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Biochemical and bioassay studies on the influence of different organic manures on the growth of Mulberry Variety V_1 and silkworm, *Bombyx mori* Linn.

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ABSTRACT
The domesticated silkworm, *Bombyx mori* Linn., is an insect of immense economic importance for sericulture industry, feeds on mulberry leaves during its larval period and spins silk cocoons. Keeping in view of the importance of silkworm, *B. mori* and its host plant mulberry the present study was carried out. The V_1 mulberry plant was grown in four different organic manures viz., poultry manure, piggery waste, goat waste and cow dung to assess the leaf biochemical evaluation and the economic characters of silkworms. Leaf biochemical analysis was carried out for leaf moisture content, carbohydrate fractions, total carbohydrate, protein and lipid. The present study reveals that the poultry manure applied mulberry variety exhibited significant difference for all the parameters studied. The moulting percentage was highest in the larvae fed on poultry manure applied V_1 mulberry leaves. The study also proved that the moulting in silkworm has an intimate relationship with nutritive value of the leaf. The nutritive qualities of the leaves of poultry manure applied V_1 plant were found superior than that of other organic manure applied for V_1 plants as the larvae fed on the poultry manure applied V_1 leaves have shown higher values for most of the characters studied.

Keywords: Biochemical composition, Organic manures, V_1 mulberry variety, *Bombyx mori*, Mulberry growth, Bioassay study

INTRODUCTION

In mulberry, chemical fertilizers are usually used to maintain and enhance the growth and leaf quality. However, frequent use of chemical fertilizers for a prolonged period deteriorates the surface soil characteristics and affects the availability and uptake of nutrients to plants (Subbaswamy *et al*., 1994). Espiritu *et al*. (1995) had shown that the addition of nitrogen fixers can enrich and enhance the nutrient value of any compost and thus facilitate supplementation or replacement of chemical fertilizers. The maximization of quality of leaf yield is one of the most important factors for successful and good quality cocoon production. The concentrated organic manures being rich in plant nutrient could replace the inorganic fertilizers on equivalent nutrient basis. Application of organic manure improves the soil physical, chemical and biological properties with direct impact on moisture retention, root growth and nutrient conservation etc. Organic matter neutralizes the rapid fall in yield due to continuous use of inorganic fertilizers. Heavy metals react with organic matter, clay exchange site, carbonates and oxide surfaces and precipitate as hydroxides, carbonates, sulphides and phosphate in the soil. Hence, organic farm agricultural produces are residue free and fetch a higher price in the market than any other chemical farm produces. Smith (1950) observed that in poultry manure, two percent of nitrogen in the form of uric acid, which changes rapidly to ammonia form for easy utilizetion by the plants. India has abundant organic manures as nitrogen sources *viz*., Poultry manure, fish meal, pig manure, farmyard manure and press mud produced by 150 million poultry, 8.6 million pigs, 182 million and 700 sugar mills which are doubled by 2000 A.D.

Poultry manure is a rich source of nutrient, besides serving as a soil conserving material as stated by Eno (1966). Application of poultry manure at higher rates increased the soluble phosphorus concentration in the soil (Warneke and Siregar, 1994). Poultry manure is a rich source of nutrient, since liquid and solid excreted together without loss of urine and it ferments quickly (Dhillon *et al.*, 1996). Wong (1985) reported that pig manure was the major source of water pollution in Hong Kong. Christie (1987) reported that pig slurry application increased the soil phosphorus than that of cow dung slurry. Mbaguru and Piccolo (1990) noticed that application of piggery manure increased the total nitrogen by 18 percent and 43 percent of available phosphorus. Krichmann and Witter (1992) estimated that pig manure, contained 29 mg of phosphorus and 17.4 mg of kg^{-1} dry matter.

It also contained appreciable amount of micronutrients. Gaur *et al.*, (1984) estimated that the sheep manure contains 0.64 percent nitrogen, 1.7 percent phosphorus and 0.5 percent potassium. Sheep manure is organic manure domestically available and its application increased the yield (Ramasamy, 1997). Dahama (1990) reported that the folding of 7000 sheep for one night adds 149.3 quintals of dung that improved the physical condition of the soil. Therefore, in this present study the organic manures were applied in the form of poultry manure, goat waste, piggery waste and cow dung.

The growth and development of the larvae and subsequent cocoon production are very much influenced by its nutritive value of mulberry leaves. It is, therefore, imperative to improve the nutritional composition of leaves which depends chiefly on the mulberry varieties and fertilizer doses (Bose *et al.*, 1991; Petkov, 1992; Sarker *et al.*, 1992). During the young larval stage, there is a large requirement of water content, carbohydrates and inorganic salts. Proteins are required in all the stages and particularly during the fifth larval stage of *Bombyx mori* Linn., a higher quantity of protein is essential for the formation of sericin and fibroin during spinning of the silk cocoons. Hence, it is very important to select mulberry, which will have the ideal composition during various stages of larval growth of *B. mori*. In the present study V_1 mulberry variety was selected as experimental plants because of the presence of certain superior qualities. Proper selection of fertilizers, depending upon soil reaction (p^H) greatly increases the fertilizer use efficiency and economic crop production. In view of this, the present investigation was undertaken to study the influence of different organic manures on the growth of V_1 mulberry variety and silkworm, *Bombyx mori* by biochemical and bioassay studies.

MATERIALS AND METHODS

Experimental Design, Fertilizers and Biochemical studies

The V_1 mulberry plant raised in bush form was used for the study. The experiment was conducted in a randomized block design with 3 replications and was raised at a plant spacing of 60cm x 60cm. The experimental plot consisted of four blocks and each block had six rows. The fertilizers were applied in the form of poultry manure, goat waste, piggery waste, and cow dung in equal split doses. The control with out applying fertilizers was also maintained separately. The leaf samples of mulberry were subjected to biochemical studies. Total protein (Lowry *et al.*, 1951), total carbohydrates (Dubious *et al.*, 1956), and total lipid (Folch *et al.*, 1957) were estimated. The moisture percentage was estimated according to gravimetric method.

Bioassay studies

The V_1 variety was subjected to bioassay studies by feeding the leaves to the silkworm larvae of *B. mori* of eight bivoltine races (Alps yellow, Meigitsu, Yaknal, King Haung, Hauchi, JD - 6, PAM -102 and CA -2). The freshly hatched young silkworm larvae from each race were grouped into four replications of 50 larvae and were fed with five times a day with fresh mulberry leaves. The larval weight, single cocoon weight, single cocoon shell weight SR% (Shell Ratio) and PECS (Percentage of effective cocoon shell weight) were recorded.

RESULTS

Nutritional level assessed through chemical analysis

The results revealed that V1 variety of mulberry grown in different organic manures under study vary in their nutritional constitutions. Among the manures, poultry manure applied V_1 mulberry plants (76.76%) exhibited higher leaf moisture content, followed by piggery waste (75.46%), cow dung (73.80%) and goat waste (73.48%) (Table 1).

Table 1 – Leaf moisture percentage in control and different treatments.

Treatments	Moisture Percentage
Control	71.25±0.28
Poultry manure	76.76±0.33
Cow dung	73.80±0.34
Goat waste	73.48±0.17
Piggery Waste	75.46±0.14

± = Standard Error.

Organic manures (poultry manure, cow dung, goat waste and piggery waste) applied V_1 plants showed very high protein, carbohydrate and lipid content when compared to the total protein, carbohydrate and lipid content in control V_1 plants (Table 2).The amount of protein, carbohydrate and lipid content in control plant were 201.5 mg/g, 36.592 mg/g and 17.123 mg/g, respectively. Different manures applied V_1 plants showed high total hexose, reducing sugar and polysaccharide values when compared to the control V_1 plants (Table 3).

Leaf quality assessed the ough bioassay study

Effect of different organic manures applied V_1 mulberry plants on the larval weight, ERR (Effective rate of rearing), single cocoon shell weight, SR % (shell ratio) and PECS (percentage of effective cocoon shell weight) showed higher values when compared to control (Table 4). Among the four organic manures applied V_1 mulberry plants, the highest mean value of larval weight (38.28 g for 10 larval weight), ERR% (86.00), single cocoon weight (1.16 gm), single cocoon shell weight (0.24g), SR% (20.94) and PECS (1.05) of eight bivoltine races was recorded in the larval fed poultry manure applied V_1 and lowest in the larvae fed with cow dung applied V_1 mulberry plants.

Table 2 - Biochemical Constituents in control and different treatments.

Manures applied	Total Protein mg/g	Total carbohydrate mg/g	Total lipid mg/g
Control	201.500± 0.16	36.592± 0.068	17.123± 0.053
Poultry manure	228.333± 2.83	39.301± 0.45	20.100± 0.27
Cow dung	210.800± 1.39	38.021± 0.48	18.932± 0.30
Goat waste	208.700± 0.59	39.378± 0.34	17.921± 0.35
Piggery manure	223.700± 1.50	38.721± 0.30	19.820± 0.28

± = Standard Error.

Table 3 - Different Carbohydrate Fractions (mg/g of fresh leaf) in control and different treatments.

Sl.No	Manures applied	Total Hexose	Reducing Sugar	Total Sugar	Polysaccharide	Total carbohydrate
1.	Control	14.53± 0.15	1.78± 0.04	12.75± 0.10	20.28± 0.079	36.59± 0.10
2.	Poultrymanure	17.02± 0.39	2.76± 0.15	14.26± 0.12	21.28± 0.13	39.30± 0.089
3.	Cow dang	16.03± 0.05	1.87± 0.05	14.16± 0.08	21.99± 0.07	38.02± 0.06
4.	Goat waste	17.32± 0.09	2.33± 0.11	14.99± 0.22	22.06± 0.22	39.38± 0.09
5.	Piggery waste	15.93± 0.10	1.91± 0.03	14.02± 0.05	22.79± 0.062	38.72± 0.063

± = Standard Error.

Table 4 - Economic Characters in control and different treatments.

Treatments	Larval weight (10larvae) (gm)	Effective rate of rearing % by Number	Single cocoon weight (gm)	Single cocoon shell weight (gm)	Shell Ratio %	Percentage of effective cocoon shell weight
Control	33.49± 0.07	79.38± 0.11	0.99± 0.004	0.18± 0.001	18.25± 0.07	0.65± 0.02
Poultrymanure	38.28± 0.06	86.00± 0.90	1.16± 0.006	0.24± 0.006	20.94± 0.03	1.05± 0.02
Cow dung	35.73± 0.06	83.38± 0.06	1.03± 0.003	0.19± 0.0004	18.61± 0.029	0.77± 0.006
Goat waste	35.01± 0.028	83.50± 0.07	1.06± 0.006	0.21± 0.006	19.56± 0.028	0.77± 0.003
Piggerywaste	36.31± 0.03	84.63± 0.06	1.11± 0.006	0.23± 0.001	20.44± 0.006	1.03± 0.003

± = Standard Error.

DISCUSSION

Nutritional studies with varying levels of leaf quality produced under different organic manures treatment of V_1 mulberry variety also enables the understanding of the precise nature of the quality requireed for optimum cocoon production. The application of the poultry manure might have increased the release of macro as well as micronutrients, which intern increased dry matter production, plant height, and nutrient uptake leading to higher mulberry leaf yield. In the present study, the results ensure that the application of poultry manure has increased the uptake of nutrients in mulberry leaf. Increased uptake might have been due to the increased total and available nitrogen of poultry manure. Increased dry matter production and nitrogen content contributed higher nitrogen uptake. Nainar and Pappiah (1999) recorded similar observation. Simth (1950) observed that 60% of nitrogen in poultry manure was in the form of uric acid, which changes rapidly to ammonical form for easy utilization by the plants.

Improvement in soil physico-chemical properties, steady and adequate supply of nitrogen might have increased the nutrient uptake by poultry manure. Similar observation was recorded by Hsieh and Hsu (1993) in different crops. Brown (1958) stated that the poultry manure increased the growth promoting substances, which might have induced the plant for better growth and higher uptake of nutrients. An important factor, which contributed or influenceed the crop to produce better growth and yield component was the high amount of phosphorus availability in the poultry manure (Ramesh, 1997).From the above findings, it is evident that the poultry manure contains the entire essential plant nutrient such as nitrogen, phosphorus, potassium, calcium, magnesium, sulphur, boron, zinc, copper, manganese iron etc, which are necessary for increasing the leaf yield and quality (Dosani *et al*., 1999). Since solid and liquid portions of the poultry are excreted together, poultry manure is a concentrated source of nitrogen and phosphorus. It is well documented to be an excellent source of fertilizer by Simpson (1990), and Edward and Daniel (1992).

The moisture content of the mulberry leaves is a genetic character and is related to the available soil moisture content and root proliferation. The moisture content of the leaf determines the digestibility of silkworm (Kasiviswanathan *et al*., 1973). Usually moisture content varies from 64-83% in mulberry leaves (Yokoyama, 1975) and moisture of 70% above is considered as optimum (Singh and Singh, 1976).

Silkworm fed on leaf with higher moisture content (75%) produced heaviest cocoon (Kataoka and Imai, 1986). In the present study, the moisture content varies from 71.25% to 76.76% in the control as well as organic manure applied plant leaves. The maximum water content was observed in silkworm larvae fed with poultry manure applied V_1 leaves (76.76%) and produced heaviest cocoon. The mean value of single cocoon shell weight of eight different bivoltine pure breeds was 0.244 gm.

Reducing and non-reducing sugar were increased due to the application of 100% nitrogen in the form of poultry and goat manures. This may be due to the cumulative effect of adequate supply of nutrients and higher total available potassium content of the poultry and goat manures which increased the potassium content and uptake, which helped the formation of starch and sugar.

The protein content varied significantly under different organic manures. Fukuda *et al*., (1960) found that about 70% of the silk protein produced by the silkworm is directly derived from the protein of the mulberry leaves and it is directly correlated with production efficiency of cocoon shells in silkworms (Machii and Katagiri, 1991). As different minerals have a role to play in silkworm nutrition, the optimum use of nutrient becomes inevitable (Singhal and Mala, 1998; Singhal *et al*., 1998).

The highest protein content (228.333 mg/g) and PECS (7.73% mean value of 8 different bivoltine races) were observed in silkworm larvae reared in poultry manure applied mulberry leaves.

Mulberry leaf with more moisture, protein, sugar and carbohydrates and less minerals and crude fibre content is the best from the silkworm nutrition point of view (Krishnaswami, 1978). However, Thangamani and Vivekanandan (1984) observed that the silkworm which feed on leaves having 70.6% moisture, 29.46% crude protein, 17.93% minerals and 9.78% total sugar showed better cocoon result reflected in greater filament length and higher effective rate of rearing (ERR), thereby suggesting that the higher leaf protein content might have contributed to greater filament length. Thus the higher leaf protein and carbohydrate and less crude fibre contents are desirable for the healthy growth of silkworm larvae and better cocoon production. The maximum ERR% by number was observed on silkworm larvae reared on poultry manure applied mulberry leaves.

Carbohydrates are utilized by the silkworm as energy sources and for the synthesis of lipids and amino acids (Horie, 1978). Hiratsuka (1971) reported that mulberry leaves contain plenty of carbohydrates, which are found to be in the silkworm mainly as glycogen. He further suggested that a greater part of the carbohydrate content of mulberry leaves is used for physiological combustion and for making of the carbohydrate content which was highest in the larvae reared on goat waste applied leaves and the highest lipid content was observed in the larvae reared on poultry manure applied leaves.

Growth and development in silkworm and cocoon characters are found to be affected by leaf quality (Krishnaswami, 1978; Koul *et al*., 1979). Das and Sikdar (1970) observed that leaf quality had little effect on cocoon characters. It is concluded from the overall analysis of the biochemical and bioassay studies that mulberry leaves plays a vital role in the growth and development of silkworm, *B. mori* larvae.

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