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Influence of Aqueous Solution of *Agaricus bisporus* (L) Treated Mulberry Leaves on the Quality of cocoons and silk filament in silkworm, *Bombyx mori* (L).

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ABSTRACT

The aqueous solution of powder of fruiting body of *Agaricus bisporus* (L) with fifty milligram per liter (50 ppm) strength and aqueous solution of powder of AB21 protein with fifty milligram per liter (50 ppm) strength were used separately for treating mulberry leaves. Such treated mulberry leaves were fed daily to the silkworm larvae of bivoltine Cross Breed [(CSR6 x CSR26) x CSR2 x CSR27)] for first four days after the fourth moult. For each day, four feedings were supplied at the rate 100 grams of mulberry leaves for the group of hundred larvae. Larvae fed with untreated and water treated mulberry leaves were also maintained. Mature larvae were considered for the provision of mountage for spinning the cocoon. The cocoons were harvested on fifth day after the provision of mountage. The shell ratio of cocoons , denier scale of silk filament of the untreated control group; water treated group; AB21 protein treated group and fruiting body treated group were found measured 20.241, 2.734 ; 20.241, 2.734; 28.390, 3.006 and 33.309, 3.224 respectively. The contents of powder of fruiting body of *Agaricus bisporus* (L) including AB21 protein may be serving for enhancement of metabolism in the fifth larval instars of silkworm, *Bombyx mori* (L). Through the improved rate of metabolism, powder of fruiting body of *Agaricus bisporus* (L) and it's novel protein: "AB21-protein" treatment may aiming the action of gross metabolic constituency of silkworm larvae.

Keywords : *Bombyx mori* (L); *Agaricus bisporus* (L); AB21-protein; Shell Ratio; Denier Scale

I. INTRODUCTION

Nutrition plays a pivotal role in sericulture by improving the commercial characters of silkworm. Silkworm being a monophagous insect derives almost all the nutrients required for its growth from the mulberry leaf itself (Nasreen et al., 1999). Though the silkworm nutrients are balanced in mulberry leaf, the quantity available is not sufficient for the larval growth due to variation in mulberry plant cultivable soil (Ito, 1978). The intake of nutrient by the larvae is also proportional to the availability of feed. The silkworm nutrition is considered as a major area of research in sericulture (Legay, 1958). Nutrition study on silkworm is an essential prerequisite for its proper commercial exploitation. Nutrition of silkworm is sole factor which almost individually augument quality and quantity of silkworm (Laskar and Datta, 2000). In recent year's attempts have been made in sericulture with nutrients such as proteins, carbohydrates, **amino acid**s, vitamins hormones antibiotics etc. for better performance and to get high yield and quantity cocoons (Sannapa et al., 2002; Etebari et al., 2004). The salt significantly enhanced the growth of developmental stages and decreased the developmental period. Nickel chloride significantly increased the growth of larvae (Islam et al., 2004). In addition to mulberry leaves feed supplements are also given to silkworm to enhance economic

characteristics (Jeyapaul et al., 2003; Sheeba et al., 2006).

The dimeric protein AB21 from Agaricus bisporus (L), one of the most commonly and widely consumed mushrooms in the world. The protein shares no significant sequence similarity with any protein of known function, and it is the first characterized member of its protein family. The coding sequence of the "ab21" gene was determined and the protein was expressed in E. coli in a recombinant form. Komarek, et al (2018) demonstrated abundance of protein, entitled, "AB21" in the fruiting bodies of Agaricus bisporus (L). This protein deserve a high thermal and pH stability of AB21 and proved the weak affinity of the protein to divalent ions of some transition metals (nickel, zinc, cadmium, and cobalt). The reported crystallographic structure exhibits an interesting rod-like helical bundle fold with structural similarity to bacterial toxins of the ClyA superfamily.

In a 100-gram serving, raw white mushrooms provide 93 kilojoules (22 kilocalories) of food energy and are an excellent source (> 19% of the Daily Value, DV) of the B vitamins, riboflavin, niacin, and pantothenic acid (table). Fresh mushrooms are also a good source (10–19% DV) of the dietary mineral phosphorus (https://en.wikipedia.org/wiki/Agaricus_bisporus).

While fresh A. bisporus only contains 0.2 micrograms (8 IU) of vitamin D as ergocalciferol (vitamin D2), the ergocalciferol content increases substantially after exposure to UV light (Los Angels Times, 2003 and *Koyyalamudi, et al, 2009).*

Mushrooms contain hydrazine derivatives, including agaritine and gyromitrin, that have been evaluated for carcinogenic activity (Hashida, et al, 1990). Agaritine, a hydrazine, poses no toxicological risk to humans when mushrooms are consumed in typical amounts (*Roupasa*, et al, 2010). No reports on use of edible mushroom fruiting bodies in sericulture.

The present study was therefore, undertaken to study the effect of aqueous solution of fruiting bodies of Agaricus bisporus (L) and aqueous solution of it's novel protein: "AB21" on the quality of cocoons and silk filament in silkworm, *Bombyx mori* (L).

II. METHODS AND MATERIAL

The study was carried out through the steps like: Preparation of Aqueous Solution of fruiting bodies of *Agaricus bisporus* (L) Aqueous Solution of AB21-Protein; Rearing of silkworm larval stages [Race: (CSR6 x CSR26) x CSR2 x CSR27)]; Treating the mulberry leaves and feeding larval instars; Analysis of characters of cocoons; silk filament and Analysis of the data through method of statistics.

(A). Preparation of Aqueous Solution of fruiting bodies of *Agaricus bisporus* (L) and Aqueous Solution of AB21-Protein:

The fruiting bodies of *Agaricus bisporus* (L) and the AB21-Protein were procured from Harry Organo Private Limited Ganpara, Durg - 491001, for Sericulture unit of Agricultural Development Trust, Baramati (Malegaon Sheti Farm India). Addition of known volume of water was made in the bowel containing fruiting bodies of *Agaricus bisporus* (L) weigh of which was predetermined. This subjected for uniform mixing through the use of kitchen juice grinder for fifteen minutes. The content was filtered through the common laboratory filter paper. The filtrate was equalized with acetone to get the macerative of 50 ppm (mg/lit.) strength. In the similar manner, solution of AB21-Protein was prepared using water as a solvent. The strength of water solution of AB21-Protein was also 50 ppm (mg/lit.). Both were prepared freshly before using for treating the mulberry leaves.

(B). Rearing of silkworm larval stages [Race: (CSR6 x CSR26) x CSR2 x CSR27)]:

The method of Krishnaswami, *et al* , (1992) for rearing of silkworm larvae was followed. This method is appearing in the document authorized by Khyade (2004) and Vitthalrao B. Khyade, *et al* (2005, 2006, 2007, 2008, 2009, 2010, 2011, 2012, 2013, 2014, 2015, 2016, and 2017).

The disease free layings of bivoltine race (CSR6 x CSR26) x CSR2 x CSR27) of silkworm, *Bombyx mori* (L) were procured through the "Dr. APIS" Laboratory and processed for black boxing, rearing of early instars, rearing of late age instars, provision of mountage for spinning the cocoon and cocoon harvesting through the standard methods.

(C). Treating the mulberry leaves and feeding larval instars:

The fifth instar larvae were utilized for the carrying out the attempt. Soon after the fourth moult, the fifth instared larvae were grouped into four groups (each with hundred individuals). The groups include:

1. The Group of Untreated Control;
2. The Group of Water treated control;
3. Group of larvae fed with mulberry leaves treated with aqueous solution of fruiting bodies of *Agaricus bisporus* (L) and
4. Group of larvae fed with mulberry leaves treated with Aqueous Solution of AB21-Protein.

For treatment, known quantity of mulberry leaves was kept immersed in known volume of aqueous solution (at the rate hundred grams of mulberry leaves in liter of aqueous solution) for half an hour. Such treated mulberry leaves were fed daily to the silkworm larvae of bivoltine Cross Breed [(CSR6 x CSR26) x CSR2 x CSR27)] for first four days after the fourth moult. For each day, four feedings were supplied at the rate 100 grams of mulberry leaves for the group of hundred larvae. The larvae of the group of Untreated Control were received untreated mulberry leaves. The larvae of the group of Water Treated Control were received water treated mulberry leaves. From sixth day onwards, the larvae of all the groups were fed with untreated mulberry leaves through standard methods. Rearing was carried out in the trays of wood. For each day, larvae received four feedings ((at the rate of hundred grams of mulberry leaves for the group of hundred larvae for each feeding). The mountage was made available for spinning the silky cocoon by the mature last larval stages (Khyade , 2004 and Vitthalrao B. Khyade, *et al* (2005, 2006, 2007, 2008, 2009, 2010, 2011, 2012, 2013, 2014, 2015, 2016, 2017 and 2018).

(D). Analysis of economic parameters: The cocoons were separated from the mountage. Separation of cocoons from mountages is called as harvesting. This harvesting of cocoons was carried out on fifth day after the provision of mountage for spinning. Twenty cocoons from each group were selected randomly. They were deflossed. The weight of individual deflossed cocoon was recorded. Each cocoon in the was cut vertically using the blade and weight of pupa was recorded. For knowing the shell weight of individual cocoon, the reading of the weight of pupa was subtracted from weight of respective cocoon. Weight of entire deflossed cocoon; weight of shell of cocoon and weight of pupa were noted. The silk shell percentage (correctly called as shell ratio) was calculated through the use of readings of weight of whole deflossed cocoon and weight of silk shell in cocoon. The mathematical operation of dividing the readings of silk shell weight by readings of weight of whole cocoon without floss was followed. Multiplication operation was carried with hundred with quotient obtained earlier. This yields the shell percentage. In sericulture, this silk shell percentage is called as shell ratio.

Ten cocoons per replication were used for the purpose to reel the silk filament from individual cocoon. The length in meter (A) of unbroken silk filament was

obtained by using eprouvate. Weight in gram of silk filament (B) from individual cocoon was recorded. Length (A) and weight (B) of silk filament were accounted for the calculation of Denier scale. The reading of weight of silk filament (B) was divided by the reading of length of silk filament (A). Quotient thus obtained was multiplied by 9000 for the purpose to get the denier scale of silk filament Vitthalrao B. Khyade and Abhilasha C. Bhunje, 20015 and 2016).

(E). Statistical Analysis of the data:
The experimentation was replicated for three times. This is for the purpose to get the consistent results. The data was collected and it was subjected for statistical analysis. The statistical parameters considered in the attempt include: mean, standard deviation, percent variation and student "t" - test (Norman and Bailey,1955).

III. RESULTS AND DISCUSSION

The results on the attempt on the study of influence of aqueous solution of Agaricus bisporus (L) treated mulberry leaves on the quality of cocoons and silk filament in silkworm, Bombyx mori (L) [Race: (CSR6 x CSR26) x CSR2 x CSR27)] are summarized in table-1 and presented in Fig. 1 and 2.

The weight (gm) of entire deflossed cocoon; weight of silk shell (gm) and the weight of Pupa (gm) of the Untreated Control group were measured 1.823 (±0.088); 0.369 (±0.013) and 1.454 respectively. The ratio of shell to the entire cocoon in the untreated control group was calculated 20.241. The readings 796.47 (±9.616) ; 0.242 (±0.049) and 2.734 belongs respectively to the Silk Filament Length (SFL in meters); Silk Filament Weight (SFW in grams) and the denier scale of silk filament obtained from the untreated control group cocoons (Table – 1 and Fig. 1 and 2).
The weight of whole cocoon (deflossed) , silk shell weight, weight of pupa, silk shell percentage or ratio and denier scale (unit of measurement of quality of filament) of the water treated group was found measured 1.823 (±0.095); 0.369 (±0.014) ; 1.453 ; 20.241 and 2.734 respectively (Table – 1 and Fig. 1 and 2).

First four days feeding the fifth instar larvae of silkworm, *Bombyx mori* (L) [Race: (CSR6 x CSR26) x CSR2 x CSR27)] with leaves of mulberry, Morus alba (L) treated with aqueous solution of fruiting bodies of Agaricus bisporus (L) was found resulted into spinning the cocoons weighing 2.684 (±0.235).

The weight of pupa; weight of shell and shell ratio of cocoons of this group (larvae received leaves of mulberry, Morus alba (L) treated with aqueous solution of fruiting bodies of Agaricus bisporus L) was found measured 1.922 gm; 0.762 gm (±0.067) and 28.390 respectively.

The length, weight and denier scale of silk filament reeled from cocoons of of this group (larvae received leaves of mulberry, Morus alba (L) treated with aqueous solution of fruiting bodies of Agaricus bisporus L) was found measured 1008.77 (±58.924); 0.337 gm (± 21.786) and 3.006 respectively.

Feeding the fifth instar larvae of silkworm, *Bombyx mori* (L) [Race: (CSR6 x CSR26) x CSR2 x CSR27)] (for the first four days) with mulberry leaves treated with aqueous solution of AB21 protein was found resulted into spinning the cocoons weighing 2.756 (±0.317).
The weight of pupa; weight of shell and shell ratio of cocoons of this group (larvae received leaves of mulberry, Morus alba (L) treated with aqueous solution of AB21 protein) was found measured 1.838 gm; 0.918 gm (±0.119) and 33.309 respectively.

The length, weight and denier scale of silk filament reeled from cocoons of of this group (larvae received leaves of mulberry, Morus alba (L) treated with aqueous solution of AB21 protein) was found

measured 1141.47 (±97.858); 0.409*** (±0.091) and 3.224 respectively.

The silk cocoon is foremost feature in sericultural practices. This is because, silkworm cocoons are sole source for silk commercially available. Silk cocoons are used to obtain the silk filament. The weight of cocoon weight; weight of silk shell and therein the silk shell percentage or shell ratio in present attempt of study were found effected through treating the mulberry leaves with aqueous solution of fruiting bodies of Agaricus bisporus (L) and aqueous solution of AB21 protein feeding separately to the fifth instar larvae of silkworm. The mathematical range of increase in percentage of the cocoon weight and silk shell weight in the experimental (treated) groups was 47.229 to 51.179 and 106.50 to 148.780 respectively.

Silk shell percentage or shell ratio of the cocoons was found improved in the corresponding groups of treatment. Both, AB21 Protein, a known and novel mushroom protein and agaricus fruiting body, used for treating mulberry leaves before feeding fifth instar larvae in the present attempt were found suggestively the most significant ($p<0.001$) with reference to the yield of the silk cocoons through silk shell percentage or shell ratio.

The growth and development of silkworm is under the continuous influence of factors operating within and outside the body (Murugan et al., 1998). Ascorbic acid had effect on the growth of silkworm (Javed and Gondal, 2002) and combination of 0.2% of N which enhances the growth of silk production (Hussain and Javed, 2002). It is evident from the mean data of the experiments that, AB21-Protein treated leaves fed larvae showed a significant enhancement in reeling performance and bioenergetics. Sarkar et al. (1995) reported that growth of larvae B.mori significantly improved when they were fed on mulberry leaves supplemented with different nutrients such as Soya milk, Milk powder, Sugars, vitamins and amino acids. Rate of feeding influences the synthesis of total DNA, RNA and protein synthesis (Chavancy and Fournier, 1979). According to Soo-Hoo and Frankel (1966) the diminishing consumption rate of less preferred food was partially compensated by increased assimilation efficiency. However, according to Mathavan and Krishnan (1976) assimilation efficiency did not vary significantly as a function of reduced **food consumption**. Verma and Atwal (1963) observed that feeding leaves supplemented with distilled water alone slightly increased the weights of larva, pupa and silk shells. The significant results obtained in the attempt may be through the integrative action of the contents of fruiting bodies of Agaricus bisporus (L) and it's novel protein: AB21. This attempt on the use of AB21 Protein and the agaricus fruiting bodies through water for treating mulberry leaves before feeding silkworm larvae is much more easy method and may open a new avenue in the sericulture.

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Table-1: The quality of the cocoons and silk filament spinned by mature fifth instar larvae of silkworm, *Bombyx mori* (L) [Race: (CSR6 x CSR26) x CSR2 x CSR27)] separately received mulberry leaves treated with aqueous Solution of *Agaricus bisporus* (L) and aqueous solution of dimeric protein "ab21" a novel mushroom protein.

Parameters→ Group↓	Cocoon Weight (gm)	Shell Weight (gm)	Pupal Weight (gm)	Shell Ratio	S F L (m) (A)	S F W (gm) (B)	Denier Scale of S F = (B÷A) x 9000
Untreated Control (UT)	1.823 (±0.088) 00.000	0.369 (±0.0013) 00.000	1.454 00.000	20.241 00.000	796.47 (±9.616) 00.000	0.242 (±0.049) 00.000	2.734 00.000
Water Treated Control (WTC)	1.823 (±0.095) 00.000	0.369 (±0.014) 00.000	1.454 00.000	20.241 00.000	796.47 (±13.788) 00.000	0.242 (±0.055) 00.000	2.734 00.000
Treated with aqueous solution of dimeric protein "ab21" (ab.21.T)	2.684** (±0.235) 47.229	0.762** (±0.067) 106.50	1.922* 32.187	28.390** 8.149	1008.77* (±58.924) 26.655	0.337 ** (± 21.786) 29.752	3.006** 09.948
Treated with aqueous solution of Fruiting Body of *Agaricus bisporus* (ABFBT)	2.756*** (±0.317) 51.179	0.918*** (±0.119) 148.780	1.838*** 26.409	33.309*** 13.068	1141.47*** (±97.858) 43.316	0.409*** (±0.091) 69.008	3.224*** 17.922