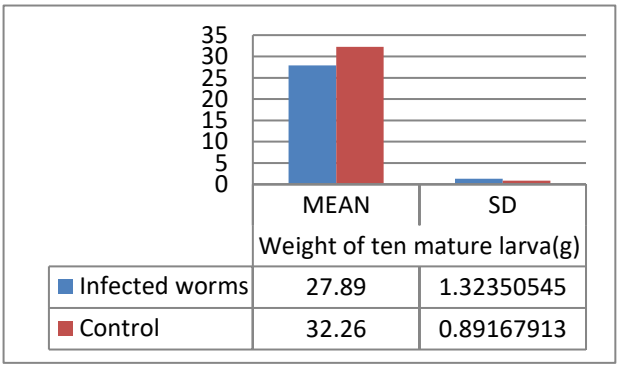


Graph 2: Total larval duration of Infected and Control worms

Weight of ten mature larvae

Breed(FC1XFC2)	Weight of ten mature larva(g)	
	MEAN	SD
Infected worms	27.89	1.32350545
Control	32.26	0.89167913

Table 3: Weight of ten mature larvae of Infected and Control worms



Graph 3: Weight of ten mature larvae of Infected and Control worms

Size of mature larva (cm)

Breed(FC1XFC2)	Size of mature larva(cm)	
	MEAN	SD
Infected worms	7	0.40824829

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Control	8	0.40824829

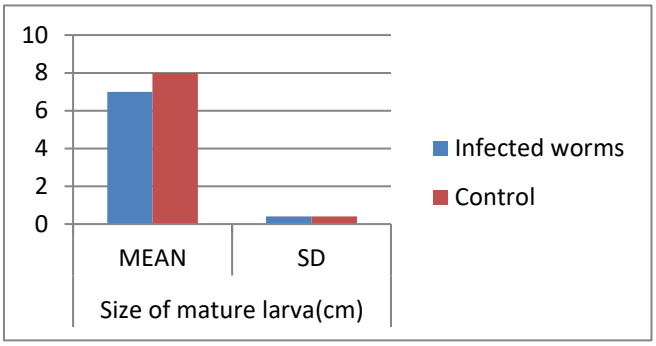
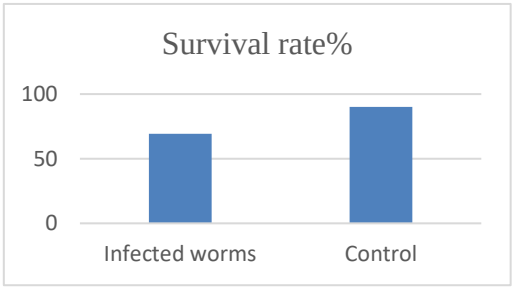


Table 4**:** Size of mature larva of Infected and Control worms. Graph 4: Size of mature larva of Infected and Control worms

Survival Rate (%)

Breed(FC1XFC2)	Survival Rate%	
	MEAN	SD
Infected worms	69.33	4.109609 67
Control	90	1.632993 16

Table 5: Survival Rate of Infected and Control worms

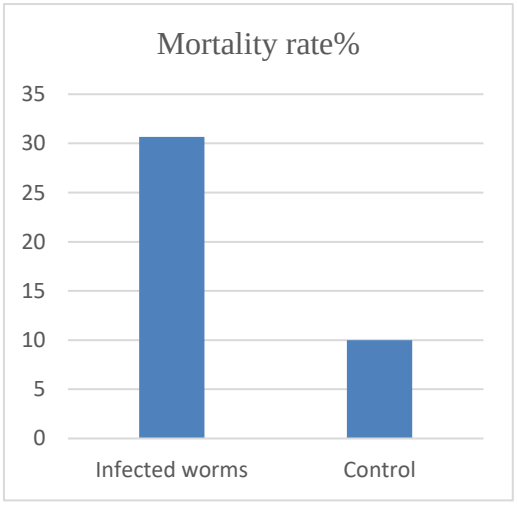


Graph 4: Survival Rate of Infected and Control worms

Mortality Rate (%)

Breed(FC1XFC2)	Mortality Rate%	
	MEAN	SD
Infected worms	30.66	0.4109611
Control	10	0.01632993

Table 6& Graph 5: Mortality rate of Infected and Control worms



Economic parameters of Cocoon

Following parameters have been observed in Cocoon stage.

Single Cocoon weight (g)

Table 7: Single cocoon weight of Infected and Control worms

Breed(FC1XFC2)	Single Cocoon Weight(g)	
	MEAN	SD
Infected worms	1.1	0.03095696
Control	1.34	0.03095696

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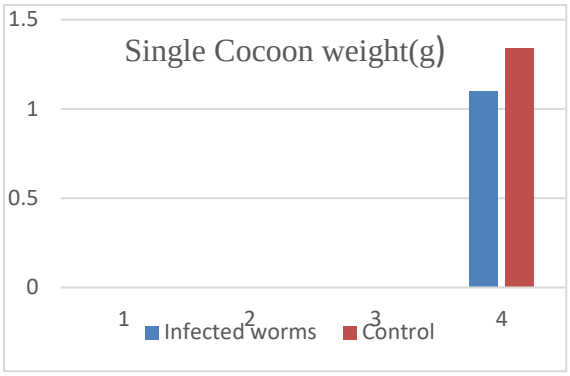
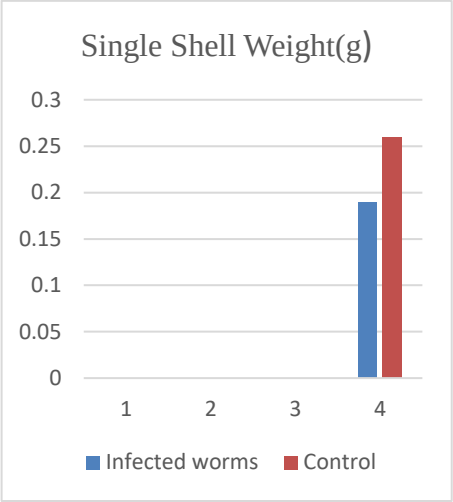


Graph 6: Single Cocoon Weight of Infected and Control worms

Single Shell weight (g)

Breed(FC1XFC2)	Single Shell Weight(g)	
	MEAN	SD
Infected worms	0.19	0.0057735
Control	0.26	0.005

Table 7: Single Shell weight of Infected and Control worms



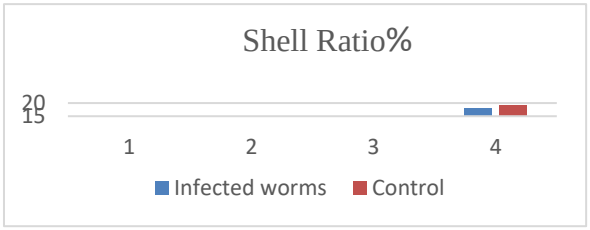
Graph 7: Single Shell weight of Infected and Control worms

Shell Ratio (%)

Table 8: Shell ratio of infected and control worms

Breed(FC1 XFC2)	Shell Ratio%	
	MEAN	SD
Infected worms	17.83	0.791328
Control	19.35	0.18627936

Table 8: Shell ratio of Infected and Control worms

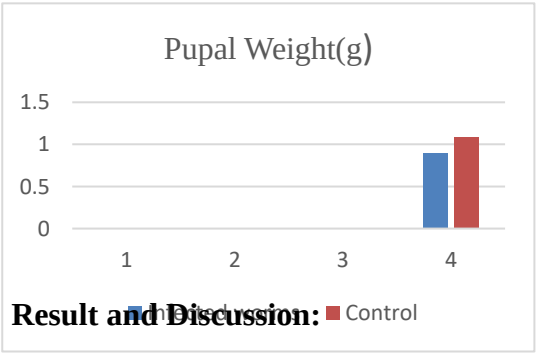


Graph 8: Shell ratio of Infected and Control worms

Pupal Weight (g)

Breed(FC1XFC2)	Pupal Weight(g)	
	MEAN	SD
Infected Worms	0.9	0.03316625
Control	1.08	0.02828427

Table 9 & Graph 9: Graph 9: pupal weight of Infected and Control worms



Result and Discussion:

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The larval duration of infected and control breeds. Statistical analysis of the data showed that the significantly maximum larval duration was seen in infected worms (8.33). In case of control the Mean of larval duration was recorded as 7.33, where as in case of infected worms it was recorded as 8.33. Larval duration of control worms SD was 0.47140747 and infected worms SD was 0.47140747. Total larval duration of infected and control breed. Statistical analysis of the data showed that the significantly maximum total larval duration was seen in infected worms (28.66). In case of control the Mean of total larval duration was recorded as 26.33, where as in case of infected worms it was recorded as 28.66. Larval duration of control worms SD was 0.47141631 and infected worms SD was 0.47140747. The weight of ten mature larva of infected and control breed. Statistical analysis of the data showed that the significantly maximum weight of ten mature larva was seen in control worms (32.66). In case of control the Mean value of weight of ten mature larva was recorded as 32.66, where as in case of infected worms it was recorded as 27.89. Weight of ten mature larva of control worms SD was 0.89167913 and infected worms SD was1.32350545.length of full grown 5^{th} instar larva given in Table 5. Analysis of the data showed that the maximum mean size of larva of control worms was 8, where as in infected worms the size was 7. In case of control the Mean value of size of mature larva was recorded as 8, where as in case of infected worms it was recorded as 7. Size of mature larva of control worms SD was 0.40824829 and infected worms SD was 0.40824829. the survival rate of infected and control worms. The highest survival rate was shown by the larva of control worms. In case of control the Mean value of survival rate was recorded as 90, where as in case of infected worms it was recorded as 69.33. Survival rate of control worms SD was 1.63299316 and infected worms SD was 4.10960. The mortality rate of Silkworm larva was seen least in Control worms. In case of control the mortality rate was 10, where as in infected worms the mortality rate was 30.66. In case of control the Mean value of mortality rate was recorded as 10, where as in case of infected worms it was recorded as 30.66. Survival rate of control worms SD was 0.01632993 and infected worms SD was 0.4109611.The result concerning the Single cocoon weight of infected and control breeds. Statistical analysis of the

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data showed that the significantly highest single cocoon weight was seen in control worms (1.34). In case of control the Mean of single cocoon weight was recorded as 1.34, where as in case of infected worms it was recorded as 1.1. Single Cocoon weight of control worms SD was 0.03095696 and infected worms SD was 0.03095696.Statistical analysis of the data showed that the significantly highest single shell weight was seen in control worms (0.26). In case of control the Mean of single shell weight was recorded as0.26, where as in case of infected worms it was recorded as 0.19. Single shell weight of control worms SD was 0.005 and infected worms SD was 0.0057735. Statistical analysis of the data showed that the highest shell ratio was seen in control worms (19.35). In case of control the Mean of shell ratio was recorded as 19.35, where as in case of infected worms it was recorded as17.83. Shell ratio of control worms SD was 0.18627936 and infected worms SD was 0.791328.The maximum mean pupal weight was observed in Control worms 1.08. In case of control the Mean of pupal weight was recorded as1.08, where as in case of infected worms it was recorded as 0.9. Pupal weight of control worms SD was 0.02828427 and infected worms SD was 0.03316625.

CONCLUSION:

The experimental results showed that the mulberry silkworm, domesticated and mass reared for several centuries, presumably has weakened immune system which has made the insect highly vulnerable to viral infections. Silkworm viral diseases are the major cause for cocoon crop loss. The course of infection by these viruses is largely influenced by environmental, nutritional and rearing managerial practices. Information on the aspects like causative agents, incidence and occurrence, infection and transmission, symptoms and detection, and management, in the silkworm with reference to four major viral infections in particular is scanty. Prevention of viral infections during silkworm rearing helps to increase the silk productivity, because if the diseases are controlled below the economic threshold level then there will be an increase in silk production.

Another most effective solution is timely detection of viral infections in silkworm rearing to stop further spread of the disease. The present information may prove vital in identifying the suitable symptoms in the silkworm larvae during viral infections and to protect the commercial characteristics of cocoon yield in addition to suggest suitable measures in regulating the disease. This will help in the restoration of sericulture output and will

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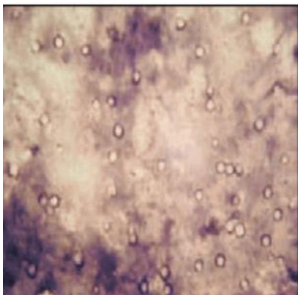
safe guard the interest of the farmers involved in sericultural practices. Crop loss percentage due to this disease was 30% to 40% average crop loss in silkworm rearing. The present study revealed that the crop loss percentage is 30.66, conducted at Poonch Campus during spring rearing.

Disease larva

Crushing of worms



Polyhedral bodies showing viral disease

Chopping of leaves for feeding

mature larvae

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