Appendix-A

CHEMICALS USED

A

Abscisic acid

ABTS [2,2′ azinobis-(3-ethylbenzthiazoline-6-sulfonic acid]

Acryl amide

Aluminium chloride

Ammonium per sulphate

Anthrone reagent

Ascorbic acid

B

Benzidine

Bis-acrylamide

Bovine serum albumin

Bromocresol blue

C

1-Chloro-2,4-dinitrobenzene

Chloroform

Copper sulphate

D

DNS (3,5-dinitrosalicylic acid) reagent

Di-potassium hydrogen phosphate

Di-sodium hydrogen phosphate

5,5′-dithiobis-(2-nitrobenzoic acid)

DPPH (2,2-diphenyl-1-picrylhydrazyl)

E

Ethanol

F

Folin-Ciocalteu reagent

G

Gallic acid

Glacial acetic acid

Glutathione

Glutathione reductase

Glycerol

Glycine

Guaiacol

H

Hydrochloric acid

Hydrogen peroxide

L

Liquid nitrogen

M

Methanol

N

Napthylethylenediamine dihydrochloride

Nicotinamide-adanine dinucleotide phosphate (NADPH)

Ninhydrin

Nitro-blue tetrazolium

O

Oxidized glutathione

P

Polyvinyl pyrrolidone

Potassium dihydrogen phosphate

Potassium bromide

Potassium ferricyanide

Potassium hydroxide

Potassium iodide

Potassium persulfate

Potassium sulphite

Proline

Putrescine

Q

Quercetine

R

Rochelle salt

Riboflavin

S

Schiff's reagent

Sodium acetate

Sodium carbonate

Sodium dodecyl sulphate

Sodium dihydrogen phosphate

Sodium hydroxide

Sodium nitroprusside

Spermidine

Spermine

Starch

Sulfanilamide

Sulfosalicylic acid

Sulphuric acid

T

Temed

Thiobarbituric acid

Thiourea

Toluene

Trichloroacetic acid

Tween 20

Tris base

Tris-HCL

Triton

T

Appendix-B

ABBREVIATION AND SYMBOLS USED

˚C	Degree centigrade
·OH	Hydroxyl
$^1O_2$	Singlet oxygen
AA	Ascorbic acid
AAE	Ascorbic acid equivalent
abs.	Absorbance
ABTS	2,2′ azinobis-(3-ethylbenzthiazoline-6-sulfonic acid)
ANOVA	Analysis of variance
APX	Ascorbate peroxidase
ATP	Adenosine triphosphate
CAT	Catalase
CON	Control
DMRT	Duncan's Multiple Range Test
DP	Digestive gland protein
DPPH	2,2-diphenyl-1-picrylhydrazyl
DTNB	5-5′-dithiobis-2-nitrobenzoic acid
FWT	Fresh weight tissue
g	gram
GAE	Gallic acid equivalent
GPX	Guaiacol peroxidase
GR	Glutathione reductase
GSH	Glutathione
GSSG	Glutathione oxidized
GST	Glutathione-S-transferase
h	Hour
H2O2	Hydrogen peroxide
IC50	50% inhibition concentration
M	Molar
mg	Milligram
mM	Millimolar
MDA	Malondialdehyde
min	Minute
ml	Millilitre
NA	Not applicable
NADH	Nicotinamide-adanine dinucleotide reduced
NADPH	Nicotinamide-adanine dinucleotide phosphate
NBT	Nitro-blue tetrazolium
NOX	NADPH oxidase
$O^{2\cdot-}$	Superoxide
O_3	Ozone
OH	Hydroxyl radical
PAGE	Polyacrylamide gel electrophoresis
PC	Principal component
PCA	Principal component analysis
PMS	Phenazine methosulphate
POD	Peroxidase
Put	Putrescine
QE	Quercetin equivalent
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RP	Reducing power
rpm	Revolutions per minute
SDS	Sodium dodecyl sulphate
SEE	Standard error of estimates
SO	Superoxide
SOD	Superoxide dismutase
Spd	Spermidine
Spm	Spermine
SPSS	Statistical package for the social sciences
TBA	Thiobarbituric acid
TCA	Trichloroacetic acid
TCC	Total carotene content
TLC	Thin layer chromatography
TRS	Total reducing sugar
TSS	Total soluble sugar
µl	Micro liter
µM	Micro molar

Appendix-C

List of Publications

1. **Jha S**, Bhattacharyya P, Ghosh A and Mandal P (2016). Feeding preference of silkworm larvae depending on biochemical attributes related to mulberry genotypes. *International Journal of Pharmacy and Pharmaceutical Sciences*. 8(4): 307-314.

2. Bhattacharyya P, **Jha S**, Mandal P and Ghosh A (2016). Artificial Diet Based Silkworm Rearing System-A Review. *International Journal of Pure & Applied Bioscience,* 4(6): 114-122.

3. **Jha S**, Bhattacharyya P, Ghosh A and Mandal P (2015). A comparative study of silkworm (*Bombyx mori* L.) rearing under different sources of peptides isolated from Dudhiya and S1 mulberry leaves. *NBU Journal of Plant Sciences*, 9(1): 98-106.

4. **Jha S,** Gupta PD, Bhattacharyya P, Ghosh A and Mandal P (2015). Impact of feeding of low molecular weight mulberry peptides on cocoon and silk development by *Bombyx Mori* L. (Bombycidae). *Indian Journal of Sericulture* [Accepted].

5. **Jha S**, Mandal P, Bhattacharyya P, Ghosh A (2015). Influence of antioxidant rich mulberry peptides on the growth rate pattern and economic attributes of silkworm. *International Journal of Pure & Applied Bioscience,* 3(2): 63-71.

6. **Jha S**, Mandal P, Bhattacharya P and Ghosh A (2014). Free-radical scavenging properties of low molecular weight peptide(s) isolated from S1 cultivar of mulberry leaves and their impact on *Bombyx mori* (L.). (Bombycidae). *Journal of Animal Science and Biotechnology.* 5:16.

Appendix-D

JOURNAL OF ANIMAL SCIENCE AND BIOTECHNOLOGY

RESEARCH Open Access

Free-radical scavenging properties of low molecular weight peptide(s) isolated from S1 cultivar of mulberry leaves and their impact on *Bombyx mori* (L.) (Bombycidae)

Suchisree Jha[1], Palash Mandal[1], Phalguni Bhattacharyya[2] and Amitava Ghosh[3*]

Abstract

The mulberry leaves have been considered as a sole food source for silkworm, *Bombyx mori* (L.). In present work an attempt was made to investigate the role of low molecular weight peptide(s) isolated from mulberry leaves on silkworm rearing. Also we have tried to find out the role of free-radical scavenging activities of isolated peptide(s) on silkworm growth. Larval growth rate was found effective under the influence of peptide(s). Consumption rate of larvae after peptide(s) treatment on mulberry leaves was significantly enhanced over control. High antioxidant activity was found in Low molecular weight peptide(s) which have an effect on silkworm.

Keywords: Antioxidant, HPLC, Low molecular peptide(s), Mulberry leaf, Silkworm

Background

Bombyx mori L. also commonly known as silkworm is a monophagous insect reared in captivity (sericulture). India is the second largest producer of silk and also the largest consumer of silk in the world followed by China [1]. Human beings have benefited by the silkworm in various ways and scientists have been continuously trying to improve the techniques of silkworm rearing. The mulberry leaves have considered as a sole source of food for silkworm, especially at larval stage. The quality and quantity of mulberry leaves determine the growth and development of silkworm and subsequently cocoon production [2]. Plants are considered as a richest resource of phytochemicals and these phytochemicals have been reported to manipulate the life cycle and activity of different insects [3,4]. The effects of different types of dietary protein on silkworm growth were resolute by using semi-synthetic diets. Some workers have clearly described that protein acts as an essential ingredients in silkworm diet for their growth and silk production. Several reports stated that the soybean meal as a protein source in silkworm diet can significantly increase the weight of silkworm larvae and fresh silk glands [5,6]. Since smaller proteins have also been considered as peptides, therefore it may be predicted that these peptides may also have significant effect on the growth and development of silkworm. In present study, a scientific attempt was made to figure out the effect of peptide(s) of two different molecular ranges (0.5-3 kDa and 3-10 kDa) isolated from mulberry (S1 cultivar) leaves of different maturation stages on silkworm growth and silk production.

Material and methods

Plant culture

Leaves of S1 cultivars of mulberry leaves were collected from sericulture farm of Malda, West Bengal, India at same season and same time. Leaves were selected at different maturity status e.g. young, mature and senescence leaves and was weighed out (1 kg each). Young mature

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INTERNATIONAL JOURNAL OF PURE & APPLIED BIOSCIENCE

Research Article

OPEN ACCESS

Influence of antioxidant rich mulberry peptides on the growth rate pattern and economic attributes of silkworm

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ABSTRACT

Bombyx mori L. is a monophagous insect and it totally depends on the mulberry leaves at their larval stage. It was recognized that dietary proteins had an effect on silkworm growth and silk production. But until now, no work was achieved on the role of the peptide(s) isolated from mulberry leaves on silkworm rearing. In present work an effort was made to investigate the role of low molecular weight (LMW, 0.5-3 KDa) peptide(s) on silkworm rearing. Role of antioxidant activities of isolated peptide(s) on silkworm rearing system also was evaluated. For that purpose, mulberry leaves (S1 cultivar) was introduced in different peptide(s) solution and fed by silkworm larvae (Nistari variety). Larval growth rate at 5th instar stage was found effective under the influence of peptide(s). In peptide(s) treatment consumption rate of the larvae was significantly high over control. Economical parameters like shell weight and shell ratio was enhanced by peptide treatment than control. Apart from this, LMW peptide(s) exhibited high scavenging activity than high molecular weight (HMW, 3-10 KDa) peptide(s). HPLC analysis of bioactive peptide fraction was performed and the peptide with maximum abundance was sequenced. The findings from our study may perhaps assist the sericulture industry for enhancing silk productivity.

Keywords: *Bombyx mori (L.), HPLC, DPPH, Nitric oxide, Low molecular weight peptide.*

INTRODUCTION

The physiology of *Bombyx mori* (L.), a monophagous lepidopteran insect has been studied comprehensively due to economically valuable silk production. Optimal nutrient and favorable physical features are required for growth and silk production. Mulberry leaves is a traditional food for silkworm larvae as a source of nutrition. Mulberry leaves contain several chemicals like 80% water, 27% proteins, 11% carbohydrates, vitamins and different minerals[1]. Recently many works were completed on the supplemented diet of mulberry leaves for silkworm. Several reports stated that protein acts as an essential ingredient in silkworm diet for their growth and silk production. Numerous studies have clearly described that soybean meal as a protein source in silkworm diet can increase the larval weight and fresh silk glands[2,3].

Since smaller proteins have also been considered as peptides, therefore it can be predicted that these peptides may also have significant effect on the growth and development of silkworm[4]. In present study, a scientific attempt was made to figure out the effect of peptide (s) of two different molecular ranges (0.5-3 kDa and 3-10 kDa) isolated from mulberry (S1 cultivar) leaves of different maturation stages on silkworm growth and silk production.

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Original Article

FEEDING PREFERENCE OF SILKWORM LARVAE DEPENDING ON BIOCHEMICAL ATTRIBUTES RELATED TO MULBERRY GENOTYPES

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ABSTRACT

Objective: The silkworm rearing was influenced by different mulberry cultivars according to the biochemical properties of leaves. In this present study, a comparative analysis was made to investigate feeding preference of silkworm larvae depending on biochemical attributes of mulberry genotypes.

Methods: For this purpose, seven different mulberry cultivars and one germ plasm namely Dudhiya was selected. F1 hybrid (Nistari × bivoltine) of silkworm larvae was reared under selected cultivars of mulberry leaves at different seasons. Biochemical assessment of all leaves was also done.

Results: Among these, S1, V1 and S1635 mulberry cultivars showed higher amount of total protein, total sugar and chlorophyll, also exhibited better feeding response on economic attributes of silkworm. Maximum accumulation of ascorbic acid and glutathione was recorded during winter in Dudhiya leaves. The accumulation of H_2O_2, superoxide and lipid peroxidation was comparatively higher than other cultivars during stress period in Dudhiya. Statistical analysis revealed that larval growth and economical parameters depend on biochemical properties of leaves and inversely associated with excessive production of Reactive oxygen species (ROS).

Conclusion: The scavenger and ROS ratio was properly maintained in S1, V1 and S1635 leaves which might help leaf metabolic homeostasis. Proper metabolic activities of leaves possibly will produce higher proteins and carbohydrates which were required for larval growth and silk production as established from the PCA plot analysis. Therefore S1, V1 and S1635 might be recommended for silkworm rearing or commercial cultivation purpose throughout all season.

Keywords: Silkworm, Mulberry cultivars, Proline, Reactive Oxygen Species (ROS), MDA, Single cocoon weight.

INTRODUCTION

Silkworm, *Bombyx mori* L. is a domestic lepidopteron insect. Silkworm larval growth depends on the nutritive value of mulberry leaves [1]. The Foliar nutritional value of leaves and biomass production depends on the weather and agricultural practices [2], and it was also different according to cultivars. On the other hand, Susheelamma *et al.* [3] reported that mulberry genotypes produced high biomass and due to more rapid growth rate and higher metabolic activities, mulberry cultivars had a fabulous water demand. Water scarcity can clutch mulberry plant growth and metabolism. Therefore plants experience oxidative stress that reduces plant primary and secondary metabolite production [4, 5] and reduction of plant production directly affects larval development and silk production. Various reports were published on oxidative stress of mulberry plant, and scientists have concentrated on the responses of enzymatic antioxidants [5, 6 and 7]. Kotresha *et al.* [8] investigated some of the most important non-enzymatic antioxidants in different mulberry leaves in response to drought and high-temperature stress. Guha *et al.* [9] analyzed non-enzymatic antioxidative defense under water and drought stress. It was hypothesized that foliar production of mulberry leaves differ under various stress periods. Silkworm larvae may choose superior mulberry cultivars on the basis of their nutritional values. Therefore, in the present work, an attempt was made to find out superior mulberry genotypes on the basis of biochemical leaf quality and feeding response of silkworm from Malda district of West Bengal, India. For the said purpose, seven different cultivars of mulberry leaves were selected namely S1, V1, K2, S1635, Mandalaya, Jaysree and Bombay along with primitive germplasm Dudhiya as a feeding source for 5th instar larvae. Also, we worked on different biochemical attributes of selected leaves which might assist in determining the partial role of antioxidants in leaves related to the larval choice of feeding. Our observation might assist farmers involved in sericulture for selection of mulberry cultivars to rear silkworm larvae at a different season.

MATERIALS AND METHODS

Study location

The study area, Malda district of West Bengal is located at 25.00 ˚N and 88.15 ˚E. The weather is usually extremely humid and tropical. Temperatures can reach as high as 46 ˚C during the day in May and June and fall as low as 4 ˚C overnight in December and January. The winter season arrives in Malda district in the middle of November and continues till the last of February. Winter is succeeded by summer in the months from March to May. After the summer season, the city witnesses a rainy season that begins in the month of June and ends by the middle of September. The rains in this city are the result of the south-west monsoons. Normally, the rainfall in the area is 1453.1 mm. The brief season after rains and before the arrival of winter is the period referred to as the post-monsoon period. This season lasts for about one and a half month and is characterized by cool weather.

Study methods

Feeding experiment

We conducted overall rearing procedure under an optimal temperature (27˚-29 ˚C), humidity (70±5%) and overall sterilized environment in our laboratory. Feeding trial with these eight selected cultivars of mulberry leaves was conducted at three different seasons, spring, summer and autumn. Larvae were fed with young, mature and senescent leaves of all selected cultivars of mulberry. According to Gangwar [10], larval weight, mortality percent, single cocoon weight, single shell weight and other economic parameters were calculated at three different seasons separately.

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A comparative study of silkworm (*Bombyx mori* L.) rearing under different sources of peptides isolated from Dudhiya and S1 mulberry leaves

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Abstract

Silkworm is a domestic monophagous insect, produces only natural animal fibre. Growth of silkworm larvae depends on the nutritional components of mulberry leaves. In present study one attempt was made to investigate the role of low molecular weight (0.5-3 kDa) mulberry peptides on silkworm rearing. For peptide extraction, two different types of mulberry leaves were chosen, one from S1 which was preferred by larvae for feeding, another is a germplasm named Dudhia, refused by larvae. Peptides isolated from young S1 leaves showed higher larval growth followed by peptides isolated from mature and senescence leaves. High ERR% along with enhanced weight of single cocoon and single shell was observed in silkworm fed with S1 peptide treated mulberry leaves as compared with the same by Dudhia peptides. Elevated antioxidant activities were exhibited by S1 peptides than Dudhia at all maturity stages (young, mature and senescence). Significant correlation was obtained between antioxidant activities of S1 peptides and economical attributes of silkworm rearing such as ERR%, weight of single cocoon, weight of single shell etc. From our observation it might be stated that the farmers would have been benefited if they could use mulberry leaves treated with antioxidant enriched peptides as a food for silkworm rearing.

Keywords: Oligopeptides, *Bombyx mori,* HPLC, antioxidant, Mulberry leaf.

Introduction

The *Bombyx mori* L. is an important sericigenous insect due to their golden fibre and it plays an important component of sericulture industry which contributes to the economic development of India and Bangladesh. The nutritional quality and quantity of mulberry leaves have a direct consequence on silkworm growth and development and subsequent cocoon production (Seidavi *et al.* 2005). Recently scientists are trying to improve silkworm rearing by feeding them different mulberry leaf supplementary products. The effects of different types of dietary protein on silkworm growth were determined by using semi-synthetic diets. Several reports stated that protein acts as an essential ingredient in silkworm diet (Horie and Watanabe 1983; El-Sayed and Nagda 1999). Smaller proteins less than 10 kDa have also been considered as peptides, therefore it can be predicted that these peptides might also have significant impact on the growth and development of silkworm. In present study, a scientific attempt was made to find out the effect of peptide(s) at low molecular weight ranges (0.5-3 kDa) isolated from mulberry leaves. Dudhia is a germplasm of mulberry and S1 is a cultivar used for peptides extraction at different maturation stages. As silkworm larvae have feeding preference on leaves of S1 cultivars than Dudhia germplasm (primitive), this study was undertaken by comparing the rearing efficiency and antioxidant activity of oligopeptides isolated from the two above mentioned sources of mulberry leaves.

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Material and methods

Plant culture

Leaves of S1 cultivars of mulberry and Dudhia were collected from Sericulture Farm of Malda, West Bengal, India. Leaves were selected at different maturity stages namely young, mature and senescence leaves at same season and same time. Young, mature and senescence leaves were selected on the basis of the biochemical attributes (chlorophyll and protein content) and the morphological parameters (length and breadth) of the leaves.

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Review Article

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OPEN ACCESS

Artificial Diet Based Silkworm Rearing System-A Review

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ABSTRACT

There are about 1000 varieties of silkworm, Bombyx mori L., among them bivoltine and multivoltine races are particularly used for rearing in India. Silkworm is a monophagous insect which has special significance in sericulture industry. It totally depends on the mulberry leaves at their larval stage. Mulberry leaves are a traditional food for silkworm larvae due to presence of morin. Human beings have benefited by the silkworm in various ways and scientists have been continuously trying to improve the techniques of silkworm rearing. The physiology of Bombyx mori (L.) has been studied comprehensively due to the economically valuable silk production. Mulberry belongs to the genus Morus of the family Moraceae. Three types of mulberry are found, white (Morus alba L.), red mulberry (M. rubra) and black mulberry (M. nigra). Among them, only white mulberry is recognized as the food source for silkworm. Now a days, sericulture research developed number of supplement nutrients with mulberry leaves for silkworm rearing. There are number of foods used as an ingredient for artificial diet of silkworm. Artificial diet encourages the small landless farmers to take up sericulture and it also helps to reduce labour cost for mulberry cultivation. In this review, one attempt was made to discuss on artificial diet based silkworm rearing and different ingredients used in artificial diet preparation.

Key words: *Artificial diet, silkworm, mulberry, protein, sterol.*

INTRODUCTION

India is the second largest producer and also the largest consumer of silk in the world due to development of innovative technologies in mulberry cultivation as well as in silkworm rearing. The best silk quality is obtained from the cocoons of the larvae of mulberry silkworm *Bombyx mori* L. reared in captivity (sericulture). Sericulture is an art and science of technology fundamentally a village based and welfare oriented industry that plays an important role in our national economy. Sericulture depends on rearing of silkworm on mulberry leaves; and it can be stated that silk productions are directly correlated with larval growth and development on mulberry. The production of mulberry leaves both in terms of quality and quantity change due to different climatic factors and field practices.

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