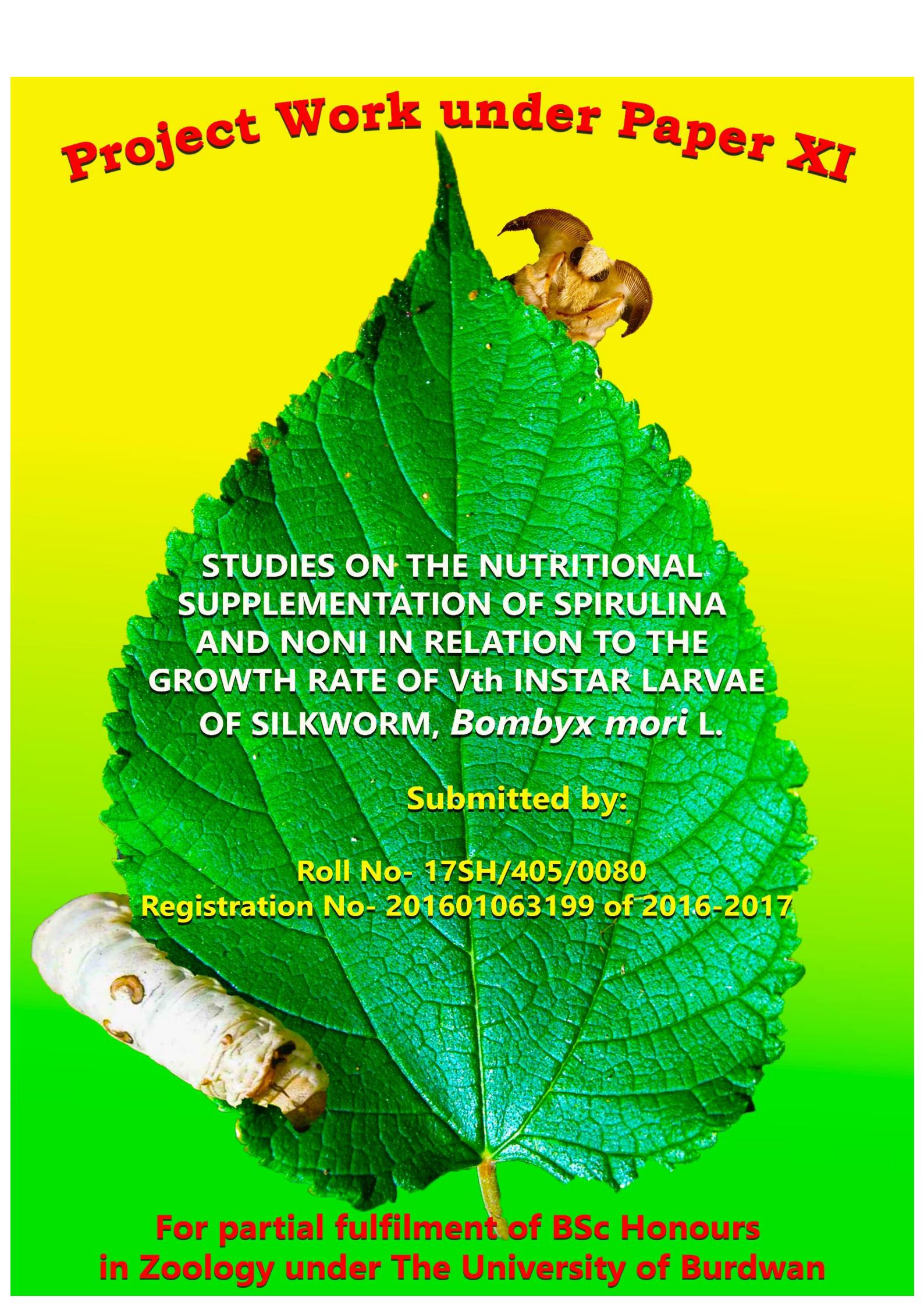


Project Work under Paper XI



**STUDIES ON THE NUTRITIONAL
SUPPLEMENTATION OF SPIRULINA
AND NONI IN RELATION TO THE
GROWTH RATE OF Vth INSTAR LARVAE
OF SILKWORM, *Bombyx mori* L.**

Submitted by:

**Roll No- 17SH/405/0080
Registration No- 201601063199 of 2016-2017**

**For partial fulfilment of BSc Honours
in Zoology under The University of Burdwan**



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CERTIFICATE

This is to certify that the student bearing the **University Roll: 17SH/405 No.- 0080** and **Registration No.- 201601063199 of 2016-2017** has carried out project work in original under my supervision for last 3 months (January '19 to March '19) for the academic year 2018-2019. The student became acquainted with the data handling & processing as well as with the presentation of scientific report while carrying out the project entitled "**STUDIES ON THE NUTRITIONAL SUPPLEMENTATION OF SPIRULINA AND NONI IN RELATION TO THE GROWTH RATE OF VTH INSTAR LARVAE OF SILKWORM, *Bombyx mori* L.**" This work was done successfully gathering knowledge and keeping the Under Graduate Honours course under consideration.

Signature

ACKNOWLEDGEMENT

I would like to express my special thanks of gratitude to my respected teacher **Dr. Dipanjan Dutta**, Assistant Professor who gave me the golden opportunity to do this wonderful project on this topic "**STUDIES ON THE NUTRITIONAL SUPPLEMENTATION OF SPIRULINA AND NONI IN RELATION TO THE GROWTH RATE OF VTH INSTAR LARVAE OF SILKWORM, *Bombyx mori* L.**"

My sincere respect to our Associate Professor **Dr. Nilay Ray**, Assistant Professor **Dr. Silpi Acharyaa** and **Mr. Manojit Chatterjee** for their incredible contribution in developing the project and presenting it with utmost precision.

I am also grateful to the Head of the Department of Zoology, for providing me with all necessary requirements.

I am also grateful to my parents, our silkworm larva supplier to provide us good quality of instar larva.

This topic also helped me in doing a lot of research and I came to know about so many new things like feeding habit of instar larva, disease of instar larva, growth rate, rearing of silkworm etc. Secondly, I would also like to thank my project partners who helped me lot in finalizing in project within the limited time frame.

POULOMI GHOSH

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INTRODUCTION

Silkworm *Bombyx mori* is an important economic insect for silk production and also a tool to convert leaf protein into silk. The industrial and commercial use of silk, the historical and economic importance of production and its application in all over the world finely contributed to the silkworm promotion as a powerful laboratory model for the basic research in biology (Prabu G., 2011)(Ganesh Prabhu et al 2011). The leaves of Morus species are the sole source of the food for silkworm, *Bombyx mori*. L, Nutritional quality of leaves plays a vital role in determining the health and growth of the larvae. The feeding of nutritionally enriched leaves showed better growth and development of silkworm larvae, as well as directly influence on the quality and quantity of silk production (Krishnaswamy et al 1971). Nearly 70% of the silk proteins produced by silkworm are directly derived from the protein of mulberry leaves (Narayanan et al 1967). The silkworm larvae are highly sensitive and respond sharply to the changes of the leaf quality. Variations in the quality of the mulberry leaves and climatic factors are many times reflected on the performance of the cocoon production.

Feeding the Mulberry leaves and tropical application with *Spirulina* as a feed to *B. mori* L. (Lepidoptera: Bombycidae) found to be effective in enhancing the larval and cocoon characters. *Spirulina*, blue-green algae contain 18 amino acids viz., Glutamine, Glycine, Histidine, Lysine, Methionine, Creatine, Cysteine, Phenylalanine, Serine, Proline, Tryptophan, Asparagine, Pyruvic acid and vital vitamins like Biotin, Tocopherol, Thiamine, Riboflavin, Niacin, Folic acid, Pyrodozoic acid, Beta-carotene and Vitamin B₁₂ (Venkataramana et al 2003). Various researches have been carried out on the tropical application to silkworms. These supplementations include vitamins such as ascorbic acid, thiamine, niacin, folic acid and multivitamins. Nutritional study on silkworm is an essential prerequisite for its proper commercial exploitation. Nutrition of silkworm is sole factor which almost individually augment quality and quantity of silk (Laskar and Datta 2000). The current investigation highlights the effect of dietary supplementation of spirulina and noni on the quantitative cocoon parameters viz., larval weight, cocoon weight, shell weight and shell ratio.

SYSTEMATIC POSITION:

Phylum – Arthropoda

Class – Insecta

Sub-Class – Pterygota

Division – Endopterygota

Order – Lepidoptera

Family – Bombycidae

Genus – *Bombyx*

Specimen – *Bombyx mori*



Bombyx mori (Male & Female)

MATERIALS AND METHODS

MATERIALS- The sources of Spirulina (*Spirulina* sp.) and Noni (*Morinda citrifolia*) were capsules manufactured by Vestige India Ltd.

ANIMAL STUDIES- Experiments were conducted in accordance with the internationally accepted laboratory animal use and care.

A. Specimen: Disease free, three breeds of 5th instar larvae of mulberry silkworm - *Bombyx* sp. were taken, viz. Bivoltine (SK6xSK7), Multivoltine (Nistari plain) and F1 (hybrid of bivoltine (SK6xSK7) and multivoltine (Nistari plain)). Twenty larvae were reared per treatment group. The experiment was conducted for 5 days in the month of January and February 2019.

B. Supplementation Dose: The doses of application of Spirulina and Noni were 100mg/day/20 larvae topical application once in a day (every morning it was applied after 30 minutes of taking it out of refrigerator). In the combined supplementation group equal dose of spirulina and noni was taken to adjust the net dose to 100mg/day/20 larvae. The control group was devoid of any supplementation.

C. Feeding: Disease free, 2 days old Fifth instar larvae were procured from extension farms of state sericulture directorate and cultured on large plastic trays at the Sericulture Research Laboratory, Hooghly Mohsin College. The larvae of all the treatment groups were fed normal diet of fresh mulberry leaves (3 times /day) procured from mulberry plantation, Hooghly Mohsin College.

DATA COLLECTION

1. Ten Mature Larval Weight (gm)
2. Single Cocoon Weight (gm)
3. Single Shell Weight (gm)
4. Shell Ratio (%)

SHELL RATIO DETERMINATION

SHELL RATIO = (Shell weight / Cocoon weight) x 100

SUPPLEMENTATION

| Picture | Name | Amount |
|---|---|---|
|  | Spirulina (Manufactured by Vestige India Ltd.) | 100mg/day/20 larvae |
|  | Noni (Manufactured by Vestige India Ltd.) | 100mg/day/20 larvae |
|  | Spirulina & Noni (Combinedly) (Manufactured by Vestige India Ltd.) | Net Dose 100mg/day/20 larvae |

FEEDING



Mulberry plants being cultivated
Location : Hooghly Mohsin College



Larvae eating the mulberry leaves

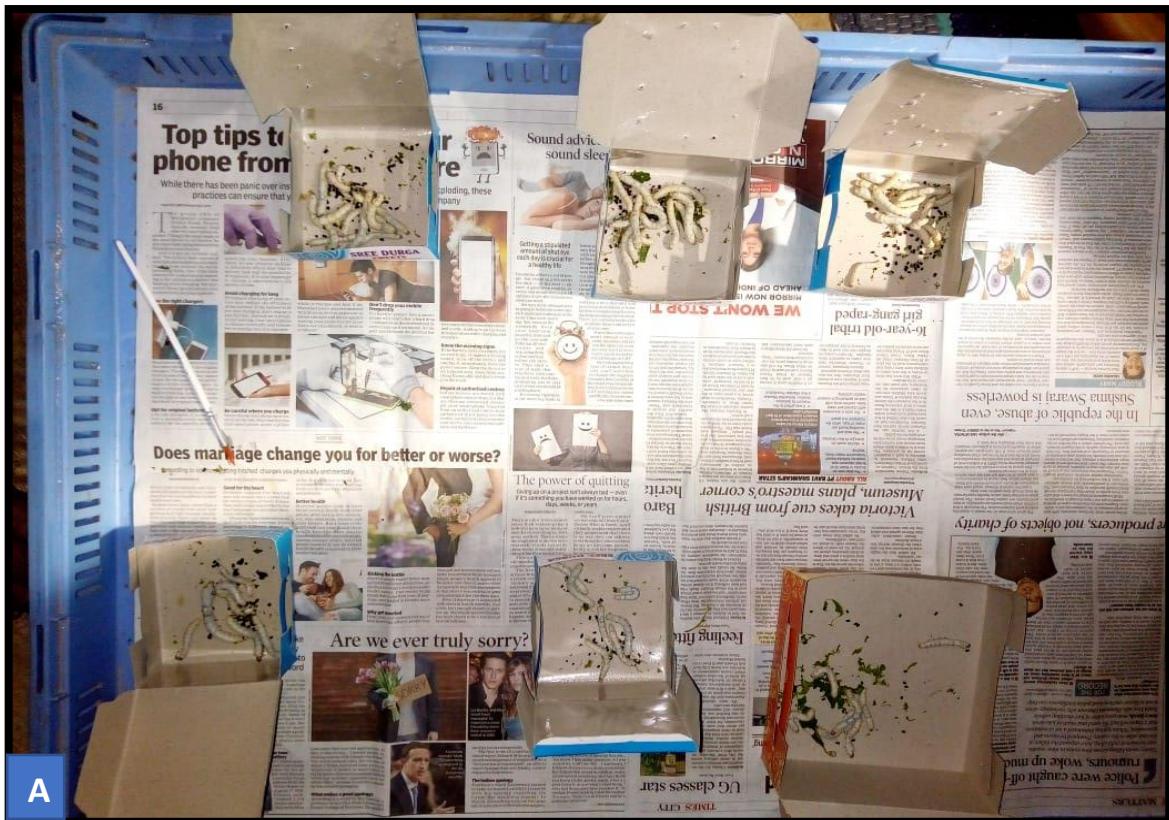
INSTRUMENTATIONS USED IN THE PRESENT STUDY



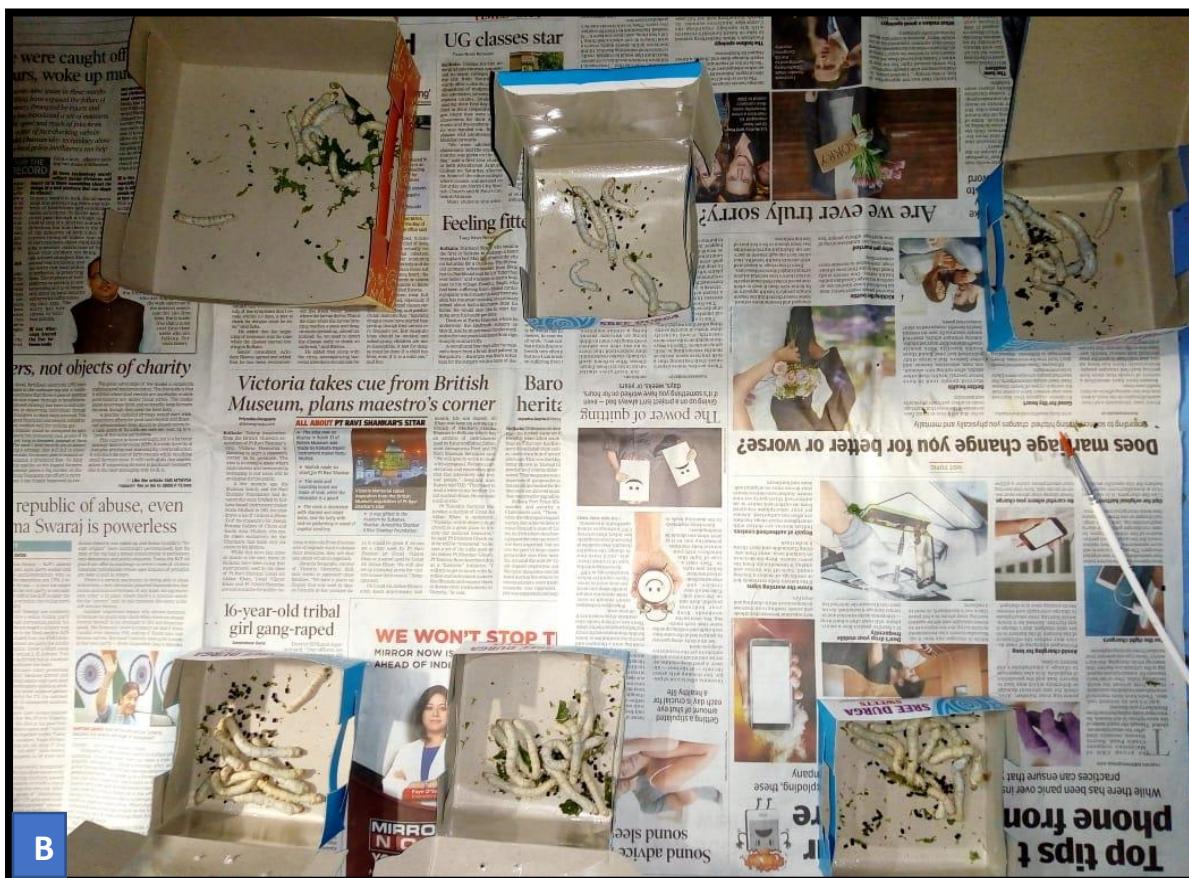
Plastic trays (Medium & Large)



Digital balance for estimation of different parameters



Vth Instar Larvae A. Before Supplementation B. After Supplementation





Larvae Before Supplementation



Larvae Eating the Mulberry Leaves



Larvae After Supplementation

RESULTS AND DISCUSSION

1. TEN MATURE LARVAL WEIGHT :

In silkworm race Bivoltine maximum weight was recorded in no diet supplementation (19.9 gm) However, mature larva with diet supplementation Spirulina recorded the least weight (10.6 gm)

In silkworm race Multivoltine maximum weight was achieved where the mature larva supplemented with Spirulina (21.75 gm) However, mature larva with no diet supplementation recorded the least weight (18.16 gm). Nevertheless, in mature larva supplemented with Spirulina and Noni combinedly, better weight gain was recorded (20.38 gm) in comparison to diet supplementation Noni (18.85 gm)

In silkworm race F1 Hybrid maximum weight gain was achieved where the mature larva with no diet supplementation (32.5 gm) However, mature larva with diet supplementation Noni recorded the least weight gain (11 gm). Nevertheless, in mature larva supplemented with Spirulina, better weight gain was recorded (12.9 gm) in comparison to diet supplementation Spirulina and Noni combinedly (12.09 gm).

In an earlier study by Kumar and Balasubramanian (2014), the larva achieved an individual growth of 3.87 gm on 5% supplementation of Spirulina. In another study by Vanmathi (2016), maximum larval weight (4.59 gm) was observed in 5th instar larvae in 10% treatment of *Zea mays* flour.

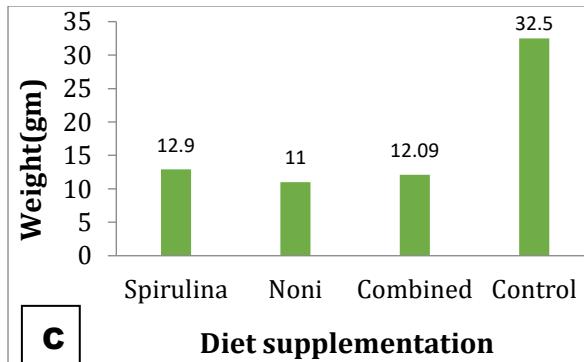
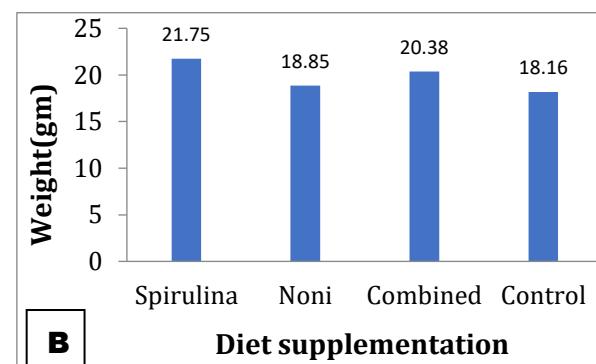
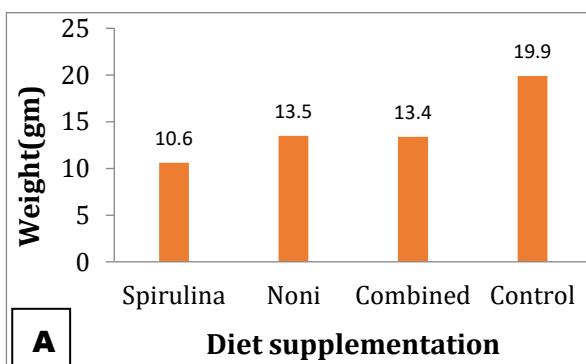


Fig 1: Ten mature larval weight after dietary supplementation for 5 days (A) Bivoltine (B) Multivoltine (C) F1 hybrid.



Bivoltine Life Cycle



2. SINGLE COCOON WEIGHT:

In silkworm race Bivoltine maximum single cocoon weight recorded where larva supplemented with Spirulina and Noni combinedly (1.607 gm). However, on administration of Noni as diet supplementation the cocoon weight was drastically reduced (1.187 gm). Nevertheless, the single cocoon weight recorded better result in no diet supplementation (1.389 gm), in comparison to diet supplementation Spirulina (1.234 gm)

In silkworm race Multivoltine maximum single cocoon weight recorded where larva supplemented with Spirulina (1.126 gm). However, on administration of Noni as diet supplementation the cocoon weight was drastically reduced (1.022 gm). Nevertheless, the single cocoon weight recorded better result in no diet supplementation (1.123 gm), in comparison to diet supplementation Spirulina and Noni combinedly (1.048 gm)

In silkworm race F1 Hybrid maximum single cocoon weight recorded where larva supplemented with Spirulina and Noni combinedly (1.504 gm). However, on administration of Spirulina as diet supplementation the cocoon weight was drastically reduced (1.305 gm). Nevertheless, the single cocoon weight recorded better result in diet supplementation Noni (1.449 gm), in comparison to no diet supplementation (1.442 gm).

In a study by Ananda Kumar and Michael (2011), maximum cocoon weight achieved in 5th instar was 1.89 gm on single application of Serifeed per day. However, Kumar and Balasubramanian (2014) achieved a meagre 0.84 gm shell weight on 5% supplementation of Spirulina.

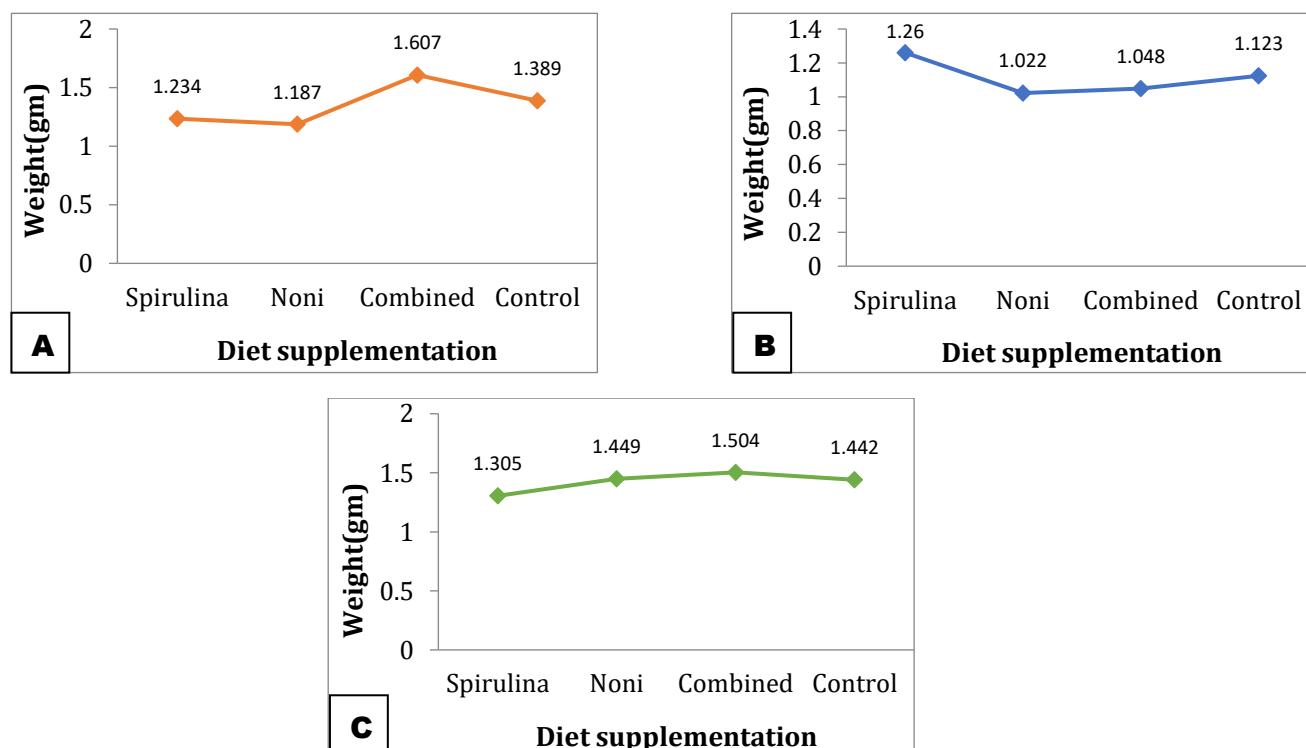
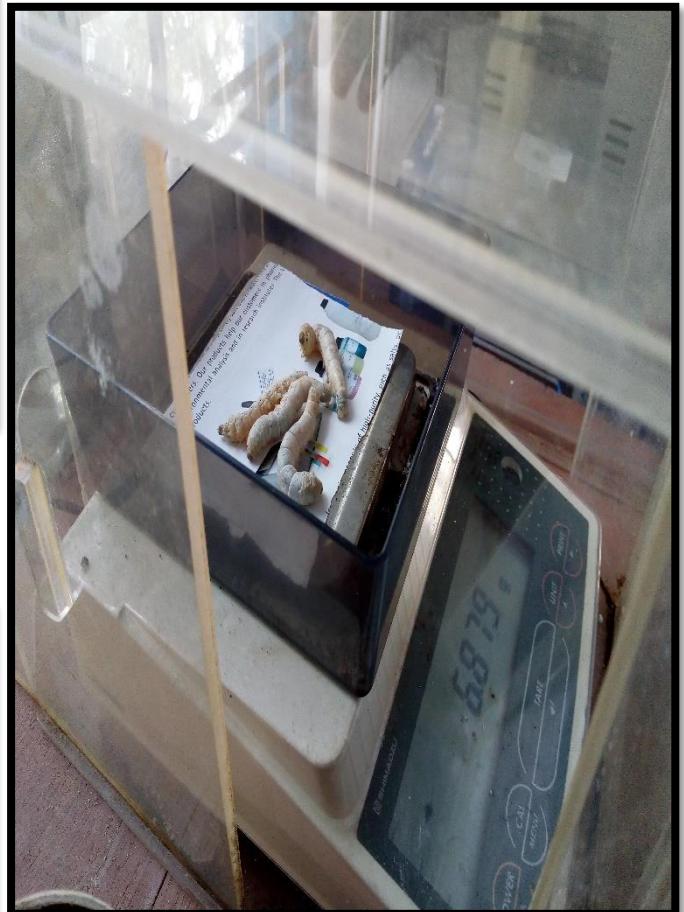


Fig 2: Single cocoon weight after dietary supplementation for 5 days (A) Bivoltine (B) Multivoltine (C) F1 hybrid.



Measurement of Cocoon weight



Measurement of larval weight



Measurement of Cocoon weight

3. SINGLE SHELL WEIGHT :

In silkworm race Bivoltine maximum single shell weight recorded where larva supplemented with Spirulina and Noni combinedly (0.213 gm). On other hand the shell weight was drastically reduced in no diet supplementation (0.153 gm). Nevertheless, the single shell weight recorded better result in diet supplementation Noni (1.389 gm), in comparison to diet supplementation Spirulina (1.234 gm).

In silkworm race Multivoltine single shell weight recorded maximum in both diet supplementation with Spirulina and Noni combinedly and no diet supplementation (0.159 gm). On the other hand, administration of Noni as diet supplementation the shell weight was drastically reduced (0.146 gm). Nevertheless, the single shell weight recorded better result in Spirulina diet supplementation (0.158 gm).

In silkworm race F1 Hybrid maximum single shell weight recorded where larva supplemented with Noni (0.249 gm). However, on administration of Spirulina as diet supplementation the shell weight was drastically reduced (1.305 gm). Nevertheless, the single shell weight recorded better result in diet supplementation with Spirulina and Noni combinedly (0.167 gm), in comparison to no diet supplementation (0.153 gm).

Vanmathi (2016) achieved 0.42 gm shell weight in 5th instar on 10% supplementation of *Zea mays* flour protein which is. Kumar and Balasubramanian (2014) achieved a tremendous high shell weight of 0.84 gm in 5th instar on 5% supplementation of Spirulina. The shell weight achieved in both these studies was much higher than the shell weight achieved in the present study.

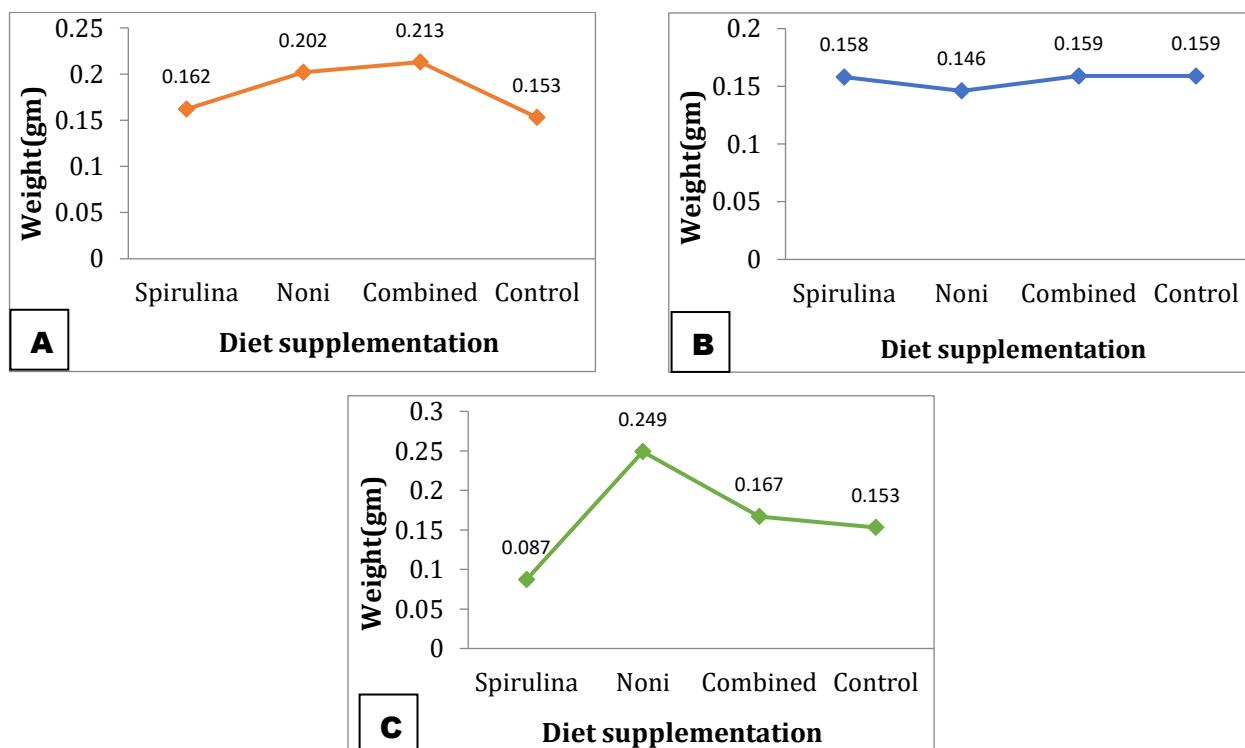


Fig 3: Single shell weight after dietary supplementation for 5 days (A) Bivoltine (B) Multivoltine (C) F1 hybrid.



Eggs



Adult (Copulation stage)



Vth instar larva

Multivoltine Life cycle



Pupa



Larva started cocoon formation



Cocoon (Yellow)

4. SHELL RATIO

In silkworm race Bivoltine (Prabu G., 2011) highest shell ratio recorded in Noni diet supplementation (17.01 %) However the shell ratio with no diet supplementation recorded lowest (11.01 %). On other hand shell ratio recorded better result with diet supplementation Spirulina and Noni combinedly (13.25%) in comparison to Spirulina diet supplementation (13.12%).

In silkworm race Multivoltine highest shell ratio recorded in diet supplementation Spirulina and Noni combinedly (15.17%) However the shell ratio with Spirulina diet supplementation recorded lowest (14.03%). On other hand shell ratio recorded better result with diet supplementation Noni (14.25%) in comparison to no diet supplementation (14.16%).

In silkworm race F1 Hybrid highest shell ratio recorded in Noni diet supplementation (17.18%) However the shell ratio with Spirulina diet supplementation recorded lowest (6.6%). On other hand shell ratio recorded better result with diet supplementation Spirulina and Noni combinedly (11.10%) in comparison to no diet supplementation (10.61%).

Ananda Kumar and Michael (2011) reported a maximum shell ratio of 18.25 & in 5th instar on single application of Serifeed. In another study by Vanmathi (2016), 24.55% shell ratio was achieved on 10 % supplementation with *Zea mays* flour protein.

On the other hand, Kumar and Balasubramanian (2014) achieved a 33% shell ratio in 5th instar on 5% supplementation with Spirulina.

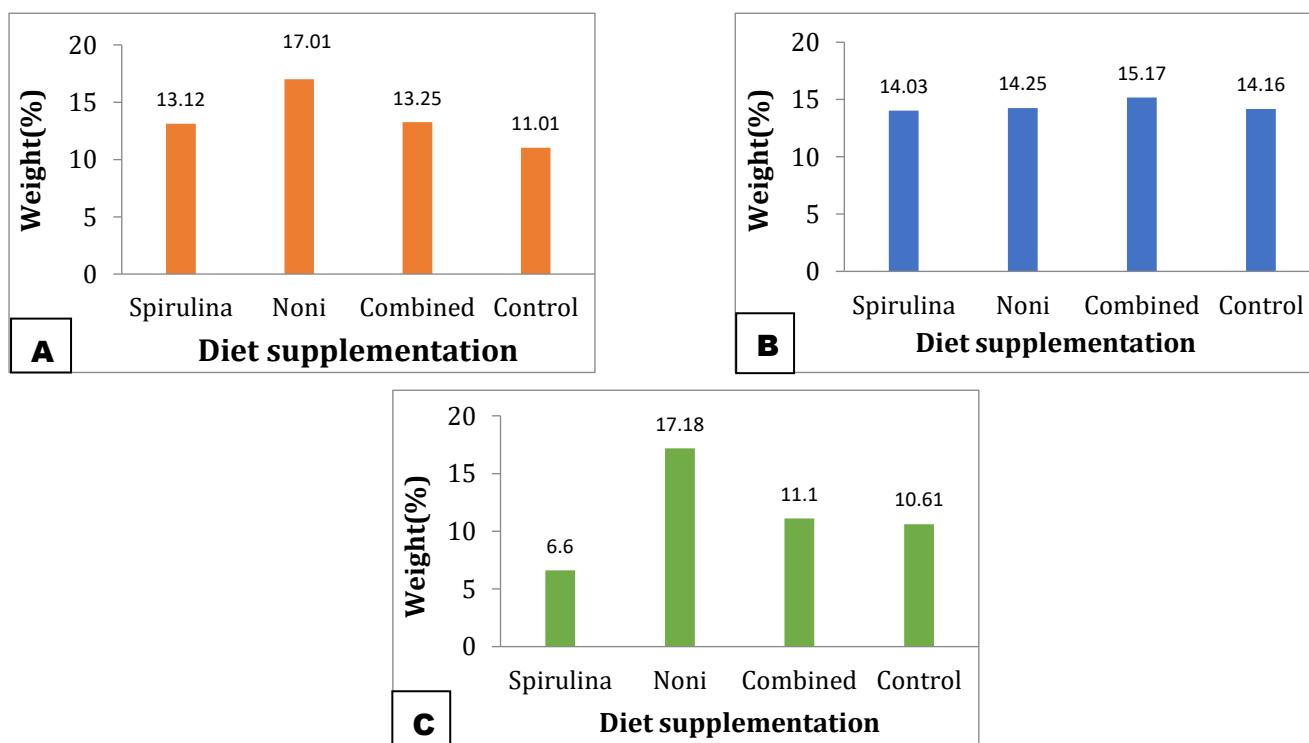


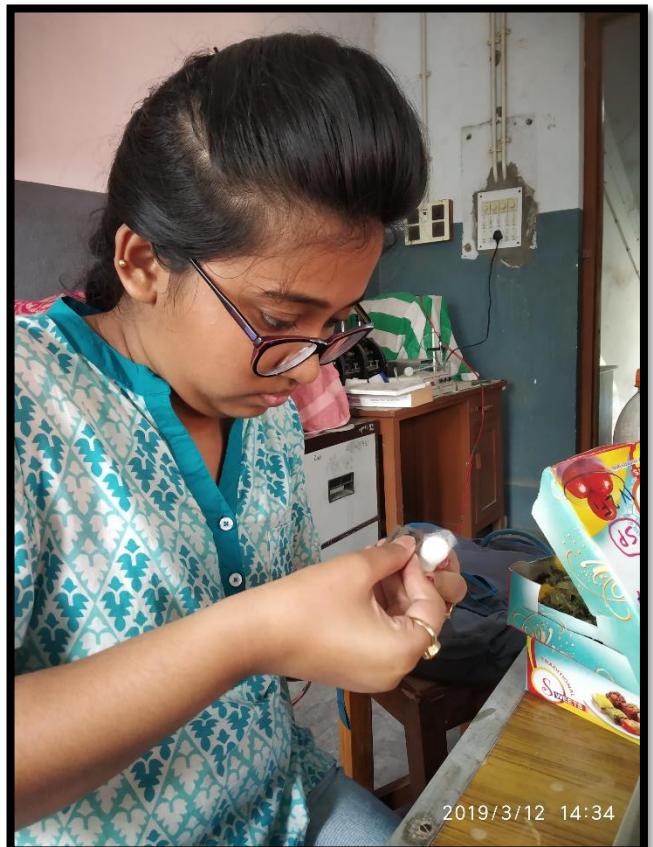
Fig 4: Single ratio after dietary supplementation for 5 days (A) Bivoltine (B) Multivoltine (C) F1 hybrid.



Measuring The Larval Weight



Measuring The Cocoon Weight and Taking Out the Pupa from Shell



Measuring The Shell Weight and Taking Out the Pupa from Shell





Group work

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