Project ID-518

Competitive Research Grant (CRG)

Sub-Project Completion Report

On

IDENTIFICATION OF DIFFERENT SPECIES OF BACTERIA CAUSING BACTERIAL DISEASESS OF SILKWORM (*BOMBYX MORI* L.) AND THEIR CONTROL.

Project Duration

May 2016 to September 2018

Pathology Section, Bangladesh Sericulture Research and Training Institute
Rajshahi

Submitted to
Project Implementation Unit-BARC, NATP-2
Bangladesh Agricultural Research Council
Farmgate, Dhaka-1215

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Citation
Identification of different species of bacteria causing bacterial diseases of silkworm (*Bombyxmori*) L. and their control Project Implementation Unit
National Agricultural Technology Program-Phase II Project (NATP-2)
Bangladesh Agricultural Research Council (BARC)
New Airport Road, Farmgate, Dhaka – 1215
Bangladesh

Edited and Published by:
Project Implementation Unit
National Agricultural Technology Program-Phase II Project (NATP-2)
Bangladesh Agricultural Research Council (BARC)
New Airport Road, Farmgate, Dhaka – 1215
Bangladesh

Acknowledgement

The execution of CRG sub-project- **Identification of different species of bacteria causing bacterial diseases of silkworm (*Bombyxmori*) L. and their control(ID-518)**.Has successfully been completed by Bangladesh Sericulture Research and Training instiiute,Rajshahi using the research grant of USAID Trust Fund and GoB through Ministry of Agriculture. We would like to thanks to the World Bank for arranging the grand fund and supervising the CRGs by BARC. It is worthwhile to mention the cooperation and quick responses of PIU-BARC, NATP 2, in respect of field implementation of the sub-project in multiple sites. Preparing the project completion report required to contact a number of persons for collection of information and processing of research data. Without the help of those persons, the preparation of this document could not be made possible. All of them, who made it possible, deserve thanks. Our thanks are due to the Director PIU-BARC, NATP 2 and his team who given their whole hearted support to prepare this document. We hope this publication would be helpful to the agricultural scientists of the country for designing their future research projects in order to technology generation as well as increasing production and productivity for sustainable food and nutrition security in Bangladesh. It would also assist the policy makers of the agricultural sub-sectors for setting their future research directions.

Published in: September 2018

Printed by: [Name of press with full address]

Acronyms

BSRTI	Bangladesh Sericulture Research and Training Institute
BSDB	Bangladesh Sericulture Development Board
DG	Director General
BARC	Bangladesh Agricultural Research Council
BM	Bangladesh Mulberry
MS	Mulberry Silkworm

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Executive Summary

Environmental condition of our country, especially during hot and humid month silkworm rearing is very much apt to attack of bacterial diseases; there causing a substantial loss of the cocoon growers of the country also the silk industry. Through careful observation in different rearing season, some of bacterial diseases affecting silkworm were detected. There are though many bacteria have been isolated from the infected silkworm are *Bacillus subtilis Streptococcus pnumoniae, Staphylococcus aureus,* and *Bacillus thuringiensis*. In this period four season's data was collected on the basis of spacing and feeding for silkworm rearing. The effect of different spacing on the incidence of bacterial diseaseof silkworm. This indicated that crowded rearing reduced spacing of T4-(600sq2/100) worms caused highest mortality due to bacterial diseases of silkworm. The effect of feeding tender, mature, water-soaked, One-day preserved and Normal mulberry leaves to the silkworm larvae and the result mortality due to bacterial diseases. The highest bacterial mortality observeT4- (One day preserved) mulberry leaves. To prevent the loss, disease management strategies are essential and in the present study an experiment was conducted to study the efficacy of various combinations of bed disinfectants against bacterial disease. Locally available There are some identified effective chemicals composite to control finely powdered Calcium carbonate($Caco_3$) was the base material, which was mixed with different chemicals such as, Bleaching powder, Paraformaldehyde, Ammonia, Benzoic Acid and Sodium carbonate. Silkworms after third moult were surface inoculated with the suspension of bacteria inoculation bodies of silkworm. Bed disinfectants of various formulation applied half hour before feeding, uniformly on the silkworm, twice during fourth instar and thrice during fifth inster after through bed cleaning. The results showed that bed disinfectants significantly reduced the mortality of silkworms in contrast to treatments where no bed disinfectants were applied. The formulations comprising of Bleaching powder, Paraformaldehyde, Ammonia, Benzoic Acid and Sodium carbonate showed the highest survival rate Bleaching powder(2%), Paraformaldehyde(2%), Ammonia(2%), Benzoic Acid(2%), Sodium carbonate(2%,3%), No adverse effect on larval and cocoon characters of silkworms due to the application on these was observed.

CRG Sub-Project Completion Report (PCR)

A. Sub-project Description

1. Title of the CRG sub-project: Identification of different species of bacteria causing bacterial diseases of silkworm (*Bombyx mori* L.) and their control.

2. Implementing organization: Bangladesh Sericulture Research and Training Institute (BSRTI)

3. Principal Investigator:

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4. Sub-project budget (Tk):1500000.00 (Fitten Lakh)

5. Duration of the sub-project:
   5.1 Start date (based on LoA signed):17 May 2017
   5.2 End date: 30 September 2018

6. Justification of undertaking the project:
Sericulture is an agro-based enterprise highly suited to small and marginal farm holdings with less capital investment. Cocoon production involves mainly two distinct activities namely, mulberry leaf production and silkworm rearing. Silkworms are infested with a number of bacterial diseases, which affect the cocoon quality and productivity resulting in economic loss to the farmers. In Bangladesh, there are four major commercial silkworm rearing and cocoon production seasons like Jaistha, Bhaduri,Agrahyani and Chita. Bacterial disease mostly occurs in Jaistha and Bhaduriseasion. Bacterial diseases are popularly known as 'Flacherie' due to the flaccid nature of the diseased larvae. In India Chittra*et* al. (1974) reported 20-40% damage in Bangladesh. For minimization of

losses due to bacterial diseases, It is very essential to know the causedagainst bacteria and then find out their control measure so that crop stability can be ensured. Hence this project has been proposed.

7. **Sub-project goal:** Enhancement of quality cocoon production through control of bacterial disease of silkworm

8. Sub-project objective:

I. Study on different bacterial species.
II. Study on relationship between host and pathogen.
III. Study on effective chemicals to control bacterial diseases

9. Implementing location (s):

Bangladesh Sericulture Research and Training Institute, Rajshahi.

Fig: Main Signboard of the project

10. Methodology in Brief:

Sample collection: Diseased silkworm was collected from sericulture extension areas and BSRTI Lab.

Media and biochemical reagents: Brain heart infusion ager, Nutrient ager, Potato ager media and Blood ager media were used.

Isolation and purification of bacteria: Isolation and purification of bacteria was done using following procedure.

- Culture media was used to isolate and purify bacteria. The most common media nutrient ager. The media was dissolving in water and stream sterilize at 121^{o}c for 15 minute in autoclave. Specific culture media was used to specific type of bacteria.
- Suspension/homogenate collected from heamolymph, midgut external surface of silkworm was used for isolation of bacteria.
- 2 sterile nutrient ager plate and streak the suspension /homogenate will be taken. Streak on plate with the water was used in the last washing of the insect.
- Label them and was incubate for 14 h in an inverted position at room temperature:
- The amount of inoculum streaked and the type of streaking may vary with number of microorganisms present in the inoculum. If many bacteria are expected in the inoculum, a single lopful may be adequate. If the number of bacteria is expected to be few, two or more loopful may be needed.
- To streak, lift the metal cap from the tube containing the inoculum and flame the open end. Flame in inoculating loop cool and deep it into the inoculum.
- Lift the cover of Petridis containing the nutrient medium just high enough to manipulate the inoculating loop.
- Apply a lopful of inoculum at the edge of medium and streak the inoculum. Label incubated the petri plate in an inverted position.
- Isolated colony was found in great abundance in the area covered first streaking then the cover by second streaking, streak the isolated colony in separate sterile tube.
- Record the results after 48-96 hrs.
- In order to obtain the pure culture of organism, the isolated colonies are aseptically transferred on to different nutrient agar slant tubes and incubated overnight at 37 degree Celsius. It is then stored for future purpose.

Molecular Characterization: Molecular characterization of different type of bacteria in silkworm disease will be used by PCR and determined by gel electrophoresis.

Use of selected bed disinfectants:

Selected dose of different disinfectants viz; Lime, Paraformaldehyde, bleaching powder, Medicinal plants and polu powder use to disinfect the rearing bed which is one of the most possible sources of bacteria contamination. The powder disinfectants was dusted uniformly on the rearing trays after removing the larvae at another pre-disinfected tray for the moment. Different germicidal chemicals will be dusted uniformly through the fine cotton duster. All the disinfectants was applied only once a day from the 3rdinstar to 5thinstar.This experiment will consist of ten different treatments and each treatment contain three replications with 100 silkworms for each replication.

Observations on the larval mortality due to bacterial diseases and total mortality was separately recorded.

Different germicidal chemicals with appropriate percentage will be selected for formulate appropriate chemical composites as an effective disinfectant of bacterial pathogen.

T_1= Bleaching powder- 2%

T_2= Paraformaldehyde- 2%

T_3 = Ammonia- 2%

T_4 = Benzoic Acid- 2%

T_5 = Sodium carbonate- 2%

T_6= Paraformaldehyde (2%) + Bleaching (2%) + Sodium Hypochlorite (1%)

T_7 = Paraformaldehyde (2%) + Bleaching (1%) + Ammonia (1%)

T_8= Paraformaldehyde (2%) + Bleaching (2%) + Sodium Benzoate (1%) + Sodium Carbonate (1%)

T_9 = Paraformaldehyde (2%) + Sodium Carbonate (1%) + Benzoic acid (1%)

T_{10}= Paraformaldehyde (2%) + Sodium H (2%) + Ammonia (1%)

T_{11} = Benzoic 2%, Bleaching (2%), Ammonia (1%)

T_{12} = Benzoic 2%, Sodium C (2%), Ammonia 1%

T_{13} = Medicinal plants

T_{14} = Inoculated

T_0 = Normal Rearing

Identification of bacterial species causing bacterial diseases:

- The silkworm larvae showing bacterial infections were surface sterilized with 0.1 per cent mercuric chloride solution and washed thrice with sterile distilled water.
- The larvae were then triturated in sterile water blanks using glass rod. A loopful of the suspension was taken with inoculation needle and streaked on the Nutrient Agar medium and incubated at room temperature).
- Bacterial pathogens were isolated after growing them in a Nutrient Agar (NA) medium streak plate technique, most popular and easier method for isolating single colonies from large number of different bacteria was followed for further sub-culturing of isolated pathogens. The bacterial colonies were further purified by streaking on the plate. The isolated single colonies of bacteria (Plates 1 and 2) were picked up and streaked on NA slants.
- After staining good growth, slants were stored in a refrigerator at 4^0 C for further studies.

Culturing medium for spore producing bacteria:

- For bacterial species the colonies from the nutrient agar plates were further streaked in deferent medium for efficient production of spores and crystals.
- The well isolated colonies from the deferent medium plates were then taken and sub cultured until pure colonies of bacterial species were obtained.

- The suspension of bacterial species was prepared form single colony using sterile double distilled water and subjected to heat treatment at 50-60^{0} C for 15 minutes. The heat-treated suspension was streaked on the deferent medium plate for better recovery of bacterial species colonies.

Identification and characterization of bacteria:

- Morphological characteristics such as colony morphology, cellular morphology and staining techniques including simple staining, negative staining, gram staining and endospore staining were carried out for bacterial pathogens of silkworm.
- The cultural characteristics were observed using different media

Sericulture is an agro-based enterprise highly suited to small and marginal farm holdings with less capital investment. Cocoon production involves mainly two distinct activities namely, mulberry leaf production and silkworm rearing. Silkworms are infested with a number of bacterial diseases, which affect the cocoon quality and productivity resulting in economic loss to the farmers. In Bangladesh, there are four major commercial silkworm rearing and cocoon production seasons like Jaistha, Bhaduri, +

Agrahyaniand Chita. Bacterial disease mostly occurs in Jaistha and Bhaduri seasion. Bacterial diseases are popularly known as 'Flacherie' due to the flaccid nature of the diseased larvae. In India Chittra,et. al, (1974) reported 20-40% damage bacterial diseases of silkworm in Bangladesh. For minimization of losses due to bacterial dieases, It is very essential to know the causedagainst bacteria and then find out their control measure so that crop stability can be ensured. Hence this project has been proposed.

Results and discussion:

Experiment:1 Study on different bacterial species

In the present study, disease causing microorganisms were isolated from mulberry silkworm, *Bombyx mori* L. Figure 1 represents the life cycle of silkworm.

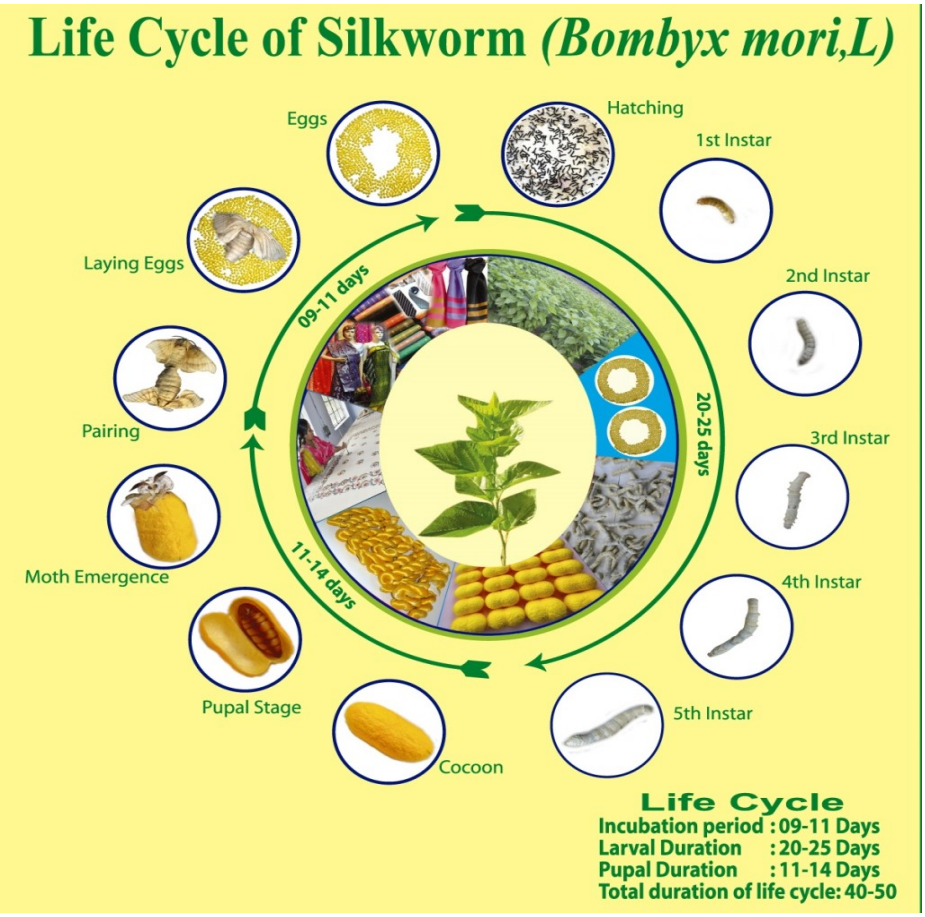


Fig: Life cycle of silkworm *Bombyxmori* L.

Diseased, dead larvae of *Bombyx mori* were used in the present study (Figure 2). A total 14 bacterial isolates (BM1-BM14) were obtained from serial dilution techniques. Distinct colonies were subculture on nutrient agar media (Figure 3).

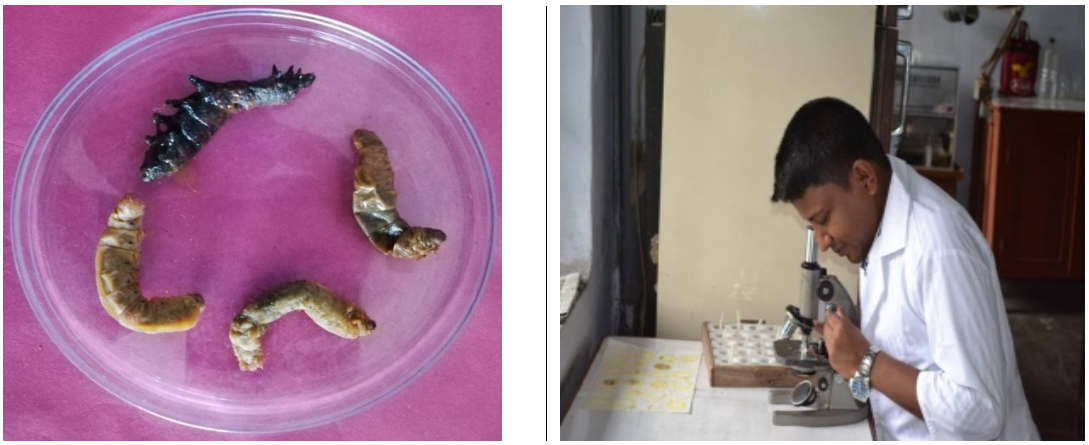


Fig 3: Diseased silkworm larvae were used for the isolation of causative microorganisms.

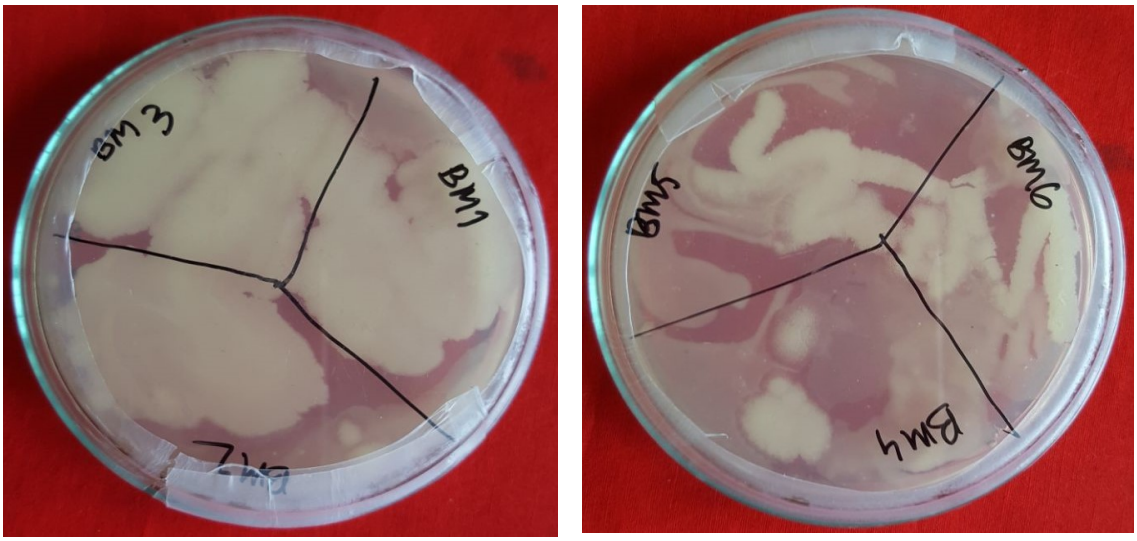


Fig 4: Pure culture of bacterial strains in nutrient agar plates

Mulberry silkworm, *Bombyxmori* L., is affected by a number of diseases but bacteria is one of the major disease causing agents. Such diseases are known to occur in almost all the silkworm rearing areas of the world causing considerable damage to the silkworm cocoon crop. Bacterial diseases affecting silkworm are collectively known as flacherie due to the flaccid nature of the disease larvae. In the present study, we isolated 14 bacterial strains from diseased silkworm in Bangladesh.

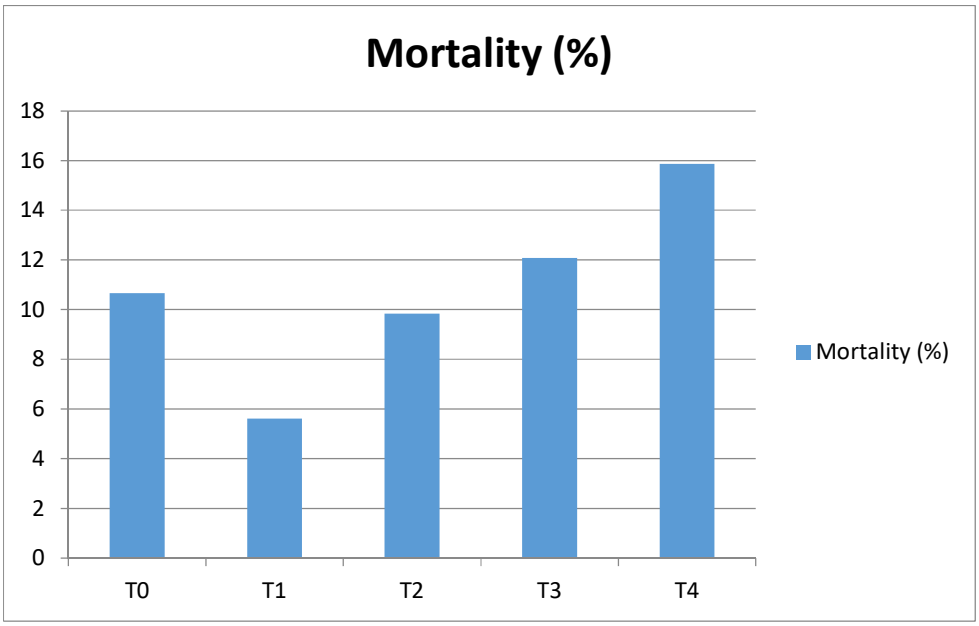
Though many bacteria have been isolated from the infected silkworm .Some isolated bacterial species given below

- *Bacillus subtilis*
- *Streptococcus pnumoniae*
- *Staphylococcussp aureus*
- *Bacillus thuringiensis.*

Experiment: 2 Effect of different quality mulberry leaves on mortality of silkworm larvae.

Four unusual qualities of mulberry leaves viz. normal (T_0), Tender (T_1), Matured (T_2) , Water soaked (T_3) and one day preserved (T_4) were fed to the silkworm through 3rd to 5thstage. The test material to local race Nistari(M) and the experiment was repeated through the year. Three replications each with 300 worms were maintained for each treatment. Incidence of bacterial diseases were separately noted every day at bed cleaning, spacing and other routine managements were equally provided to all the treatments.

Mean of larval mortality due to different quality of Mulberry leaves provided from 3rd to 5thstage of silkworm



Quality of mulberry leaves

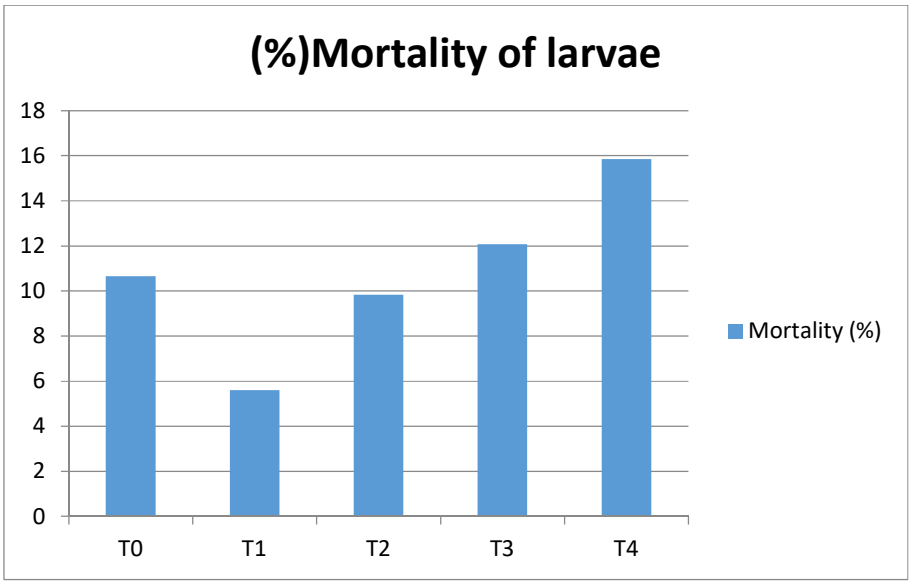
The effect on feeding tender, mature, water soaked, one day preserved and normal mulberry leaves to the silkworm larvae and the resultant mortality due to bacteria are shown in table -1 .Highest bacterial mortality was observed in case of feeding on one day preserved mulberry leaves (25.56).Followed by water soaked (22.91) and Tender (22.72). But normal laves and matured leaves resulted the lowest bacterial disease respectively.

Fig 5: Effect of different Quality leaves on bacterial incidence of silkworm

Expt-3 Effect of population of worm in rearing tray on larval mortality of silkworm.

Hard board bottomed wooden trays were used for rearing silkworms. A local race Nistari (M) considered for the experiment. Four spacing used were 300worms 1200cm^2/100worms (T_1), 400worm's 900cm^2/100worms (T_2), 500worms' 700cm^2/100worms and 600 worms' 600cm^2/100worms (T_4) per tray. All the treatments were replicated into three individual batches. Normal feeding and management were provided to all the bathes .The experiment was repeated through the year.

Mean of mortality of silkworm larvae due to different spacing provided from 3rd to 5thinster of silkworm.



Population of silkworm larvae

The effect of different spacing on incidence of bacterial diseases in shown table-2 which indicate that crowded rearing reduced spacing treatment (T_4)worms cause highest mortality due to bacterial diseases, while spaces rearing extended spacing of T_1 resulted lowest.

Fig 6: Effect of different spacing's on bacterial incidence of silkworm

Experiment: 4. Effect of bed disinfectants on growth and survivability of silkworm.

Various combinations of bed disinfectants comprising of Bleaching powder, Paraformaldehyde, Ammonia, Benzoic acid, Sodium Carbonate and Medicinal plant rewe used in the present study

Table-1: Effect of bed disinfectants on the economic characters and survival of silkworm (*Bombyx mori* L.) infected with bacterial diseases.

Treatment	Larval Weight (10 Larvae)	Single Cocoon Weight(gm)	Single Shell Weight(gm)	Shell Ratio (%)	Survival rate (%)

T_0=Normal Rearing	30.90cd	1.01a	0.11bcd	11.93ab	34.90h
T_1= Bleaching Powder- 2%	33.81ad	1.10a	0.14a	14.08a	75.52 a
T_2= Paraformaldehyde- 2%	30.68cd	1.02a	0.12bcd	11.55ab	66.94b
T_3 = Ammonia- 2%	34.91a	1.06a	0.12bcd	13.23ab	53.98cde
T_4 = Benzoic Acid- 2%	34.99a	1.06a	0.13abcd	12.47ab	45.637fg
T_5 = Sodium carbonate- 2%	32.19abc	1.03a	0.11bcd	14.11a	52.24cdef
T_6= Paraformaldehyde (2%) + Bleaching (2%) + Sodium Hypochlorite (1%)	32.75abc	1.01a	0.14a	12.52ab	54.49bcde
T_7 = Paraformaldehyde (2%) + Bleaching (1%) + Ammonia (1%)	31.75bcd	1.01a	0.12bcd	13.04ab	44.88g
T_8 = Paraformaldehyde (2%) + Bleaching (2%) + Sodium Benzoate (1%) + Sodium Carbonate (1%)	33.43abc	1.04a	0.13abcd	13.90ab	61.23bc
T_9 = Paraformaldehyde (2%) + Sodium Carbonate (1%) + Benzoic acid (1%)	31.17bcd	1.07a	0.11bcd	13.20ab	50.16defg
T_{10} = Paraformaldehyde (2%) + Sodium H (2%) + Ammonia (1%)	29.02 d	1.02a	0.13abc	13.31ab	49.20defg
T_{11} = Benzoic 2%, Bleaching (2%), Ammonia (1%)	32.70abc	1.01a	0.11cd	12.30ab	54.58bcde
T_{12} = Benzoic 2%, Sodium C (2%), Ammonia 1%	32.83abc	0.99a	0.13ab	12.15ab	55.59bcd
T_{13} = Medicinal plants(*Syzygium cumini*)	33.87ab	1.09a	0.11bcd	12.84ab	57.95bc
T_{14} = Inoculated	32.85abc	1.04a	0.10d	11.35b	34.80h

The results presented table-1 show that bed disinfectants significantly reduced the mortality of silkworm larvae due to bacterial diseases as compared to untreated control. Among various treatments the batches of silkworm treated with bleaching powder T_1 showed the highest survival rate of 75.52% followed by the T_2 and T_8 with a survival rate of 66.94b and 61bc present respectively .The least effective treatment was T_{14} with a survival rate only34.80h. The treatment did not show any adverse effect on economical characters of the silkworms. Among the treatments the larval weight was higher in T_4treated silkworms which were at per with normal rearing and that of T_0 Treatment. Cocoon weight was recorded highest (1.10) gm. in T_2 followed by T_{13} and lowest treatment T_{12}. The shell weight of (0.14) gm. was recorded maximum in T_2 and T_6 which was followed by T_4,T_8,$T_{10}$and T_{12}.The normal rearing with (0.11)gm. The shell ratio of T_5 and T_1 was maximum followed by T_4, T_8, T_{10}, T_{12} and in normal rearing (11.93%).

The above observations clearly indicate that the treatments are effective against the bacterial diseases. The combinations in different formulations with lime have also been reported as effective against bacterial diseases of silkworm. In this study it was also observed that the treatment combinations having Bleaching powder as one of the ingredients were more effective as compared to other treatments. Since the main reason of bacterial disease outbreak is the heavy pathogen load in the rearing bed, the present study provides a solution for the prevention of the bacterial diseases. Topical application of the effective bed disinfectant can be utilized as effective prophylactic measure against occurrence of these bacterial diseases.

Fig 7: preparation bed disinfectants, dusting Silkworm body and data collection

12. Research highlight/findings (Bullet point – max 10 nos.):

- There are four genera of bacteria were found to be associated with silkworm such as
  Bacillus subtilis
  Streptococcus pnumoniae
  Staphylococcussp aureus
  Bacillus thuringiensis
- The effect of different spacing on the incidence of bacterial disease. This indicated that crowded rearing reduced spacing of T4 (600sq2/100) worms caused highest mortality of bacterial diseases.

- The effect of feeding Tender, Mature, Water-soaked. One-day preserved and normal mulberry leaves to the silkworm larvae and the result mortality due to bacterial diseases of silkworm are highest bacterial mortality observeT4 (One day preserved) mulberry leaves.

- Among the adverse season (Jhaistha & Vhaduri) T_1 (Bleaching- 2%), T_4(Benzoic Acid- 2%), T_5 (Sodium carbonate- 2%),T_8 (Paraformaldehyde 2%+Bleaching2%+Sodium benzoate1% and Sodium Carbonate15) and T_{11}(Sodium carbonate- 2%) have showed the best result on survivalist of silkworm (*B. mori*).

B. Implementation Position

1. Procurement:

Description of equipment and capital items	PP Target		Achievement		Remarks
	Phy (#)	Fin (Tk)	Phy (#)	Fin (Tk)	
(a) Office equipment	Laptop-1	60000	Laptop-1	60000	All are purchased properly through RFQ
(b) Lab &field equipment	Laminar Airfow	350000	Laminar Airfow	350000	

2. Establishment/renovation facilities:

Description of facilities	Newly established		Upgraded/refurbished		Remarks
	PP Target	Achievement	PP Target	Achievement	

3. Training/study tour/ seminar/workshop/conference organized:

Description	Number of participant			Duration (Days/weeks/ months)	Remarks
	Male	Female	Total		
(a) Training					
(b) Workshop					

C. Financial and physical progress

Fig in Tk

Items of expenditure/activities	Total approved budget	Fund received	Actual expenditure	Balance/ unspent	Physical progress (%)	Reasons for deviation
A. Contractual staff salary	195679.00	195679.00	195679.00	00	100	
B. Field research/lab expenses and supplies	693936.00	693936.00	693936.00	00	100	
C. Operating expenses	102785.00	102785.00	102785.00	00	100	
D. Vehicle hire and fuel, oil & maintenance	00	00	00	00	-	
E. Training/workshop/seminar etc.	00	00	00	00	-	
F. Publications and printing	80000.00	00	00	00	-	
G. Miscellaneous	19200.00	19200.00	19200.00	00	100	
H. Capital expenses	408400.00	408400.00	408400.00	00	100	

D. Achievement of Sub-project by objectives: (Tangible form)

Specific objectives of the sub-project	Major technical activities performed in respect of the set objectives	Output(i.e. product obtained, visible, measurable)	Outcome(short term effect of the research)
Study on different bacterial species.	Procedure of bacteria culture was determined	Visible and Measurable	Quality cocoon production was improved
Study on relationship between host and pathogen.	Optimum doses of Temperature, Humidity, Feeding and spacing was determined	Visible and Measurable	Quality cocoon production was improved
Study on effective chemicals to control bacterial diseases	Optimum doses of effective chemicals was determined	Visible and Measurable	Quality cocoon production was improved

E. Materials Development/Publication made under the Sub-project:

Publication	Number of publication		Remarks (e.g. paper title, name of journal, conference name, etc.)
	Under preparation	Completed and published	
Technology bulletin/ booklet/leaflet/flyer etc.			
Journal publication			
Information development			
Other publications, if any			

F. Technology/Knowledge generation/Policy Support (as applied):

i. Generation of technology (Commodity & Non-commodity)

- 04 (Four) type bacteria genera identified from bacterial diseases of silkworm.
- There are some identified effective chemicals composite to control bacterial diseases such as Bleaching powder, Paraformaldehyde, Ammonia. Benzoic Acid and Sodium carbonate.
- 02% Benzoic acid appropriate bacterial disease control measures developed for ensuring quality and quantity cocoon production.

ii. **Generation of new knowledge that help in developing more technology in future**

- Introduction of bacterial species identified from bacterial diseases of silkworm.
- Standardization appropriate bacterial disease control measures developed for ensuring quality and quantity cocoon production.

iii. **Technology transferred that help increased agricultural productivity and farmers' income :**

1. Identified genera bacteria from bacterial diseases of silkworm.
2. Appropriate control measures developed bacterial disease for ensuring quality and quantity cocoon production.

iv. Policy Support: N/A

G. Information regarding Desk and Field Monitoring

i) Desk Monitoring [description & output of consultation meeting, monitoring workshops/seminars etc.):
ii) Field Monitoring (time& No. of visit, Team visit and output):

Team visit	Time	No. of Visit	Output
PIU-BARC,NATP-2	January,May	02	
Internal monitoring by Director (BSRTI) and DG(BSDB)	Total project period	03	
Other Visitors: Agricultural Faculty of Rajshahi University		01	

H. Lesson Learned/Challenges (if any)

i) Understanding about a successful project monitoring auditing process.
ii) Management process and techniques for executing of a project.

I. Challenges (if any)

I. Delayed due to excessive rainfall.
II. In the microbial Lab, culture of bacteria hampered due to temperature, humidity and skilled man power.
III. Sometimes financial problems faced due to fund disbursement delayed.

Signature of the Principal Investigator
Date …….

Seal

Counter signature of the Head of the organization/authorized representative
Date ……..
Seal