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STUDIES ON THE INFLUENCE OF SUPPLEMENT FEED (SPIRULINA) ON LARVAL AND PUPAL MORTALITY OF *BOMBYX MORI* (L.) (LEPIDOPTERA : BOMBYCIDAE) FED WITH MR-2 MULBERRY LEAVES

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Abstract

Sericulture is both an art and science of raising silkworms for silk production. Silkworm *Bombyx mori* is essentially monophagous insect feeding solely on mulberry leaves. The silkworm larvae are highly sensitive and respond to the changes in mulberry leaves and climatic factors. The supplementation and fortification of mulberry leaves is a recent technique on sericulture research. Fortification of the mulberry leaves by nutrient supplementation can increase the quality and productivity of silk. *B. mori* L requires specific sugar, amino acid, proteins and vitamins for normal growth and development. Various nutrient substrates and extracts of medicinal plants have been tested by supplementation in the silkworm diet and were seen to influence the body weight, silk gland height and the silk thread length in *Bombyx mori.* Multivitamin and mineral compounds could increase the food intake, growth and permission efficiency of silkworms. Again nutrient supplement is good in improving the qualities of silk fibre which can be used for yield enhancement in sericulture industry. *Spirulina*, (Blue green algae) are free living, photosynthetic, and N_2 fixing bacteria found in fresh, marine water and terrestrial environments which contains 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. Silkworm derives over 70% of the protein from the mulberry leaves and in 5^{th} instar up to 96% of ingested protein is used for silk protein synthesis and variation in the quantity or quality of nutrition have profound effect on insect development. The result obtained from the present study revealed a significant variance on nutritional traits between the different doses of spirulina treated MR_2 leaves. It can be utilized as a source of proteins, lipids, vitamins and secondary metabolites.

Introduction

Mulberry is a fast growing deciduous woody perennial plant. It has a deep-root system. The MR2 variety is moderately resistant to powdery mildew disease caused by *Phyllactinia corylea* and is very popular in the plains of Tamilnadu and better suited for high altitude areas also where high temperature prevails. It is one of the open pollinated hybrid selections from exotic mulberry varieties which were introduced in Nilgiri Hills. This variety is good in rooting, leaf yield and growth.

Chemical composition of mulberry leaf

Chemical composition of leaf varies with variety and maturity. However, on the basis of the analysis carried out at CSR and T1 Mysore, the Chemical composition of leaf is Moisture (65-78%); Protein (19-25%); Minerals (10-15%); Reducing sugars (1.22-1.90%) and Sugar (10-11%). The mulberry leave are rich in flavinoids, alkaloids and polysaccharides components, which are known as the most potent compound by chemical constituent investigation (Wang *et al.,* 2008). The anti-inflammatory antioxidant and anticarcinogenic effects of flavinoids are some of the properties that have been under consideration in view of therapeutical purposes for several human diseases. Flavinoids are thought to be one of the most critical constituents in mulberry that have therapeutic activity (Snijman, 2007).

Silkworm selects the mulberry leaf as feed because of the following reasons:

1. Leaves contain Citralvinaylacetate, Linalool, Terpenylacetate and Hexanol. The presence of this acetate adds lusture to the leaves and attracts silkworms.
2. Mulberry leaves also contain Sitosterol because of which, the softness in leaves increases.
3. Above all, it contains a mineral salt that dissolves in easily in water, act as a digestive material.

The silkworm larvae are attracted by three stimulants in mulberry leaves viz., the attractant, biting factor and swallowing factor (Hamamura and Naito, 1961). There is a need to recognize and integrate the physiological and nutritional requirements of the silkworm hybrids under ecological conditions in breeding and management

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programmes to make them need-based (Nagaraju, 2002). Therefore to boost the production of the silk, improved quality of leaf or mulberry variety has to be used for silkworm rearing. During the last three decades, silk production increase benefited, to a great extent, from the application of genetic and feeding principles in the silkworm breeding and feeding programmes (Nagaraju, 2002). Thus, for each instar, the increase in fresh and dry weight of the larvae, fresh and dry weight of food eaten and digested and dry weight of faeces produced were recorded by Rath *et al.,* 2003. Enrichment of mulberry leaves with vitamin E did not have significant effect on food consumption in silkworm larvae (Mosallanejad *et al*., 2002). The relationship between the environment and genes has considered by directional with food consumption efficiency gene expression various depending on the genetic background of an organism and expressed physiological or nutritional unit in gene regulation studies (Giacobino *et al.,* 2003; Milner, 2004; Kang, 2008; Ogudanwo and Okanlawon, 2009). Further, the ingesta and digesta required for producing one gram of cocoon and shell (I/g and D/g) were worked out as described by standard gravimetric method (Waldbauer, 1968; Scriber and Feeny, 1975). The total annual demand for silk yarn and fresh cocoon is approximately 655 and 6,500 MTs, respectively, valued about 5000 million rupees.

Materials and Methods

Supplement feed preparation

Studies were carried out on enriching the mulberry leaves with *Spirulina* in different concentrations to improve the silkworm nutrition. The experiments were conducted with silkworm race L **X** CSR 2 (multivoltine). *Spirulina* was dissolved in distilled water and diluted into 100ppm, 200ppm, 300ppm and 400ppm concentrations. The IIIrd, IVth and Vth instar larvae were utilized for the experiment. Third instar larvae were divided into five experimental groups including control, each group consisting 20 larvae. Four replications were maintained for each of the treatments. Fresh mulberry leaves were sprayed with aqueous extract of *Spirulina*, and then leaves were dried under fan before feeding to the silkworms till end of the fifth instar. During this period the worms were fed four times a day and maintained with necessary disinfection conditions. The feeding was maintained up to the cocoon stage of the silkworm. The mature larvae from both control and experimental groups were isolated and mounted on separate plastic mount age (Netrika).

Mortality analysis

Larval mortality (%)

The larval mortality incurred during the entire rearing was noted. The larval mortality at the end of the larval period was calculated by using the following formula and expressed in percentage.

Table: Influence of supplement feed on larval and pupal mortality of *Bombyx mori*

Treatments	Mortality (%)	
	Larval Mortality **	Pupal Mortality **
T_1-MR2 mulberry leaves + 100 ppm *Spirulina*	6.80^d (15.10)	5.90^d (14.05)
T_2-MR2 mu lberry leaves + 200 ppm *Spirulina*	6.65^c (14.93)	4.75^c (12.58)
T_3-MR2 mulberry leaves + 300 ppm *Spirulina*	5.75^b (13.86)	3.25^b (10.34)
T_4-MR2 mulberry leaves + 400 ppm *Spirulina*	4.50^a (12.22)	2.50^a (9.04)
T_5-Control	7.15^e (15.48)	6.53^e (14.79)
SED	0.1309	0.1047
CD (p=0.05)	0.2851	0.2281

Values are means of Four replications: ** values in the parentheses are arc sine transformed values; in a column, means followed by common letter(s) are not significantly different (P = 0.05) by Duncan's Multiple Range Test

Larval mortality

The results revealed that the least per cent of larval mortality (4.50) was found in mulberry treated *Spirulina* T_4 400ppm followed by 300ppm (5.75) and 200ppm (6.65). In contrast, the highest percent mortality was recorded in 100ppm (6.80) followed by untreated control (7.15). With regard to pupal mortality, the least per cent mortality was recorded in mulberry treated *Spirulina* T_4 400ppm with 2.50 per cent followed by 300ppm with 3.25 per cent. The highest per cent mortality was found in untreated control with 6.53 followed by 100ppm had 5.90 per cent.

Nutritional and its conversion efficiency, in sericulture, contribute directly or indirectly on the cost benefit ratio of silkworm rearing. In silkworm, 97% of the total food intake is during the last two instars and the feed utilization study confined to 5th instar larvae as 80-85% of the total food is consumed at this stage (Rahamathulla *et al*., 2005).

The result obtained from the present study revealed a significant variance on nutritional traits between the different doses of spirulina treated MR_2 leaves and exhibits least larval and pupal mortality. Similar results have been reported by Magadum *et al*. (1996) and Gokulamma and Reddy (2005). Efficiency of the nutrition gets nullified by the increase in consumption resulting in increased production of cocoon, shell and it is understand that dietary factors and related metabolic interactions has either a direct or indirect bearing.

Cocoon and shell, which otherwise be referred as leaf-cocoon and leaf-shell conversion rate are ultimate indices to evaluate nutritional efficient silkworm breed in terms of the production of cocoon/shell. (Mariba shetty *et al*., 1991; Ding *et al*., 1992; Junliang and Xiaofeng, 1992). Previously, Vijayshanti and Subramanyan (2002) have stated that multivoltine silkworms had significantly higher rates of feeding, assimilation and conversion with increased efficiency of conversion of ingested and digested food to body substance when compared with bivoltine silkworms. The nutritive value of mulberry leaves depends on various agro-climatic factors and any deficiency of nutrients in leaves affects silk synthesis by the silkworm. Nutritional management directly influences the quality and quantity of silk production (Hiwari, 2006). Therefore, in recent years, attempts have been made to fortify the leaves with nutrients, spraying with antibiotics, juvenile hormones, juvenile-hormones mimicking extracts of plants etc., to reduce larval and pupal mortality and improve the quality, quantity of silk.

Bombyx mori feeding behaviour also depends on niche, amount of food offered, quality of food, age and health of larvae. As most phytophagous Lepidoptera are voracious feeders any imbalance in the inputs from various factors affect food intake and result in poor larval development (Vijaysanthi and Subramanyan, 2002).

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