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The Effect Of Copper Nanoparticles (CUNPS) In Food On The Feed Efficacy Of The Silkworm, *Bombyx Mori* (L.) (Lepidoptera: Bombycidae)

V.Rajathi[1*], M. Veerappan[2], H. Manikandan[3]

[1]Assistant professor, Department of Zoology, Government Arts College (Affiliated to Thiruvalluvar University), C. Mutlur , Chidambaram - 608 102, Tamil Nadu, India.
Email: rajathiv2007@gmail.com
[2]Ph.D Research Scholar, Department of Zoology, Government Arts College (Affiliated to Thiruvalluvar University), C. Mutlur , Chidambaram - 608 102, Tamil Nadu, India.
Email: veerapanmsc@gmail.com
[3]Associate professor, Department of Chemistry, Annamalai University , Annamalai Nagar, Chidambaram - 608 002, Tamil Nadu, India.
Email: profmani.au@gmail.com

Corresponding Author: V.Rajathi
Assistant professor, Department of Zoology, Government Arts College (Affiliated to Thiruvalluvar University), C. Mutlur , Chidambaram - 608 102, Tamil Nadu, India.
Email: rajathiv2007@gmail.com

ABSTRACT

Bombyx mori, the silkworm, is a significant economic insect as well as a tool for converting leaf protein into silk protein. The purpose of this study was to compare the feed efficacy of silkworm *B. mori* (V instar larvae) fed by V-1 mulberry (*Morus indica*) leaves and different concentrations of Copper nanoparticles treated V-1 mulberry (*Morus indica)* leaves to physiological parameters such as Food Consumption (FC), Food Utilization (FU), Approximate Digestibility (AD), Consumption Index (CI), and Co-efficient of Food Utilization (CFU). Green synthesis of Copper nanoparticles employing Copper precursor ($CuSo_4$) and *Morus indica* leaf extract as a decrease and stabilize agent. The larvae were exposed to varied concentration (25 %, 50 %, 75 %, & 100 %) of produced Copper nanoparticles. The feed efficiency of larva (5^{th} instar) was found to be 25 % higher in the Copper nanoparticles treated group than in the control and additional treated groups (50%, 75%, and 100%) in the current study. According to this study, Copper nanoparticles have some growth stimulating action and can be employed to boost silk give up in marketable silkworm rearing with reference to Sericulture.

Key words: *B. mori*, *M. indica*, Copper Nanoparticles, Mulberry leaves

INTRODUCTION

Sericulture is the art and science of rearing silkworms for silk manufacture. India's traditional and culture-bound domestic market, as well as an astounding array of silk outfits reflecting 'regional distinctiveness,' have elevated India to the 2^{nd} largest producer of raw silk after China. Feeding is critical to the development and growth of the silkworm, *Bombyx mori* L., and silk production is dependent on larval nutrition and the nutritional value of mulberry leaves, as well as the formation of high-quality cocoons (M Younus Wani *et al.*, 2018).

The silkworm, *B.mori* L, has been cultivated purely for its high-quality silk material (A. Yerranna et al., 2019). The silkworm, *B.mori*, is a monophagous lepidopteran insect that has been cultivated for almost 5000 years. Because of the economic significance of silk production over the years, the physiologies of this group have been intensively investigated. Further research into dietary preferences and suitable nutrient level for utmost larval growth and silk production resulted in the creation of a non-natural diet. Due to the growth of cutting-edge technologies in mulberry farming and silkworm rearing, India is both the world's second-largest producer and consumer of silk. The cocoons of the larva of the captive-raised mulberry silk worm *B. mori* L. yield the highest-quality silk (sericulture). Sericulture is a technology-based art and science that is mostly rooted in rural communities and is welfare-oriented. It is a significant sector of the national economy. A key component of sericulture is the rearing of silkworms on mulberry leaves, and it can be said that the amount of silk produced is straight proportional to the development and growth of the larva on the mulberry. Different environmental elements and agricultural techniques affect the number and quality of mulberry leaves produced (Phalguni Bhattacharyya, Suchisree Jha, Palash Mandal and Amitava Ghosh, 2016).

Bombyx mori can now be reared wholly on non-natural diet from the 1st to the last larval instar. Mulberry leaves suitable for silkworm food must contain several chemical constituents such as carbohydrates (11%), proteins (27%), water (80%), other extracts, mineral matters, vitamins, and so on, as well as favorable physical characteristics such as suitable softness, thickness, and tightness, in order to be easily eaten by silkworms (Koul, 1989). Because of the presence of morin, mulberry (*Morus* species) leave is the sole food and source of sustenance for the silkworm, *B. mori* (Tribhuwan and Mathur, 1989). According to Ewa Sawosza *et al.*, (2018), copper nanoparticles (NanoCu) can be employed in considerably smaller amounts than bulk copper due to their strong physicochemical reactivity and bioavailability, which reduces the excretion of copper into the environment. To determine the ideal level of CuNps food supplementation, the study's goal was to assess the effects of various CuNps levels on the development and growth of broiler chickens. Copper (Cu), a trace element, is crucial to the nutrition of chickens. This study's goal was to assess how different quantities of CuNps, which replaced CuSO4, affected chicken growth, oxidative state, tissue and excrement Cu content.

The mulberry silkworm, *B. mori*, only feeds on mulberry leafs (*Morus alba* L). During the final two instars (i.e., the fourth and fifth instars) of the silkworm life cycle, 97% of total food intake and 80-85% of feed utilisation in the fifth instar larva of the total leaves ingested were metabolically active (Rahamathulla *et al.*, 2003; Rahamathulla and Suresh, 2012). Furthermore, foliar vitamin supplementation has been proven to boost mulberry leaf productivity and quality, and hence the healthy growth of silkworms, resulting in increased cocoon yield and quality (Geetha *et al.*, 2016).

Foliar micronutrient supplementation has increased mulberry leaf output and quality (Geetha *et al.*, 2016). The presence of all micronutrients in the leaves may improve plant health, quality, and yield, resulting in healthier silkworm growth and greater cocoon yield with quality. Because nanoparticles are smaller in size and have a larger surface area, foliar supplementation with nano micronutrients can result in quick absorption and utilization, meeting the majority of nutritional requirements. Nano fertilizers, specifically micronutrient combinations, are gaining popularity. Mulberry, as a foliage crop, responds well to timely nutrition delivery via foliar sprays (Geetha *et al.*, 2016).

Much research has recently been conducted on the diet supplementation of mulberry leaves fed to silkworms. Vitamins such as ascorbic acid, thiamin, niacin, folic acid, multivitamins, and vitamin C are included in these supplements (Nirwani and Kaliwal, 1998; Saha and Khan, 1996; Etebari and Fazilati, 2003; Etebari *et al.*, 2004 and Balasundaram *et al.*, 2008). Although some of the chemicals had considerable outcomes, enrichment did not always result in improved biological properties of the silkworm. When the content of ascorbic acid in the silkworm diet is increased, the yield decreases, according to Etebari *et al.*, (2004). Copper sulphate, nickel chloride, and potassium iodide supplementation enhanced the physiological parameters of the silkworm (Magadum, 1987).

A silkworm *Bombyx mori* rearing is a traditional enterprise in Asia, and many people rely on it for survival. Increased larval growth, as well as cocoon quality and quantity, would effect in better economics for this industry, as well as meeting production needs. As a result, enriching mulberry leaves with additional substances in order to increase cocoon production is a critical factor. The addition of AgNps to mulberry leaves increased larval and cocoon length, breadth, and weight in these insects, which was linked to metabolisms other than protein metabolism. It is hypothesized that diet fortification aids in the metabolism of carbohydrates and fats. In conclusion, AgNps may raise some biological qualities in silkworm, but this augmentation may benefit the Sericulture goals economically (Ponraj Ganesh Prabu, Selvi Sabhanayakam, Veeranarayanan Mathivanan, and Dhananjayan Balasundaram, 2011).

Delighta Mano Joyce, M.I., and Mohamed Ramlath Sabura, S. (2020) have been demonstrated that feeding mulberry leaves that have been coated with silver nanoparticles improves rearing performance. The nutritional stimulation of larvae with Ag nanoparticles of two species of mushroom extract significantly influenced all the energy parameters governing the formation of high-quality silk. Therefore, the growers could be advised to use this supplement to produce more silk. As a result, this crude extract approach is an alternative to chemical methods since it is affordable, pollutant-free, and environmentally beneficial, but it also requires a thorough investigation using pure cultures.

The larval eating behavior of *Bombyx mori* was observed continuously during larva growth. In addition to this investigation, starved larvae's behaviors were monitored to evaluate how nutrition deprivation influences feeding behavior. Finally, the researchers looked into faces and physical stimulus in *Bombyx mori* larva as potential feeding triggers (Simpson, 1995). The addition of vitamin E to mulberry leaves had no effect on food consumption in silkworm larvae (Mosallanejad *et al.,* 2002). In gene regulatory studies, the interaction between the environment and genes has been considered bidirectional, with food consumption efficiency on gene expression varying based on an organism's genetic background and expressed physiological or nutritional unit (Giacobino *et al.,* 2003; Milner, 2004; Kang, 2008; Ogunbanwo and Okanlawon, 2009). According to Ganesh Prabu.P *et al.*,(2012) silver nanoparticles have been shown to have specific growth-stimulating properties and can be employed to boost silk production in marketable silkworm rearing with reference to sericulture.

According to Sudip Some *et al.,* (2019), AgNps produced utilizing the mulberry, *Morus indica* V-1, leaf extract had strong antibacterial efficacy against silkworm infections. In addition, the synthesized AgNps boosted larva, pupa, and cocoon weights and raised silkworm survivability rates. At concentrations utilized for antibacterial action and therapeutic benefits on silkworms, these nanoparticles interestingly showed no discernible harmful effect against either cell line. The mulberry leaf extract-mediated produced AgNps have a remarkable possible in biomedical applications, as alternative materials have been exposed to have weaker antibacterial activity and high toxicity at the quantities utilized in this investigation. The quantity and quality of mulberry leaves provided during rearing determine the success of the silkworm crop. As a result, one of the most critical criteria in Sericulture is selecting mulberry leaves that are suited for healthy silkworm growth. It eats continually during the larval period's five instars in order to spin a cocoon. The quantitative and qualitative characteristics of cocoons are heavily influenced by the quality and amount of leaves (Koul, 1989; Chenthilnayaki *et al.,* 2004; Balasundaram *et al.,* 2008).

The current study aims to determine the feed efficiency of copper nanoparticles treated V-1 mulberry leaves in terms of food utilization by larva and ultimate effects on silkworm cocoon parameters in order to identify the most nutritive one for bivoltine silkworm in Tamil Nadu climatic conditions. The research on the growth rate of *Bombyx mori* fed with control and copper nanoparticles treated V-1 mulberry leaves is partial. As a result, this study was conducted to determine the effect of copper nanoparticles on the feed efficacy of *Bombyx mori*.

MATERIALS AND METHODS

Rearing of Silkworm

The 1st day of the fifth instar of the popular Indian bivoltine cross (CSR2×CSR4) silkworm *Bombyx mori* (Local Bivoltine) race was taken from the Silkworm Culture Centre in Salem, Tamilnadu. In the laboratory, the larvae were grown simultaneously in control and experimental groups on mulberry leaves dipped in silver nanoparticles solution. The silkworms were given proper climatic conditions, including a photoperiod of 12:12 h light and darkness, as advised by Krishnaswamy *et al.,* (1973). The 1st day of fifth instar larva was spent in an environment with an ambient temperature of 25±27°C and relative moisture of 70 to 80%. The larvae were raised in cardboard boxes 22×15×5 cms long, covered with nylon mesh, and set in an iron stand with ant well (Govindan, *et al.,* 1981).

Preparation of *Morus indica* Leaf Extract

Thoroughly cleaned *Morus indica* leaves were then dried in the sun for 5-7 days. Fine powder was made by grinding dried leaves. 30 gm of leaf powder and 300 ml of distilled water were refluxed in a soxhlet device at 100°C for five hours to create the aqueous extract. The extracts were then gathered and stored for later use in a deep freeze in an airtight bottle (G.Valli and S.Geetha, 2016).

Green Synthesis Copper Nanoparticle from *Morus indica* Leaf Extract

A 40 ml aqueous solution of 10 mM Copper sulphate was combined with 10 ml of *Morus indica* leaf extract for reduction into Cu+ ions, which was then stored at room temperature for incubation (in the dark). For 10 mM of $CuSo_4$, the filtrate here serves as a reducing and stabilize agent. Throughout the studies, appropriate controls (40 ml of distilled water plus 10 ml of plant extract in a different test tube) were kept. The extract's color changed from bright yellow to dark brown after the reduction of copper sulphate to copper ions. Additionally, a control setup was kept in place without Copper sulphate being added to the plant extract. Further research was done on the creation of copper nanoparticles (G.Valli and S.Geetha, 2016).

Feed Efficacy (physiological Traits)

The amount of V-1 mulberry leaf given to each group was similar, and *Bombyx mori* larvae were fed five times per day. Every day, the mulberry leaves and debris were weighed and documented. Similarly, the beginning and final weights of 5^{th} instar larva in the control and Copper nanoparticles treated groups were reported. Fresh leaves were cut in half and the initial water content was determined using one half. There 5^{th} instar larva from the control and Copper nanoparticles treatment groups were dried to constant weight in a hot air oven to decide the dry weights. Physiological characteristics such as Food Consumption (FC), Food Utilization (FU), Approximate Digestibility (AD), Consumption Index (CI), and Coefficient of Food Utilization (CFU) were calculated using these weights (Arsenev & Bromlei, 1957).

The following formula was used to compute Food Consumption (FC).

FC = Dry weight of offered leaves- Dry weight of remaining leaves

The following formula was used to compute food utilization (FU).

FU = weight of food consumed - Weight of faecal matter

Approximate digestibility (AD) was calculated by following formula

$$AD = \frac{\text{Dry weight of food eaten} - \text{Dry weight of faecal produced}}{\text{Dry weight of food eaten}} \times 1000$$

The Food Consumption Index (FCI) was computed using the following formula:

$$FCI = \frac{E}{T \times A}$$

Where,

E = Dry weight of food consumed,

T = Denotes the length of the experimental period.

A = Average dry weight of the animal during the experiment.

The following formula was used to compute the Coefficient of Food Utilization (CFU).

Choosing the Most Effective Concentration of Copper Nanoparticles

The colloidal solution of Copper nanoparticles was dilute to 25%, 50%, 75%, and 100% concentrations. Fresh V-1 mulberry leaves were soaked in each concentration for fifteen minutes before drying in air for ten minutes. Copper nanoparticles-treated leaves were fed to silkworm *B. mori* 5^{th} instar larva (Suleman, 1999).

B. mori larvae were separated into two groups (Control and Treated). The treated group was divided into four subgroups (T1, T2, T3, and T4), and each subgroup was treated with a different concentration of silver nanoparticles (25%, 50%, 75%, and 100%). The efficacy of these concentrations was compare to the control group, and the physiological traits were determined. The silkworm *B. mori* was fed five times per day on the control and Copper nanoparticles-treated V-1 mulberry (*Morus indica*) leaves.

Groups of Experimenters

Bombyx mori larvae in the V instar were fed the following V-1 mulberry leaves. Control (C) larvae were fed normal mulberry leaves, T_1 larvae were fed 25% Copper nanoparticles treated V-1 mulberry leaves, T2 larvae were fed 50% Copper nanoparticles treated V-1 mulberry leaves, T_3 larvae were fed 75% Copper nanoparticles treated V-1 mulberry leaves, and T_4 larvae were fed 100% Copper nanoparticles treated V-1 mulberry leaves. Rasool (Rasool, 1995).

Observation of Larvae Feeding Behavior

Only synchronously growing larval populations were detected. Each larva was observed in a plastic container facing a 3 cm^3 block of artificial nutrition. Larvae were placed in the container in such a way that they did not interfere with the eating behavior of other animals (Shinji Nagata and Hiromichi Nagasawa, 2006).

Statistical Evaluation
Using a commercially available statistical software tool (SPSS ® for Windows, V. 16.0, Chicago, USA), all data were evaluated using one way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT). The data was given as mean standard deviation (SD). P

Table 1 shows the feed efficacy (physiological features) of *Bombyx mori* larvae of the 5th instar fed with control and varied doses of Copper nanoparticles treated V-1 mulberry.

Experimenral group and Concentration	Food Consumption (gm)	Food Utilization (gm)	Approximate Digestibility (%)	Food Consumption Index (%)	Co-efficient of Food Utilization (%)
Control	48.3214±1.4112	45.6132±1.1262	87.3042±0.6093	39.6781±1.4286	86.5408±1.1212
CuNps (T_1) 25%	52.9432±0.5425	49.6932±1.4138	90.6345±0.8800	43.7981±0.7403	91.5012±0.8613
CuNps (T_2) 50%	48.0432±1.8848	42.2567±1.0906	86.7884±1.0636	38.1844±1.0436	86.0534±0.6619
CuNps (T_3) 75%	47.1941±0.6831	43.1452±2.0787	86.9266±1.7059	36.3962±1.4055	85.0472±1.0633
CuNps(T_4) 100%	46.7621±1.0641	45.2971±1.3297	86.4921±1.1447	35.9391±1.5186	84.9855±0.9598

The values represent the mean and standard deviation of 6 observations. Values in the same column with different superscript letters differ significantly at $P<0.05$ (DMRT).
< 0.05 was considered statistically significant (Sokal and Rohilf, 1981).

RESULTS

Food Consumption (FC), Food Utilization (FU), Approximate Digestibility (AD), Food Consumption Index (FCI), and Co-efficient of Food Utilization (CFU) data of 5th instar larva of *B. mori* feed with control V-1 mulberry leaves and different concentrations of Copper nanoparticles treated V-1 mulberry leaves were represented in Table 1.
Table 1 displays the Food Consumption (FC) statistics of *B. mori* 5th instar larva fed control and Copper nanoparticles treated V-1 mulberry leaves. Food consumption (gm) of group 'C' larvae (48.3214±1.4112 gm), group T_1 larvae (52.9432±0.5425 gm), group T_2 larvae (48.0432±1.8848 gm), group T3 larvae (47.1941±0.6831 gm), and group T_4 larvae (46.7621±1.0641 gm), accordingly. The 25% (group T_1) Copper nanoparticles treat larva Food Consumption (gm) was considerably higher than the other 4 groups ('C', T_2, T_3, and T_4) in these 5 observations.
Table 1 displays the Food Utilization (FU) data of *B. mori* 5th instar larvae fed control and Copper nanoparticles treated V-1 mulberry leaves. Food utilization (gm) of group 'C' larvae (45.6132±1.1262 gm), group T_1 larvae (49.6932±1.4138 gm), group T_2 larvae (42.2567±1.0906 gm), group T_3 (43.1452±2.0787 gm), and group T_4 larvae (45.2971±1.3297 gm). In these 5 observations, the food utilization (gm) of the 25% (group T_1) Copper nanoparticles treated larvae was considerably higher than the other 4 groups ('C', T_2, T_3, and T_4).
Table 1 displays the Approximate digestibility (AD) statistics of *B. mori* 5th instar larva fed control and Copper nanoparticles treated V-1 mulberry leaves. The Approximate Digestibility (%) of group 'C' larvae (87.3042±0.6093 %), group T_1 larvae (90.6345±0.8800 %), group T_2 larvae (86.7884±1.0636 %), group T_3 larvae (86.9266±1.7059 %), and group T_4 larvae (86.4921±1.1447 %). In these 5 observations, the approximate digestibility (%) of the 25% (group T_1) Copper nanoparticles treated larvae was significantly higher than the other 4 groups ('C',T_2, T_3, and T_4).
Table 1 displays the Food Consumption Index (FCI) data of *B. mori* 5th instar larva fed control and Copper nanoparticles treated V-1 mulberry leaves. The Food Consumption Index (%) of group 'C' larvae (39.6781±1.4286 %), group T_1 larvae (43.7981±0.7403 %), group T_2 larvae (38.1844±1.0436 %), group T_3 (36.3962±1.4055 %), and group T_4 larvae (35.9391±1.5186 %), in that order. The 25% (group T_1) Copper nanoparticles treated larvae Food Consumption Idex (%) was considerably higher than the other 4 groups ('C', T_2, T_3, and T_4) in these 5 observations.

Table 1 displays the Co-efficient of Food Utilization (CFU) data of *B. mori* 5th instar larva fed control and copper nanoparticles treated V-1 mulberry leaves. Food utilization co-efficients (%) of group 'C' larvae (86.5408±1.1212 %), group T_1 larvae (91.5012±0.8613 %), group T_2 (86.0534±0.6619 %), group T_3 (85.0472±1.0633 %), and group T_4 (84.9855±0.9598 %), respectively. The 25% (group T_1) Copper nanoparticles treated larval Co-efficient of Food Utilization (%) was considerably higher than the other 4 groups ('C', T_2, T_3, and T_4) in these five findings.

DISCUSSION

Copper have been shown to affect nearly all biochemical and macronutrient concentrations in mulberry leaves. Thus, nitrogen levels increased as treatment Cu concentration increased (Prince, 1999). The findings of this study are strongly corroborated by the findings of Shaikh *et al.,* (2013) and Yerranna *et al.,* (2018), who found that the detrimental effects of copper on shoots are directly related to their toxicity on shoots and roots. Furthermore, sprouting did not develop at extremely high Cu concentrations (Yerranna *et al.*, 2018), showing the relative relevance of Cu for shoot formation and leaf functions. According to Sridhara and Bhat (1966), trace elements Zn, Cu, Mn, Mo and Co have varied impacts on silkworm growth and trace element composition. The findings suggest that manganese plays a vital part in the insect's regular metabolism. Cobalt has been demonstrated to have a very positive impact on development and silk output.

Minerals in the form of $ZnSo_4$, $MgSo_4$, and KCl were administered to silkworm larvae at various concentrations, namely 10, 25, 50, 100, and 200 ppm, during the 3rd, 4th, and 5th instars, and significantly better *Bombyx mori* growth and cocoon economic parameters (Murugesh K A *et al.,* 2020). According to Sarkar and Fujita (1994), a low food intake results in a shorter larval stage. This research clearly demonstrates that cultivars with high exchange efficiency may lower larval span and, as a result, require less food to ensure optimal growth. The study's findings demonstrated a significantly significant difference in nutritional features between the control KANVA-2 leaf and KANVA-2 treated with B-complex vitamins. Feed exchange efficiency contributes directly and indirectly to the majority of the price advantage percentage of silkworm rearing and is regarded as a significant physiological parameter for determining silkworm breed superiority. The efficiency of nutrition is almost nullified by increasing consumption, which results in increased synthesis of cocoon, shell, and recognized that dietary variables and interrelated metabolic communications have straight and indirect power on exact gene expression (M.Meeramaideen *et al.,* 2017).

According to Soo-Hoo and Frankel (1966), decreased ingestion of less liked foods was somewhat offset by enhanced absorption efficiency. According to Mathavan and Krishnan (1976), reducing food consumption had no effect on absorption efficiency. It has been shown that cocoon heaviness and pupal heaviness were proportional to JH concentration and feed period (Akai *et al.*, 1985 and Chowdhary *et al.*, 1990). According to Ashfaq *et al.* (2001), silkworm fed with *Morus nigra* demonstrated high food consumption, coefficient of nutrition utilization, larvae size, larval heaviness, and cocoon heaviness, all of which may be essential variables in enhancing silk tenacity and elongation.

Silkworm growth and development are constantly influenced by influences both inside and outside the body (Murugan *et al.*, 1998). According to Javed and Gondal, 2002, Silkworm growth was influenced by ascorbic acid. Mulberry leaves infused with nitrogen (0.2%), which promotes growth and silk production. According to Amala Rani *et al.,* 2011, Amway protein enriched mulberry leaf (10%) greatly increased silkworm larvae development and economic characteristics. Verma and Atwal (1963) discovered that feed mulberry leaves combined with distill water alone raised the weights of larvae, pupae, and cocoon shells marginally. According to Soo- Hoo and Frankel (1966), higher absorption efficiency partially compensated for declining ingestion of less liked foods.

Absorption efficiency did not differ considerably with decreasing food consumption (Mathavan and Krishnan, 1976). It was discovered that cocoon heaviness and pupal heaviness were closely proportional to JH concentration and feed period (Akai *et al.*, 1985 and Chowdhary *et al.*, 1990). Dietary or nutritional variables, as well as related metabolic interactions, have both direct and indirect effects on gene regulation and expression (Iftikhar and Hussain, 2002; Phillips *et al.*, 2008). Such nutrigenetic interactions and variations could be used to select silkworm breeds based on nutritional efficiency parameters as biomarkers.

The morphometrics of fourth and fifth instar larvae improved dramatically during the investigation. Increased larval weight could be related to the excellent quality of micronutrient-containing leaves, which makes the larvae healthier by improving micronutrient usage and assimilation (Bose *et al.*, 1994; Ramarethinam and Chandra, 2007), as well as other larval features (Sarker *et al.*, 1995; Nirwani and Kaliwal, 1995, 1996; Etebari *et al.*, 2004; Balasundaram *et al.*, 2008). The majority of silkworm germplasm breeds were examined based on their feeding habits and tolerance for commercial rising on artificial diets that lack mulberry (Mano *et al.*, 1991; Zhang *et al.,* 2002). Furthermore, it was discovered that the silkworm receives over 70% of its protein from mulberry leaves, and that in V instar, up to 96% of consumed protein is used for silk protein synthesis, and that variations in the quantity or quality of nutrition have a significant impact on insect development (Fukuda and Higuchiy, 1963). In sericulture, nutritional requirements and conversion efficiency have an impact on the cost-benefit ratio of silkworm rearing, either directly or indirectly. It was thought to be a significant physiological factor for determining the dominance of silkworm breed. The feed usage study focused to V instar larvae as 80-85% of total leaves consumed in this instar as silkworm very active metabolically at this stage (Rahmathulla *et al.*, 2005).

According to Javaid (1991) and Nadeem (1996), silkworm larvae fed on mulberry leaves enriched with mineral nutrients had high food consumption and coefficient of utilization but low mortality. Rehman (1997) discovered a comparable effect of varied nitrogen dosages and concluded that greater doses resulted in a decrease in food consumption and the coefficient of utilization.

According to Javed and Gondal (2002), larvae given mulberry leaves treated with 0.2% N + 0.150% ascorbic acid had reduced mean values of body weight, body length, food consumption, coefficient of utilization, and cocoon shell ratio but a greater mortality rate. According to Sarkar and Fujita (1994), a low food intake results in a shorter larval stage. This research clearly demonstrates that cultivars with high conversion efficiency may lower larval span and, as a result, require less food to ensure optimal growth.

Vyjayanthi and Subramanyam (2002) previously reported that when compared to bivoltine silkworms, multivoltine silkworms exhibited much higher rates of feeding, assimilation, and conversion with increased efficiency of conversion of ingested and digested food to body substance. Many insects have had their food usage factors investigated (Rath *et al.,* 2003). The nutritional value of mulberry leaves is determined by a variety of agro-climatic conditions, and any nutrient deficiency in the leaves affects silkworm silk synthesis. Nutritional management has a direct impact on the quality and quantity of silk production (Hiware, 2006). According to Vyjayanthi and Subramanyam (2002), eating behaviour in the silkworm, *Bombyx mori*, is determined by the niche, amount of food supplied, quality of food, age, and health of the larva. Because the majority of phytophagus lepidoptera are voracious eaters, any imbalance in inputs from multiple sources affects food intake and results in poor larval growth (Waldbauer, 1968; Vyjayanthi and Subramanyam, 2002). The improved nutritional status and micronutrient concentration in mulberry leaves obtained from the combined supplementation of nano ZnO + nano Cu @ 500 ppm (T11) may have stimulated the metabolic activities of silkworms, resulting in improved growth and development and, ultimately, silk production (Pramila Choudhury *et al.*, 2019).

This study suggests that the feed efficacy of *Bombyx mori* was higher when the worm was fed 25% Copper nanoparticles treated V-1 mulberry leaves rather than control V-1 leaves and other groups.

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