

**THE EFFECT OF ENRICHMENT OF MULBERRY LEAVES
WITH SOYA FLOUR, ORABOLIN, AN ANABOLIC
STEROID AND VITAMIN B COMPLEX ON THE
GROWTH AND DEVELOPMENT OF SILKWORM,
Bombyx mori L.**

BY

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Reg. No. 93 PLS 11

A THESIS SUBMITTED TO THE AVINASHILINGAM INSTITUTE FOR HOME SCIENCE
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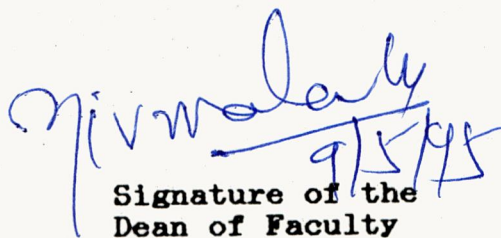
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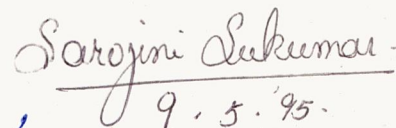
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Introduction

EFFECT OF ENRICHMENT OF MULBERRY LEAVES WITH SOYA FLOUR,
ORABOLIN, AN ANABOLIC STEROID AND VITAMIN B COMPLEX ON THE
THE GROWTH AND DEVELOPMENT OF SILKWORM, BOMBYX MORI L.

INTRODUCTION

Sericulture is one of the ancient cottage industries of India, well established from 4th century A.D. onwards. As an agrobased cottage industry, sericulture encompasses multivarious activities starting from raising of mulberry plantation, rearing of silkworms, harvesting of cocoons, reeling of silk and ending with finished silk fabric. The important factors for the success of this industry are local suitability, agronomical conditions of the place and socio economic status of the people adopting this income generating activity.

Sericulture industry in India has grown impressively over the past four decades. The total area for mulberry cultivation has increased 5.8 times, cocoon production by 10 times and raw silk production by 18.7 times. (Benjamin,1993). The export of silk from India has steadily increased at the rate of 25 percent per annum and the net income from export ~~prime~~ has increased from Rs.111 crores in 1983-84 to 440 crores in 1990-91. The raw silk output was about 15,000 tonnes in 1993 fetching about 700 crores through exports and the target set for 1994-95 is Rs.1000 crores.

Sericulture can be considered as one of the alternatives to be provided to the rural people for sustainable economy. It has the capacity to generate substantial and gainful employment in rural areas with very little capital investment. Therefore, it is considered as a sustainable economic activity positively favouring rural poor in the unorganised sector also.

In India, sericulture has grown in all dimensions covering more than 2,42,000 hectares of mulberry spread over 52,000 villages. About 5.8 million people are employed in various operations with a product value of more than 900 crores of rupees per annum.

The Government of India has accepted sericulture as a component for rural employment and rehabilitation of weaker sections, especially for scheduled castes and scheduled tribes. Therefore, efforts have been taken to popularize sericulture among small and marginal farmers and also among the unemployed educated youth.

India holds an unique place in the world silk market as it supplies all the four commercial varieties of silk- mulberry silk from the Mulberry silkworm, Bombyx mori, eri silk from Eri silkworm, Philosamia ricini, tasar silk from the Tasar silkworm, Antheraea paphia and muga silk from the Muga silkworm, Antheraea assamia.

In mulberry silk production, India maintains second position with a share of 14% of global production. In tasar silk production also, India stands as the second largest producer. With respect to golden yellow muga silk production, India monopolises the market.

Mulberry sericulture is popular in the states of Karnataka, TamilNadu, Andhra Pradesh, Jammu and Kashmir and West Bengal. With the development of new technologies and impetus given by the Central Silk Board and State Sericulture Boards, it is spreading to other states such as Maharashtra, Kerala, Gujarat, Rajasthan, Bihar and Orissa. Mulberry sericulture industry was originally concentrated in the districts of Dharmapuri, Salem, Coimbatore and Nilgris in TamilNadu. Now it is slowly spreading to other districts such as Madurai, Thanjavur, North Arcot and Anna districts (Umapathy et al., 1991).

The mulberry silkworm, Bombyx mori L. (Lepidoptera: Bombycidae) is an oligophagous insect mainly feeding on mulberry leaves (Horie and Watanabe, 1969). The ease with which this silkworm can be handled and reared makes it the most suitable material for laboratory research.

Nutrition is the most important growth regulating factor in silkworm, Bombyx mori. Being a monophagous insect, it derives all the nutrients essential for its growth from the mulberry leaves itself.

In countries like India where about 50% of the total mulberry acreage is under rainfed condition and where often rain fails or is delayed, assured irrigation becomes a distant dream of many farmers. Apart from this, the soil conditions differ from place to place in terms of available nutrients. Under such situations, quality leaf production will be hindered which in turn may affect the production of quality silk.

Therefore, attempts are made by sericulture scientists not only to increase the yield of mulberry leaves, but also to improve the quality by enriching mulberry leaves with different agents of nutritional value for providing a well balanced food for the silkworm to obtain better yield of silk.

In developed countries like Russia, Japan and China, efforts are made to formulate artificial diet for the silkworm. The initial attempt for artificial diet for the silkworm, Bombyx mori was first made in Russia in 1943. In Japan the silkworms were raised in artificial diet in the laboratory in 1960 and in the field in 1978 (Fukuda et al., 1960) in order to prevent crop (cocoon) losses and for better growth. Later, studies had been undertaken to reduce the cost of artificial diet (Horie and Watanabe, 1983 a). Many ingredients such as potato starch, soya bean powder, sucrose, cellulose etc., were abundantly used in the formulations of artificial diet.

In India, scientists have been focussing their attention on improving the quality of mulberry leaves through foliar sprays of macro and micronutrients (Murthy, 1953; Quiyyam et al., 1989; Samsijah, 1989 and Quader et al., 1990)

Several researchers have attempted to study the effect of enrichment of mulberry leaves on the growth of larvae and cocoon parameters of silkworm. Enrichment of mulberry leaves with milk powder casein, egg protein, glycine, ascorbic acid, folic acid and glucose has been tried by Sengupta et al., 1972, and with soya protein (Subba Rao et al., 1989 and Subburathinam et al., 1992). Importance of vitamins in silkworm nutrition has been reported by Legay, 1958; Horie and Ito, 1963; and Rajendran et al., 1993. A wide range of vitamins such as B₆ (Hamano, 1989 a&b), B₁₂ (Murthy, 1953) and ascorbic acid (El-Karaksy et al., 1990 and Babu et al., 1992) have been used to enrich the mulberry leaves for silk worms.

In the present investigation, an attempt was made to enrich mulberry leaves with soya flour, a combination of soya flour with graded dosages of orabolin, an anabolic steroid and a combination of soya flour, anabolic steroid and vitamin B complex. The effect of these dietary supplements on the growth and development of the silkworm, Bombyx mori L. was studied.

OBJECTIVES:

The objectives of the present study are as follows:

1. To study the effect of the soya flour on the growth and development of silkworm.
2. To assess the combined effect of the soya flour and orabolin (Ethylesterenol), an anabolic steroid in different dosages on the growth and development of silkworm.
3. To study the effect of a combination of soya flour, orabolin and vitamin B complex on the growth and development of silkworm.
4. To study the food ingestion, digestion and the approximate digestibility of silkworm.
5. To analyse and find out the optimal dose of these supplements that can be used for fortification of mulberry leaves in order to improve the quality of cocoons and enhance the silk yield.

Review of Literature

II REVIEW OF LITERATURE

Legay (1958) is of the opinion that the field of silkworm nutrition together with silkworm genetics is one of the major areas of research in sericulture, for, from the scientific point of view, the silk production in the world depends on them. While on one hand, attempts have been made to develop artificial and semi-artificial diet for silkworm, on the other hand studies have been carried out to enrich mulberry leaves with specific nutrients in order to improve larval and cocoon characters and thereby increase silk yield. The review of literature pertaining to the study on the effect of enrichment of silkworm feed on the growth and development is presented under the following headings:

II. 1. General.

1.1 Enrichment through foliar spray.

1.2 Enrichment through artificial diet.

II 2. Enrichment of leaves with specific nutrients.

2.1 Enrichment with aminoacids and proteins.

2.2 Enrichment with soya flour and other flours.

2.3 Enrichment with vitamins.

2.4 Enrichment with steroids and hormones.

2.5 Enrichment with other chemicals and antibiotics.

II. 3. Studies on digestibility and reference ratio.

II. 1. General:

Legay (1958), while reviewing nutrition of silkworm stated that the silk production is dependent on larval nutrition and the nutritive value of mulberry leaf plays a very effective role in producing good quality cocoons.

Petkov and Yolov (1979), observed that the lower nutrition level has a considerable effect on the genetic diversity of such characters as cocoon weight, silk cover weight, silkiness and length of cocoon thread and the value of heritability co-efficients decreased regularly with the reduction in nutrition rates.

II. 1.1. Enrichment through foliar spray:

The foliar application of plant nutrients was reported to be a reliable supplemental method by Afridi and Wasiuddin (1979).

Murthy (1953), recorded improvement in the quality of silk in foliar spray of mulberry leaves with ammonium nitrate.

Dasmahapatra et al. (1989) reported increased larval weight, silk gland weight, protein content, RNA and DNA contents in the silk glands of silkworms fed on mulberry leaves treated with potassium iodide, cobalt chloride and potassium nitrate.

Viswanath and Krishnamurthy (1981), have recorded improved cocoon weight and yield with foliar spray of magnesium.

II. 1.2 Enrichment through artificial diet:

The initial attempts to formulate an artificial diet for silkworm, Bombyx mori was made first in Russia in 1943 (Philip, 1990).

The silkworms were raised on artificial diet in the laboratory in 1960 and also in the field in 1978 in Japan (Fukuda et al., 1960) to prevent crop losses and for better growth. Later studies were taken upto reduce the cost of artificial diet (Horie and Watanabe, 1983 a).

The formulation of artificial diet included common ingredients such as potato starch, sucrose and cellulose. Fukuda (1960) succeeded in rearing B. mori upto spinning on artificial diet. This artificial diet was composed of powdered mulberry leaves, potato starch, soya bean powder and frozen soya-bean curds, with an addition of 20 percent sucrose solution and 10 ml of inhibitory solution.

Later in 1962, Fukuda et al. tried to replace leaf powder with cellulose powder, and prepared artificial diet with potato starch, sucrose and other nutrients such as amino acids, vitamins and minerals. A fourfold increase in larval weight was observed. However, Nihmura et al. (1978) was of the opinion that inclusion of mulberry leaf powder or mulberry leaf extract in the artificial diet is highly desirable to stimulate the feeding behaviour of the newly hatched silkworm larvae.

Ito (1961) reported improvement in the growth and development of larvae of silkworm by the addition of ascorbic acid to artificial diet, one of the effects being feeding stimulatory action.

Ito and Arai (1967) reported that single addition of alanine, glycine and serine to the basal diet containing essential amino acids alone, or essential acids plus aspartate, improved larval growth and development.

Watanabe and Imanashi (1980) found that the silkworm larvae fed on artificial diets rich in protein tended to be less susceptible to infection with NPV (Nuclear Polyhedrosis Virus).

Kamada and Ito (1984) pointed out that the silkworm larvae receiving a methanol extract from sea weeds (such as wakame) yielded larger cocoons. And also the addition of 0.04 ppb indole acetic acid to the artificial diet increased the body weight of the larvae.

Hou and Chen., 1984, found that the B. mori larvae fed with spiral algae, Spirulina platensis was slightly better for the efficiency of conversion of ingested diet into body substance and the cocoon production. Spiral algae is dietetically utilizable as a protein source for rearing silkworm is smaller quantities than on a soyabean meal.

Yanagawa et al. (1989) observed that the feeding ability and survival rate of polyphagous strains increased, when 5% of mulberry leaf powder was added to the artificial diet which contained fish meal, yeast, pupal powder, rape bran, gluten meal and alfa alfa.

Ovesenska et al. (1992) reported that the artificial nutrient mixture containing 40% mulberry meal and 0.15% sorbic acid promoted normal silkworm development of the hybrids.

Thakur and Harishkumar (1992) evaluated the effect of artificial diet on Bombyx mori belonging to the bivoltine race NB D X K .
4 2 A

Ito and Inokuchi (1992) while studying the nutritive effects of asparagine and glutamine on Bombyx mori found that dietary asparagine supported larval growth but, glutamine was less effective than asparagine.

II. 2. Enrichment of leaves with specific nutrients:

In nutritional studies of the silkworm, supplementation of specific nutrients has often been tried. Studies indicate that supplementation to the spring leaves scarcely improved the larval survival, cocoon quality or yield. While supplementation to autumn leaves or to shaded leaves yielded some what better results (Yokoyama,1963). Carbohydrates, amino acids, vitamins, minerals, and lipoidal substances have been used in these studies.

II. 2.1 Enrichment with amino acids and proteins:

Sharda et al., 1956, observed that glycine and chloromycetin through leaf supplementation and parenteral introduction had improved larval growth in silkworm.

Sengupta et al. (1972) reported that mulberry leaves enriched with glycine increased growth and silk production upto 40 to 60%.

Attempts were made to enrich mulberry leaf by the addition of three proteins (casein, egg substance and milk powder), one amino acid (glycine), two vitamins (ascorbic acid and folic acid) and two sugars (glucose and molasses) individually. Results indicated good effect of feeding atleast with six of them (excepting egg substances and molasses) resulting in better growth of silkworm and higher silk production (Sengupta et al., 1972).

Horie and Watanabe (1983 c) observed a rise in haemolymph protein and a drop in uric acid excretion in supplementation of gluten and zein (protein) with their limiting amino acids. The amino acid patterns in the haemolymph were found to be greatly changed according to supplementation.

Mathavan et al. (1984) reported significant improvements in the economic characters such as cocoon, pupal and shell weights in the larvae fed with mulberry leaves supplemented with Spirulina fusiformis as a source of single cell protein in comparison with the normal leaf fed larvae.

Krishnappa (1987) reported that the supplementation of glycine at 15 percent at a frequency of twice daily in fourth and fifth instars was helpful in improving the rearing, cocoon and grainage parameters.

According to Ito and Inokuchi., 1992, the amides (asparagine and glutamine) are utilized fairly well by Bombyx mori.

Roy choudhury et al. (1992) stated that the nutritional enrichment of mulberry leaves, Morus alba L. with carbohydrates, sucrose (LR) and commercial sugar to the Bombyx mori L. yielded better results.

II. 2.2 Enrichment with soya flour and other flours:

Ito and Nakasone., 1966, demonstrated that utilization of B - sitosterol by the silkworm, Bombyx mori L. was highly accelerated in the presence of a mixture of soyabean fatty acids or of stearic acid. The best larval growth and development were obtained with a combination of appropriate amounts of sterol and fatty acids.

Miyoshi (1975) reported that fortified foods based mainly on soya bean and sugar, etc., and applying them to mulberry leaves, produced high quality cocoons. He also found that soya bean flour was very effective.

According to Park and Park (1986), soya bean is the richest among pulses containing about 40 percent protein, 20 percent fat, and 4 percent minerals. The proteins of soya bean are of relative high nutritive value.

Subbarao et al. (1989) observed that mulberry leaves enriched with soyabean meal in ten different concentrations (1 to 10%) and fed to the 5th stage larvae resulted in improving qualitative and quantitative characters of cocoon and fecundity. They also found that lower concentrations (1 to 3%) of soyabean meal were found more effective than higher concentrations.

Ganga and Gowri, (1990) tried supplementation with three cereal flours viz., rice, wheat and ragi powder and recorded maximal shell ratio and larval weight in rice powder supplemented groups.

Fortification of mulberry leaves with red gram and black gram flour and its effect on larval and cocoon parameters was investigated by Alagumalai et al. (1991 a). Maximum improvement in larval and silk gland weight and cocoon parameters was recorded in red gram flour treatment. Number of eggs laid was also found to be maximum in fortification with red gram flour.

Subburathinam et al. (1992) recorded improvement in larval and cocoon characteristics in silkworm Bombyx mori in soyabean protein treatment.

II. 2.3 Enrichment with vitamins:

Murthy (1953) showed that ascorbic acid was effective for increasing the survival of B. mori.

Shymala and Bhat , 1958, from the experiment on growth inhibition with antimetabolites concluded that nicotinic acid and pyridoxine are necessary for silkworm.

Legay (1958) recorded that the mulberry silkworm needs a dietary source of vitamin C.

Ito (1961) proved that the presence of ascorbic acid (vitamin) is necessary for better growth and development of the silkworm B. mori.

Horie and Ito (1963) found that the quantity of Riboflavin, Niacin and Pyridoxine increased during autumn and the quantity of Pantothenic acid and Biotine decreased during spring season in mature mulberry leaves and any one of the above said vitamin B deficiency paved the way to cent percent mortality in I and II instar and also retardation of growth and development in late-age silkworms

Horie and Ito (1965) suggested that without vitamin B mixture in the diet all the larvae will die before or after reaching second instar, while addition of vitamin B mixture enable the larvae to reach fifth instar in 25 days.

Ito and Arai (1965) reported that the absence of ascorbic acid in the diet of first and second instars of B. mori resulted in poor growth and development from third instar onwards.

Sengupta et al. (1972) reported that the silk content was more with 1% ascorbic acid in the diet of silkworms.

Miyoshi (1975) stated that only vitamins absolutely necessary for silkworms are those of the B group.

Feeding of selected doses of thyroxine, vitamin B12 and some salts influenced the growth, metamorphosis, silk production, silk gland metabolism and egg production of silk worms, Bombyx mori L., race Nistari (Chakrabarti and Medda, 1978 & Medda et al., 1980).

Nihmura et al., 1978, found that the improvement in both mineral and vitamin B mixtures had markedly increased the percentage of reelable cocoons and the weight of reelable cocoon layers.

Gomaa et al. (1977) remarked that the presence of 90.0 mg of vitamin C in the diet gave the highest number of emerged adults, the maximum longevities, the heaviest weight of adults of both sexes and the highest number of deposited eggs.

Horie and Watanabe, 1983 b, found that under pyridoxine-deficient conditions, growth of larvae and silk glands was retarded, being accompanied by an inhibition of the increase of haemolymph protein content and an increase in uric acid excretion. The pyridoxine requirement was larger for the growth of silk glands than that for the whole body.

El-Karaksy et al. (1985) showed that vitamin C increases the silk yield of the mulberry silkworm.

Horie and Nakamura (1986) reported that the enzyme activities were increased with increasing levels of dietary pyridoxine.

Hamano (1989), pointed out that in the silkworm reared on the diets containing 10 to 50 percent of protein in combination with 0 to 10 μ g of pyridoxine (B6) per g of the dry diet, not only improved the silk production but the utilization of the diet. The highest cocoon weight was obtained with the diet containing 30 percent protein, whereas, for cocoon, shell weight and cocoon-shell ratio the 50 percent protein diet resulted in the highest value.

Hamano and Okano (1989) observed that the larval body weight of a hybrid race was the largest when reared with the diet containing 30 % protein and 10 μ g of B₆.

El-Karaksy and Idriss (1990), concluded that the fifth instar larvae of B.mori fed on mulberry leaves treated with ascorbic acid resulted in increased weights of both larvae and pupae and produced heavier fresh cocoons. Ascorbic acid proved to exhibit a significant effect on the increasing of the fecundity of emerged females.

Bajpeyi et al., 1991, reported that vitamin B, if supplemented to the diet can increase the body weight and also the disease resistance of the worms.

Babu et al. (1992), remarked that the first and second instar silkworm larvae reared on 1.5% ascorbic acid-enriched mulberry leaves resulted in higher silk length, weight and denier values.

Chauhan and Singh (1992) found that the ascorbic acid enhances the egg-laying capacity of the female mulberry silk moth when fed to silkworm larvae at a 10% concentration in later stages, in the form of an aqueous solution sprayed on mulberry leaves.

II. 2.4 Enrichment with steroids and hormones:

Literature on the effect of steroids on silkworm are limited.

Fujimoto et al., 1984, observed that all of the tested steroids effectively supported the growth and development of the silkworm and were converted into cholesterol regardless of the C-24 configuration.

Anabolic steroids are synthetic androgens with higher anabolic and lower androgenic activity and are reported to promote protein metabolism (Satoskar et al., 1995). Lawrence and Bannet (1992) described anabolic steroids as effective protein anabolic agents and these are reported to increase muscle mass, strength and endurance (Schwartz and Miller, 1990 and Tripathy, 1994).

Studies on the anabolic steroid durabolin had been carried out by Sita et al., (1981) on Dysdercus similis and this steroid is reported to have juvenoid like effects.

Thyagaraja et al. (1993) reported the ~~vertebrate~~ feeding of hormone thyroxine in silkworm improved yolk deposition and accelerated oocyte maturation.

II. 2.5 Enrichment with other chemicals and antibiotics:

Cobalt was found to exert favourable effect on the growth of silkworm (Takahashi, 1955 & Sridhara and Bhat, 1966). Cobalt as a mineral nutrient might have enhanced the silkworm growth and cocoon crop production.

Chakrabarthi and Medda (1978) observed an increase in body weight and in the number of eggs laid by the adult of B. mori when larvae were fed with mulberry leaves soaked in cobalt chloride solution.

Voronkov et al. (1978) reported that larvae feeding on leaves treated with 1-ethoxy silatran (I), 3, 7, 10 - trimethyl-1-ethoxy silastran (II) or 1- Silastranyl- methyl isothiuron - chloride or water as a control once a day from the 2nd instar responded with increased larval weight and weight of the cocoon membranes.

According to Pai et al. (1987), para amino benzoic acid (PABA) at 50,100, 200 and 400 ppm concentrations when administered to 3rd instar larvae of silkworm, (Bombyx mori L.) by leaf dip method resulted in marked improvement of the silk percentage at low concentrations of 50 - 100 ppm.

Radhakrishna Rai (1987) reported that the antibiotic treatments with chloramphenicol and tetracycline increased

the cocoon yield in terms of number and weight and also reduced the incidence of flacherie diseases other than cytoplasmic polyhedrosis.

Increase in larval and cocoon characters and fecundity was observed in silkworm fed with abrimax and doxt combinations (Alagumalai et al., 1991 b).

Magadum (1991) found that the feeding of mulberry leaves, soaked separately in 15,30 and 45ppm of potassium iodide (KI), to the silkworm larvae from the beginning of III instar to the end of feeding period in V instar significantly increased larval weight, silk gland weight and female cocoon shell weight.

According to Sailaja et al. (1991) silkworm, Bombyx mori (L) treated with antibiotic terramycin showed a stimulating effect on the growth and other economic traits except fecundity.

Srinivas and Purushotham Rao (1992) reported that phosphomidon was found to be more effective on V instar larvae followed by IV and III instar larvae. Decrease in larval weight, length, cocoon weight, pupal weight, shell weight, length of the filament, reeled silk weight, silk ratio and denier was observed in all the treated larval instars.

Chamundeswari and Radhakrishnaiah (1994) recorded an increase in the fresh and dry weights of the larvae and silk gland, cocoon length, width and weight, shell weight, cocoon-shell ratio and filament length and weight in larvae fed with zinc or nickel sprayed mulberry leaves (1mg/10g wet wt) once in each instar upto the fourth and once in alternate days in the fifth. The larval duration also decreased. The results indicate that zinc and nickel act as supplements to stimulate the silk synthesis and of the two elements, the role of zinc is more prominent.

II. 3. Studies on digestibility:

According to Legay (1958), the quality of mulberry leaves has great influence on the amount ingested. Not only the physico-chemical characteristics in relation to the age of the leaf, but also the characteristics of mulberry varieties affect the amount ingested.

Legay (1955), reported that the co-efficient of digestive utilization (C.D.U) and the ratio of gain weight to food retained weight (R.W) are very much affected by the food quality and they may be estimated in fresh or in dry weight.

Several workers (Hiratsuka, 1920; Matsumura and Takeuchi, 1950; Matsumura et al., 1955; Takeuchi et al., 1964; Ueda, 1965 and Ueda and Suzuki, 1967) have studied the food ingestion, digestion and utilization of mulberry leaves by the silkworm B.mori.

The quantity of food ingested and digested by silkworms reared on mulberry leaf and artificial diet and the efficiency of food utilization have been worked out on dry weight basis by Takeuchi and Kosaka (1962) and Horie and Watanabe (1969). Ito and Arai (1963) described the relationship between the mulberry variety and larval growth.

Remadevi et al. (1991) have evaluated the food utilization efficiency in some polyvoltine breeds of silkworm, Bombyx mori.

Paul et al. (1992) reported on the impact of dietary moisture on nutritional indices and growth of Bombyx mori and found a positive correlation between dry matter consumed and digested, efficiency of conversion of ingested food (ECI), efficiency of conversion of digested food (ECD) and larval weight and increased moisture content of the leaves.

Remadevi (1993) observed mutual correlation between the nutritional and economic characters of multivoltine silkworms.

Anantharaman et al. (1993) reported on the nutritional efficiency in silkworm through their studies on consumption, digestion, utilisation, ECI and ECD values.

Sarkar and Fujita (1994) studied the utilization of different mulberry varieties by 5th instar silkworm larvae and recorded the digestibility, ECI and ECD for different

varieties of mulberry and evolved a formula for calculating PECS (Production Efficiency of Cocoon Shell) which is considered to be the final indicator for the evaluation of the value of the mulberry leaves.

Methodology

III MATERIALS AND METHODS

An investigation was undertaken to find out the effect of enrichment of mulberry leaves on the growth and development of silkworm, Bombyx mori L. [Lepidoptera : Bombycidae]. The methods involved and the materials used in this study are given in detail here:

III 1. Test Insect:

Egg cards of Bombyx mori L. (PM x NB D) obtained from the State Sericulture Board, Coimbatore - 37, were used in this study.

III 2. Silkworm rearing:

The rearing room along with the rearing appliances were thoroughly disinfected using 2 percent formalin and bleaching powder. An optimum temperature of 25° C and 75 percent relative humidity were maintained throughout the experimental period. Mild dettol solution was used to wash the hands before and after handling the worms during rearing.

The eggs were kept for incubation and the newly emerged larvae were fed with Kanva - 2 (M5) variety mulberry leaves.



Fig.1 Field View

Box-rearing method was adopted for rearing the larvae upto the 3rd instar. The floor of the rearing trays were lined with paraffin paper and the edges with wet foam rubber strips. The rearing trays were covered with paraffin sheets in order to maintain the required relative humidity and temperature and to keep the leaves fresh and moist. These rearing trays were in turn placed on the rearing stand with their legs resting in ant wells. When the larvae reached the fourth instar, the paraffin paper and foam strips were removed and the larvae were transferred to bamboo trays lined with newspaper. During the entire procedure of rearing, fresh mulberry leaves of M variety were collected in the early morning and evening from the field (Fig.1) and stored in wet gunny bags. The larvae were fed four times a day, at 6 hours, 11 hours, 15 hours and 20 hours with chopped mulberry leaves according to the age of the larvae. Enough care was taken to maintain the optimum temperature and humidity. Bed cleaning and spacing were adopted carefully following the methods of Krishnaswami (1978).

III 3. Experimental set up:

The newly moulted larvae do not feed vigorously for 24 hours. The enriched mulberry leaves were supplied to the fifth instar larvae, everyday at 11'0 clock feeding.



Fig.2 Wooden stand with Bamboo trays



Fig.3. Control & treated leaves

The experiment consisted of six treatments, each treatment with five replications of thirty worms. Each replication was maintained in small circular bamboo trays which in turn were placed in bigger circular bamboo trays and kept on wooden stand (Fig.2).

Soya flour, orabolin (anabolic steroid) and vitamin B complex tablets were used for this study. The treatments consisted of 5th instar larvae, fed with the following:

Control: Fresh mulberry leaves (Fig. 3).

Treatment - 1 : Mulberry leaves dusted with soya flour (Fig.3).

Treatment - 2 : Mulberry leaves dipped in 20 ppm orabolin solution, partially air dried and dusted with soya flour (Fig.3).

Treatment - 3 : Mulberry leaves dipped in 40 ppm orabolin solution, partially air dried and dusted with soya flour.

Treatment - 4 : Mulberry leaves dipped in 60 ppm orabolin solution, partially air dried and dusted with soya flour.

Treatment - 5 : Mulberry leaves dipped in 60 ppm orabolin and 20µg of vitamin B complex, partially air dried and dusted with soya flour.

III. 4 Mode of application:

The experiment bed was cleaned thoroughly before feeding. The approximate quantity of leaves required for a single feed was weighed and used for the experiment. 1 gm of

soya flour was dusted through fine cloth on to the leaves of treatment-1. For the other treatments, the weighed leaves were dipped in different concentrations of orabolin solutions and orabolin and vitamin B complex solution and dusted with 1 gm of soya flour.

III. 5 Observation on approximate digestibility and reference ratio:

Every day at 11.00 hours, known quantity of control mulberry leaves and treated mulberry leaves were provided to the 5th instar larvae. Equivalent dry weights of treated and untreated leaves were determined by oven drying at 60 °C for 24 hours. Before 15.00 hour feeding, the residual leaves and excreta were removed and oven dried. The method of Nath et al. (1990) and Paul et al. (1992) was followed, for studying the ingestion, digestion of food and approximate digestibility. For calculating the approximate digestibility, the following formula was used

$$A.D = \frac{100 (E - F)}{E}$$

E - Dry weight of ingested food

F - Dry weight of faeces left

Reference ratio was calculated by the formula:

Ingesta
.....
Excreta

III. 6 Observations on the larval and cocoon parameters:

III. 6.1 Larval weight:

The weight of ten randomly selected larvae from each replication was taken on the sixth day of fifth instar using an electronic balance.

III 6.2 Cocoon Characters:

The mature 5th instar larvae were picked up from rearing trays and released on chandrikas for spinning the cocoons. The cocoons were harvested after 5 to 6 days of spinning. Assessment of various cocoon parameters were made.

a. Cocoon weight:

Ten randomly selected cocoons were taken and weighed using an electronic balance and the weight was expressed in grams.

b. Cocoon length:

The length of the cocoon was measured by using the vernier caliper and the length was expressed in centimeters.

c. Cocoon width:

The width of the cocoon was taken for ten randomly selected cocoons from each replication using the vernier caliper. The measurement was expressed in centimeters.



Fig. 4. Mating behaviour of the silkworm, B.mori

d. Floss weight:

The floss of the cocoon was removed from the cocoons which were taken randomly from each replication. Then the floss was weighed in an electronic balance and the weight was expressed in grams.

e. Pupal weight:

After removing the floss, the cocoons were cut open and the pupae were taken out without causing any damage to them. Then the pupae were weighed using the electronic balance.

f. Shell weight:

The shell of the cocoon after removing the floss and pupae was weighed using the electronic balance.

g. Silk ratio:

Silk ratio was calculated using the following formula and expressed in percentage:

$$\text{Silk ratio} = \frac{\text{Shell weight}}{\text{Cocoon weight}} \times 100$$

III 6.3 Fecundity:

In order to assess the fecundity, the remaining cocoons in the chandrika were allowed to mature and develop. The male and female moths emerging out of the cocoons were collected and allowed to mate (Fig.4).

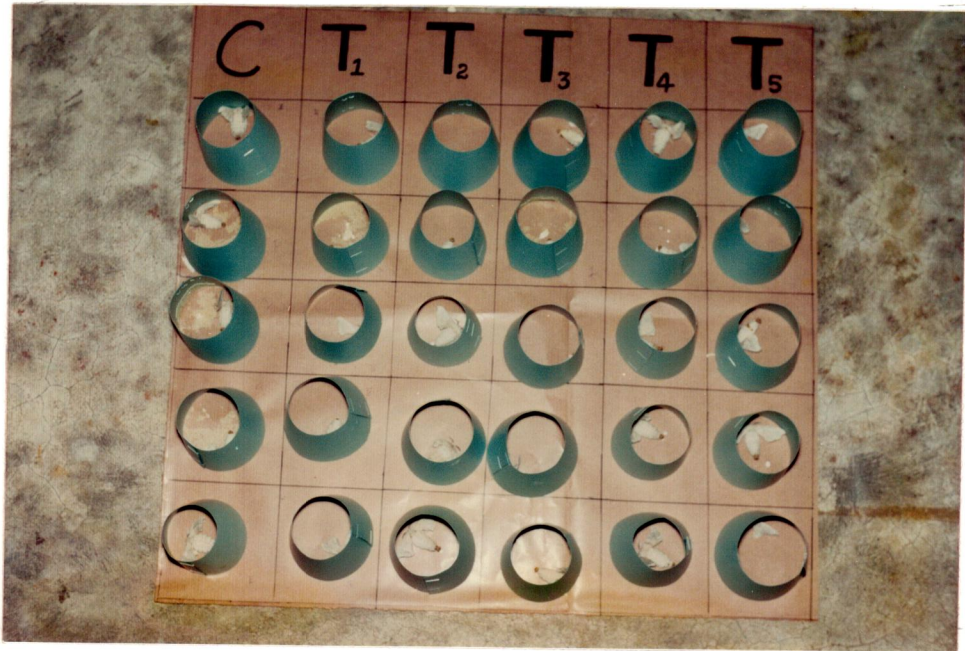


Fig. 5. Moth funnels with individual female moths



Fig. 6. Eggs laid by individual female moth

After 3 hours of mating, the female moths were separated from the male moths and left on waste papers individually to urinate. After urination, each female moth was left in individual moth funnels (Fig.5) on the egg laying sheets, undisturbed for 2 days. After 2 days, the moths were removed and the eggs laid by individual females (Fig. 6) were counted.

III 6.4 Statistical analysis:

The data collected from various observations were statistically analysed using the method of analysis of variance suggested by Panse and Sukatme (1985).

Results and Discussion



Fig. 7. Larvae fed with control mulberry leaves



Fig. 8. Larvae fed with soya flour treated mulberry leaves

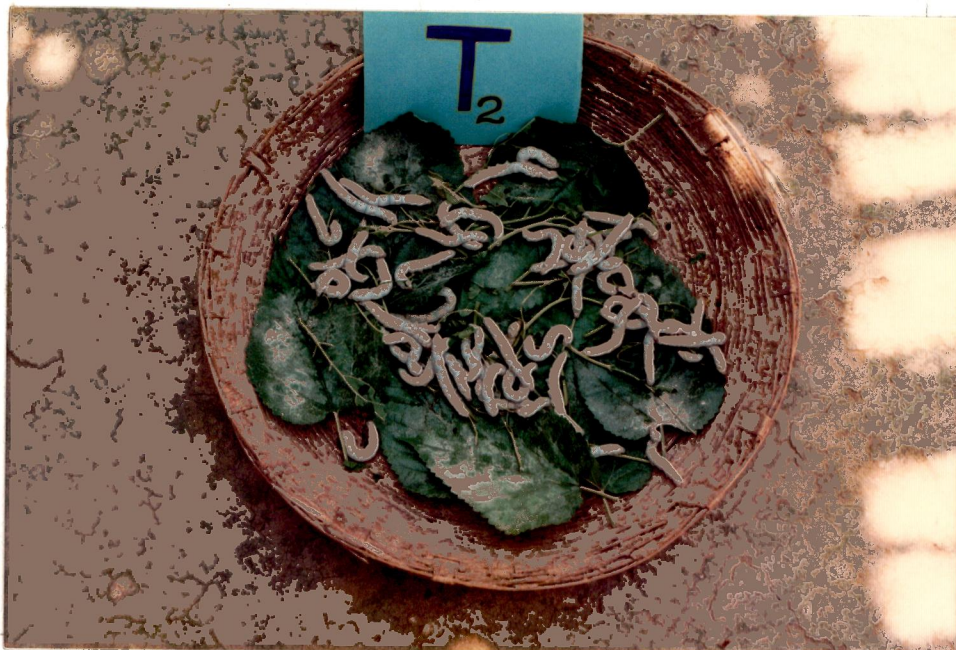


Fig. 9. Larvae fed with soya flour + 20 ppm orabolin treated mulberry leaves



Fig.10. Larvae fed with soya flour + 40 ppm orabolin treated mulberry leaves



Fig.11. Larvae fed with soya flour + 60 ppm orabolin treated mulberry leaves



Fig.12. Larvae fed with soya flour + 60 ppm orabolin + vitamin B complex treated mulberry leaves

IV RESULTS

The results pertaining to the present study, "The effect of enrichment of mulberry leaves with soya flour, orabolin, an anabolic steroid and vitamin B complex, on the growth and development of silkworm, Bombyx mori L. are presented here. (Fig. 7-12).

IV.1. Effect on larval weight:

Statistical analysis of the data on larval weight was found to be highly significant. (Table I and figure 14). The mean larval weight was maximum (3.84 gm) in treatment with soya flour +40 ppm orabolin, followed by treatment with soya flour +60 ppm orabolin (3.78 gm) and treatment with soya flour +60 ppm orabolin +B complex (3.71 gm). Minimum larval weight was recorded in treatment with soya flour alone (3.41 gm).

IV.2. Effect on cocoon weight: (Fig.13)

The results pertaining to cocoon weight was found to be statistically significant (Table I and fig.15). Maximum cocoon weight (2.03 gm) was observed in treatment with soya flour +20 ppm orabolin, followed by control (1.94gm) and treatment with soya flour +60 ppm orabolin +B complex (1.91 gm).

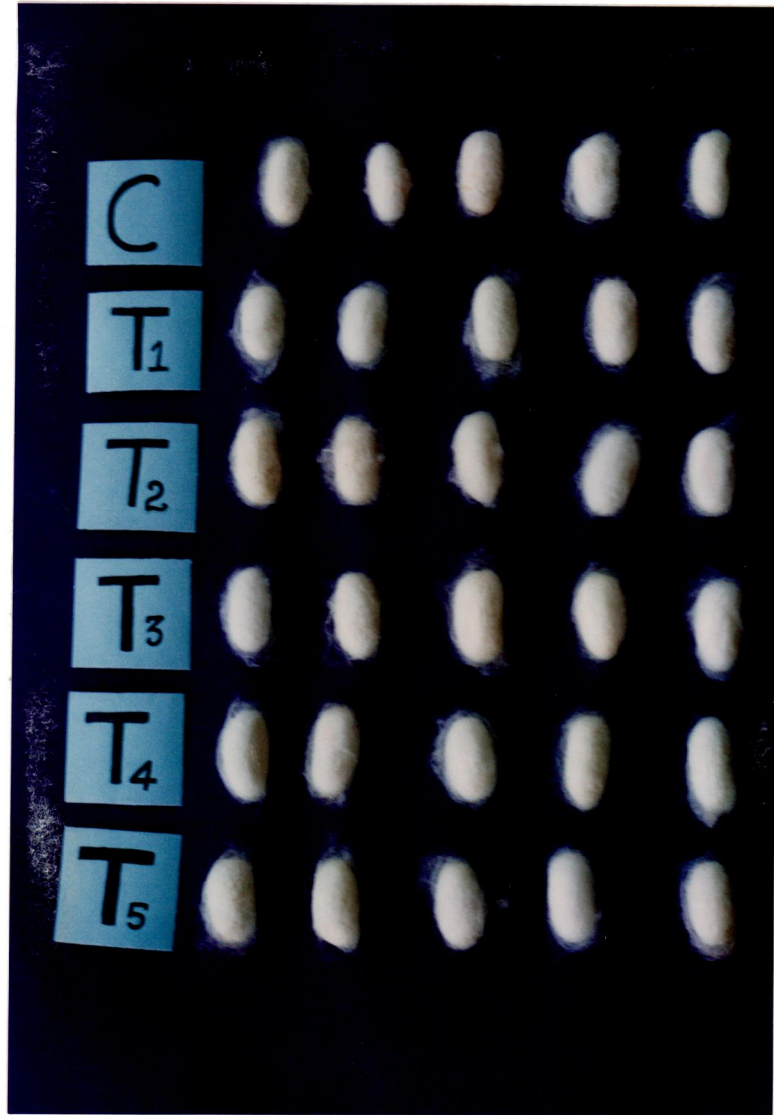


Fig.13. Cocoons harvested from larvae of control and different treatments

TABLE I

EFFECT OF ENRICHMENT OF MULBERRY LEAVES WITH SOYA FLOUR AND TEST
CHEMICALS ON LARVAL WEIGHT AND COCOON PARAMETERS OF SILKWORM, ^Bombyx
moriL.

TREATMENT	MEAN LARVAL WEIGHT (g)	MEAN COCOON WEIGHT (g)	MEAN COCOON LENGTH (cm)	MEAN COCOON WIDTH (cm)
C	3.69	1.94	3.67	1.88
T1	3.41	1.88	3.66	1.82
T2	3.70	2.03	3.61	1.88
T3	3.84	1.74	3.62	1.81
T4	3.78	1.72	3.63	1.84
T5	3.71	1.91	3.57	1.87
F	**	**	N.S	N.S
S.E _±	0.091	0.058	0.067	0.050
CD	0.247	0.156	0.181	0.135
(P=0.05)				

** : Significant at 1% level

N.S: Non-Significant

Fig. 14. Effect of enrichment of mulberry leaves with soya flour and test chemicals on larval weight of silkworm, Bombyx mori L.

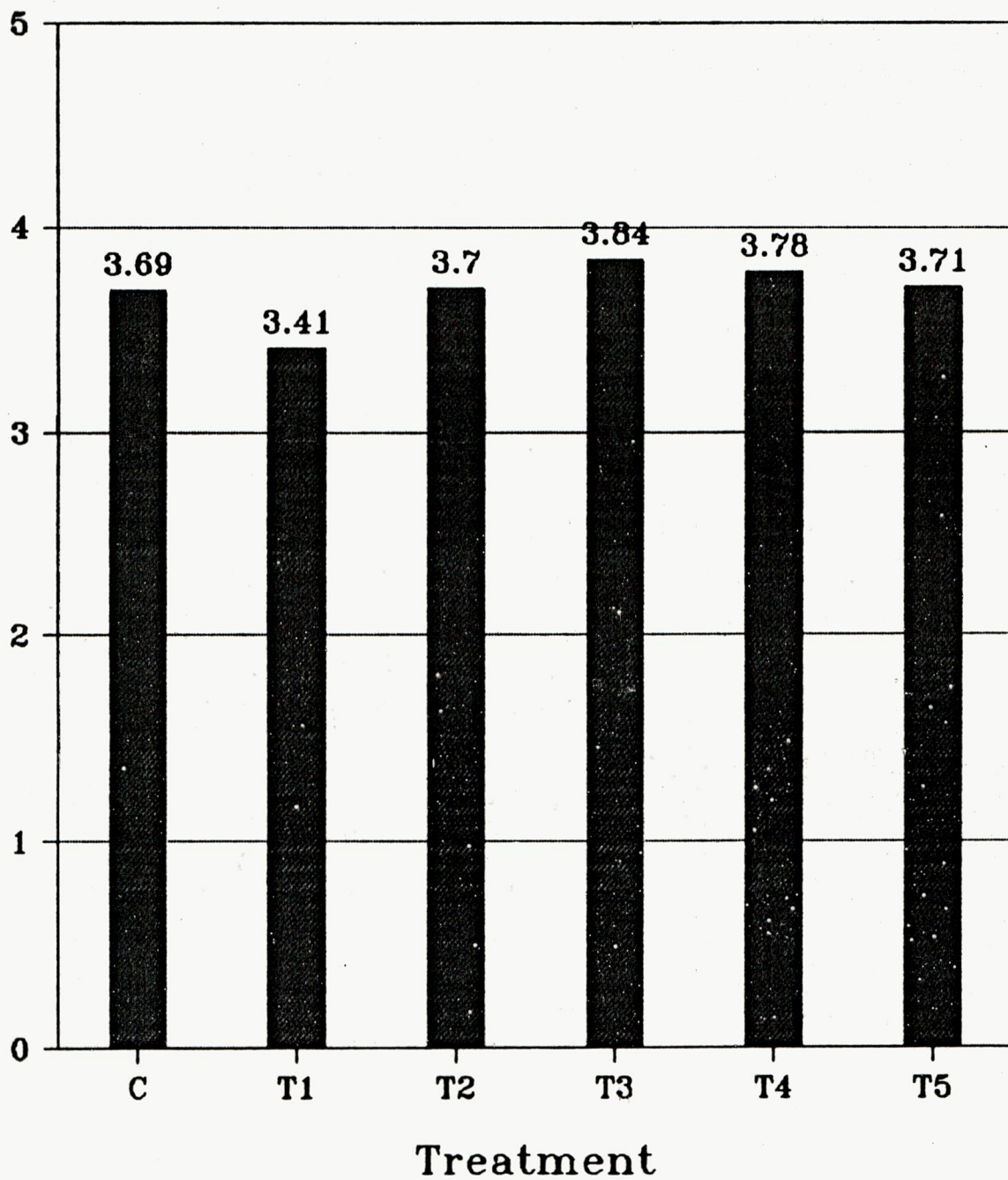


Fig. 15. Effect of enrichment of mulberry leaves with soya flour and test chemicals on cocoon weight of silkworm, Bombyx mori L.

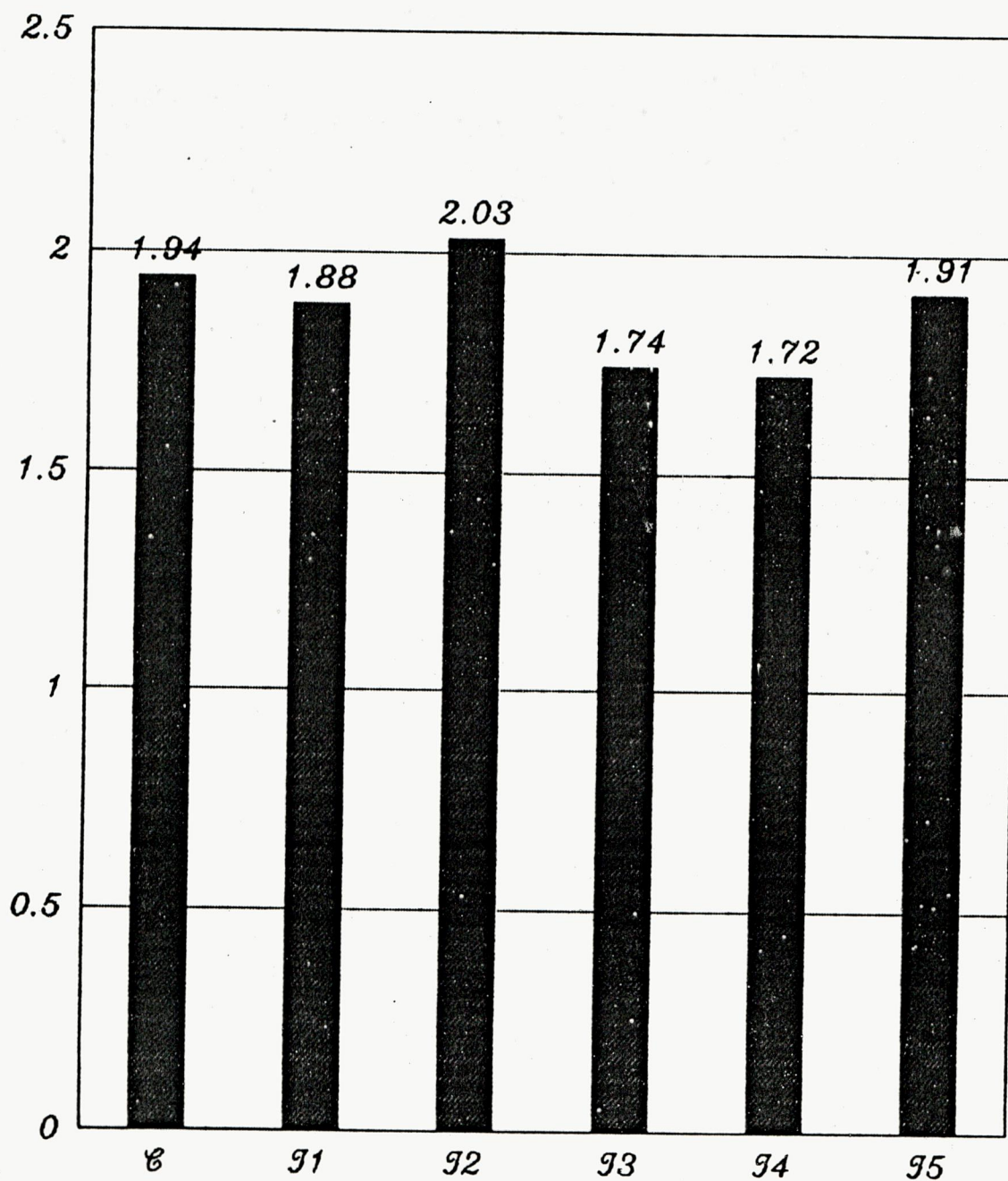


Fig. 16. Effect of enrichment of mulberry leaves with soya flour and test chemicals on cocoon length of silkworm, Bombyx mori L.

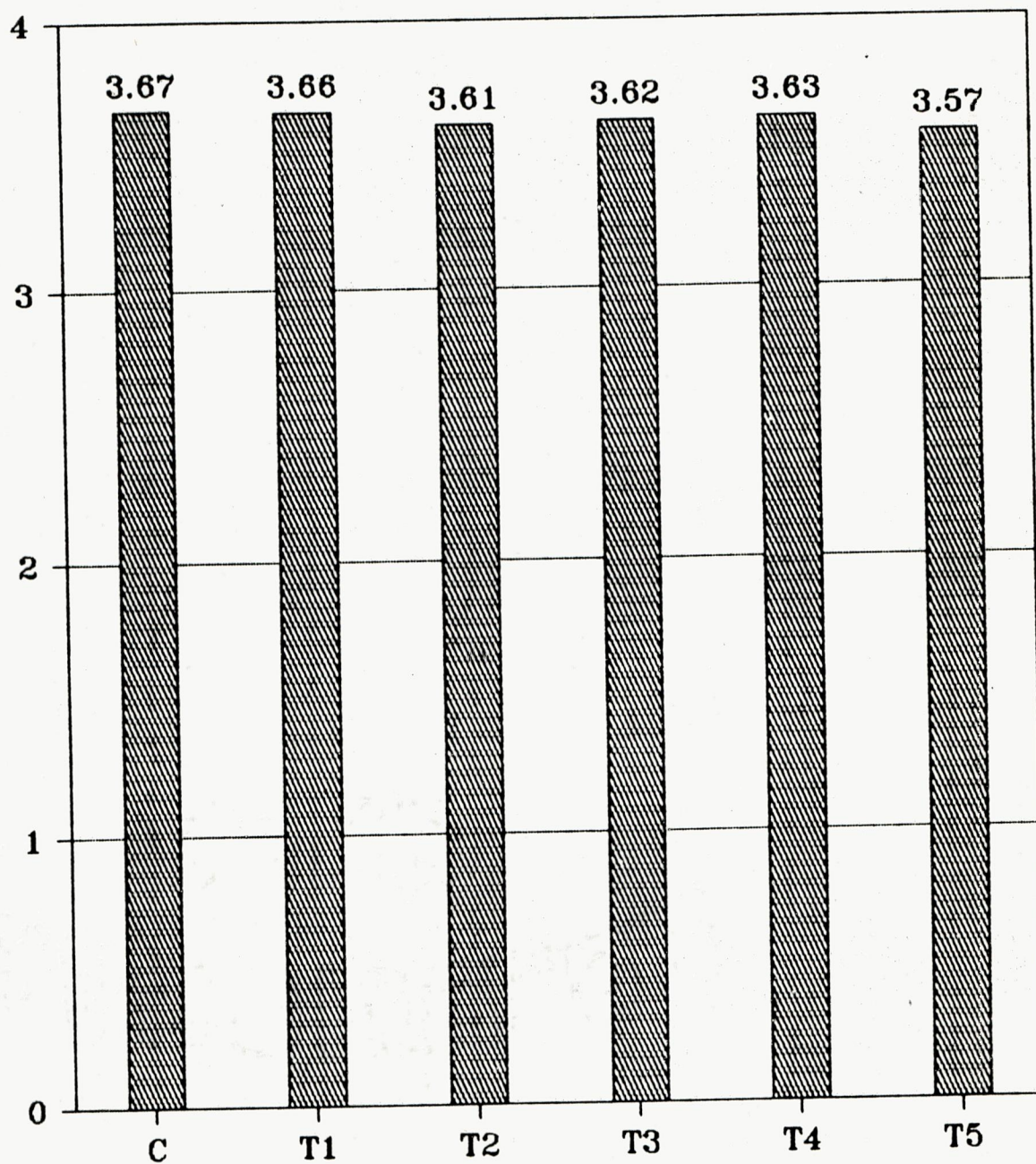
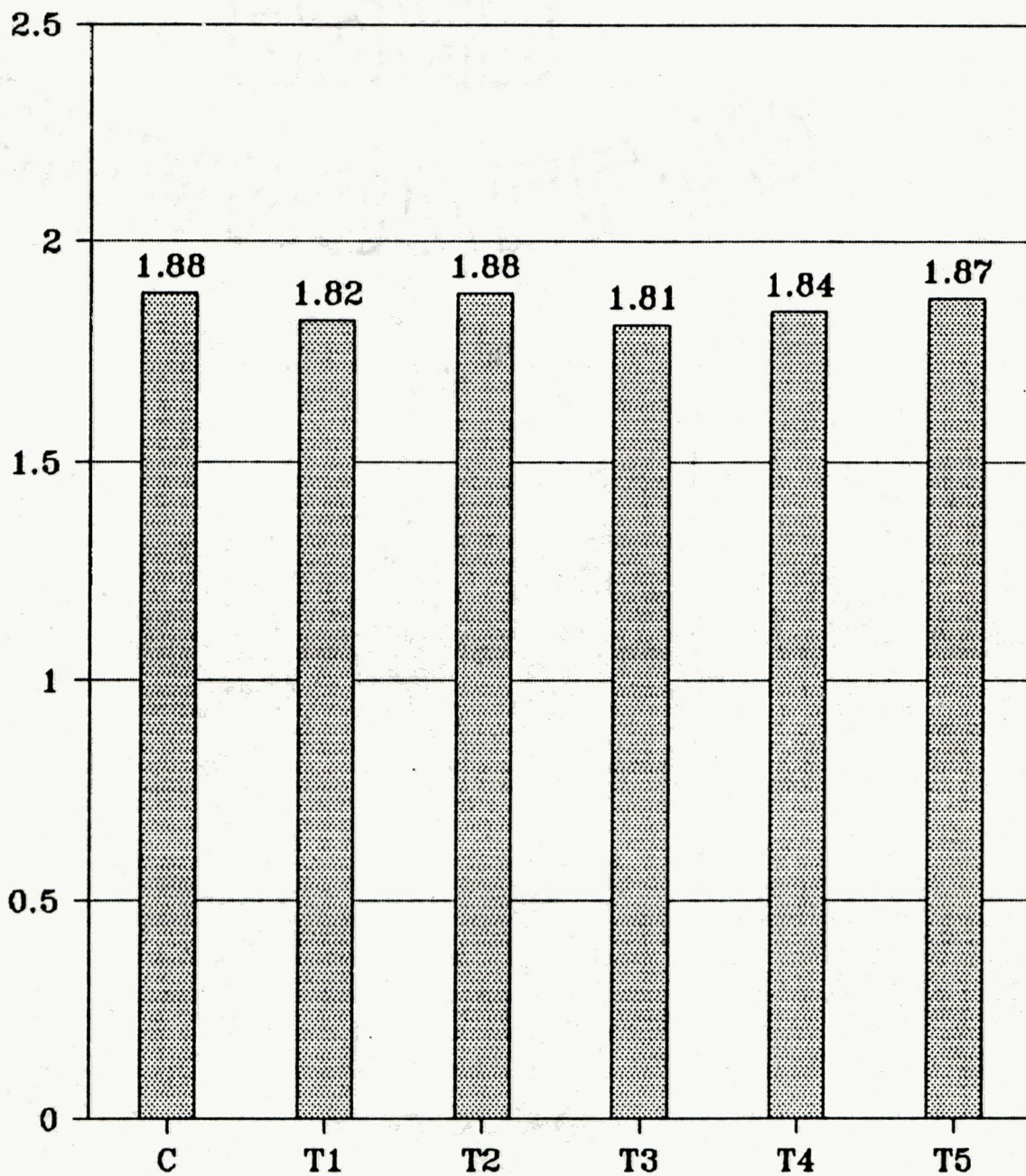


Fig. 17. Effect of enrichment of mulberry leaves with soya flour and test chemicals on cocoon width of silkworm, *Bombyx mori* L.



IV.3. Effect on cocoon length:

The results obtained with respect to this parameter were found to be statistically non-significant (Table I and Fig.16). The mean cocoon length was maximum (3.67 cm) in control which was on a par with soya flour treatment (3.66 cm) alone and soya flour +60 ppm orabolin (3.63 cm). The minimum cocoon length of 3.57 cm was obtained in treatment with soya flour + 60 ppm orabolin +B complex.

IV.4. Effect on cocoon width:

The data pertaining to the cocoon width are presented in Table I and figure 17. The mean cocoon width was maximum in treatment with soya flour +20 ppm orabolin and control (1.88cm). This was followed by treatment with soya flour +60 ppm orabolin +B complex (1.87 cm). The minimum cocoon width of 1.81 cm was recorded in treatment with soya flour +40 ppm orabolin. Statistical analysis proved this result to be non-significant.

IV.5. Effect on floss weight:

The mean floss weight was maximum (0.03 gm) in treatment with soya flour +60 ppm orabolin +B complex. (Table II and figure 18) which was on a par with control (0.02 gm) and also with all the other treatments. The results were found to be statistically non-significant.

TABLE II

EFFECT ON ENRICHMENT OF MULBERRY LEAVES WITH SOYA FLOUR AND TEST
CHEMICALS ON COCOON PARAMETERS AND FECUNDITY OF SILKWORM, Bombyx
moril.

TREATMENT	MEAN FLOSS WEIGHT	MEAN PUPAL WEIGHT	MEAN SHELL WEIGHT	MEAN SILK RATIO	MEAN FECUNDITY
C	0.02	1.48	0.26	13.60	539
T1	0.02	1.42	0.25	13.59	500
T2	0.02	1.63	0.28	13.58	557
T3	0.02	1.41	0.27	15.26	598
T4	0.02	1.42	0.24	13.93	559
T5	0.03	1.48	0.24	13.01	581
F	N.S	N.S	**	*	N.S
S.E ±	0.036	0.077	0.072	0.604	0.536
CD (P = 0.05)	0.097	0.210	0.016	1.636	1.453

* : SIGNIFICANT AT 5% LEVEL

** : SIGNIFICANT AT 1% LEVEL

N.S : NON-SIGNIFICANT

Fig. 18. Effect of enrichment of mulberry leaves with soya flour and test chemicals on floss weight of silkworm, Bombyx mori L.

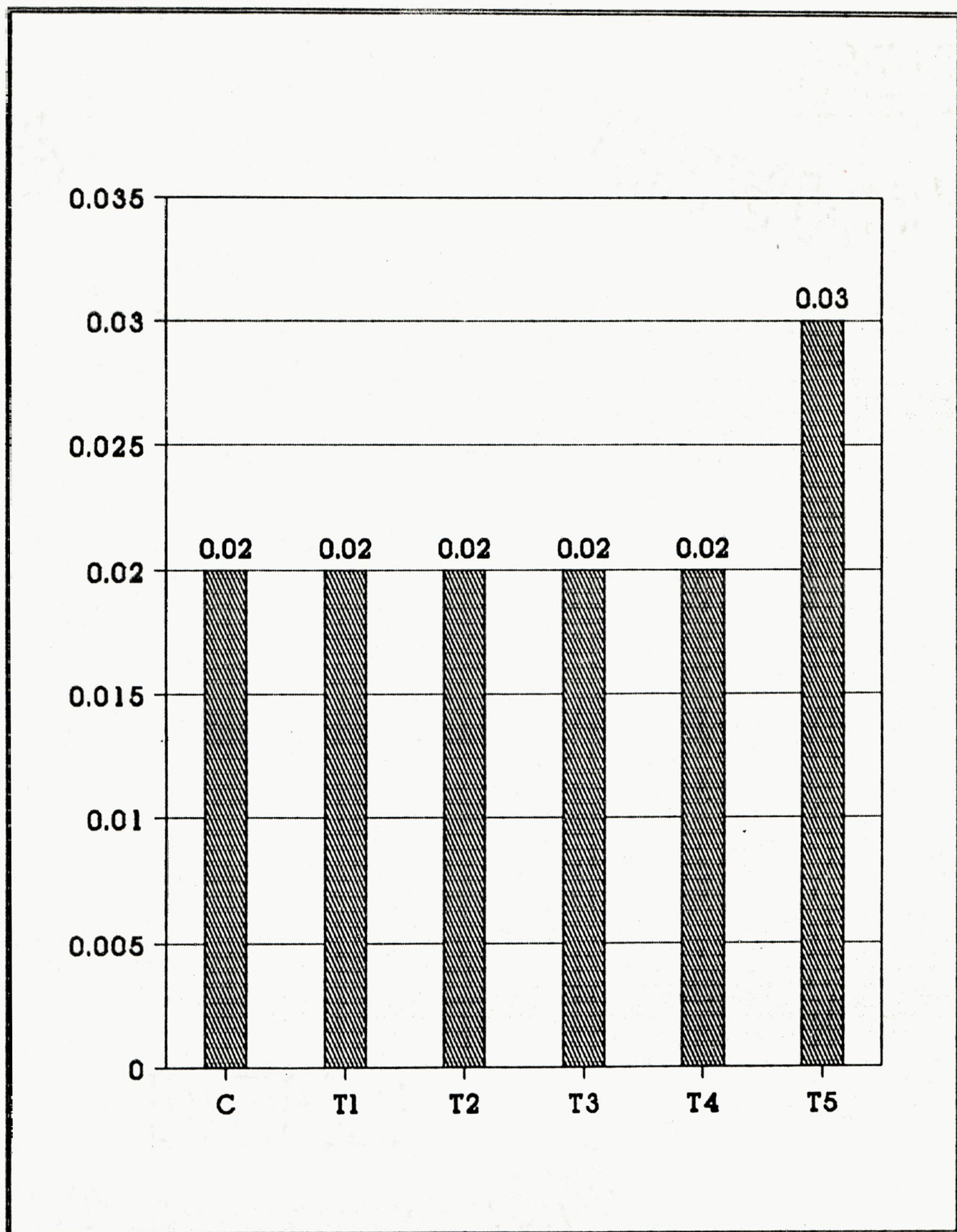


Fig. 19. Effect of enrichment of mulberry leaves with soya flour and test chemicals on pupal weight of silkworm, *Bombyx mori* L.

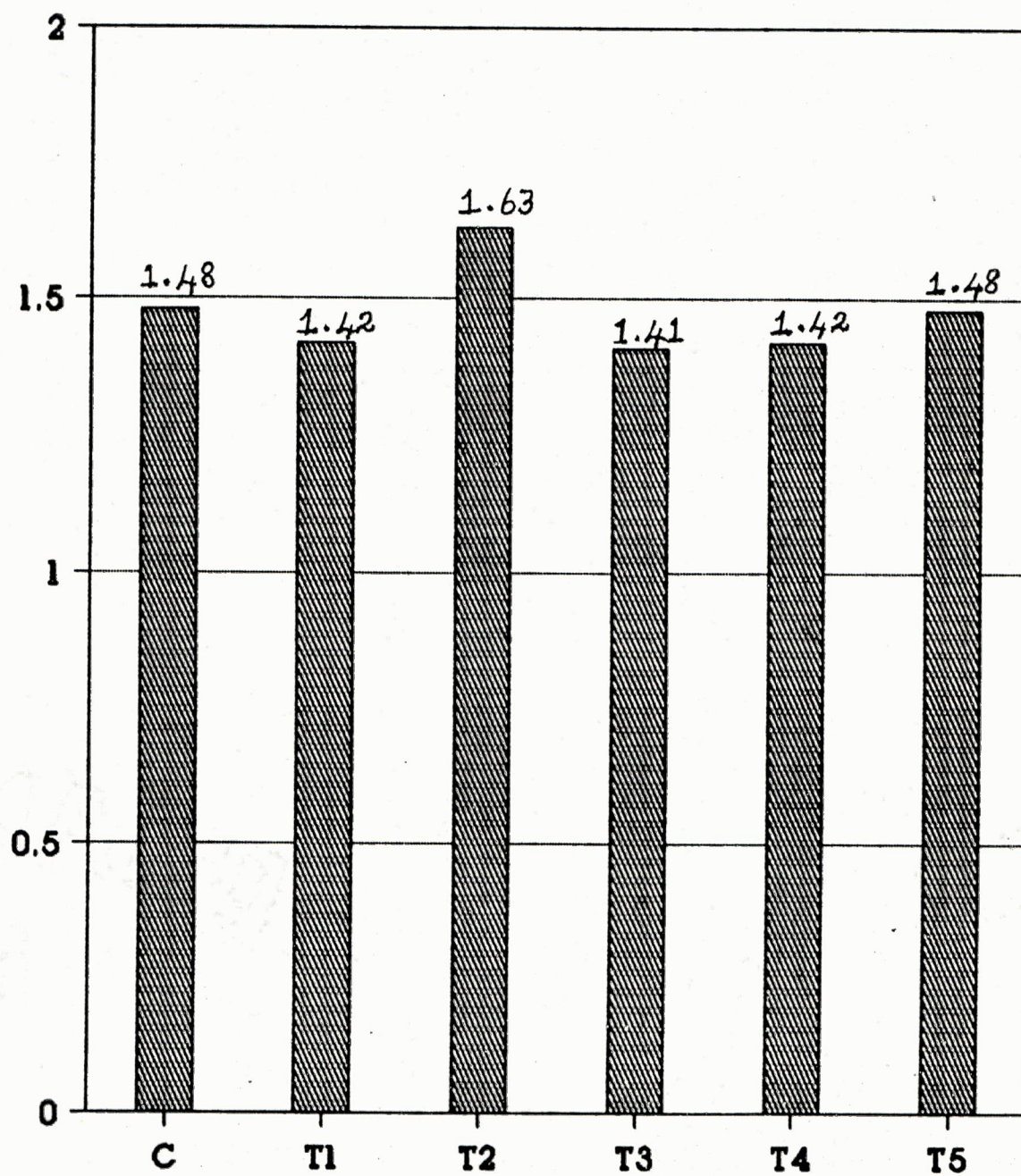


Fig. 20. Effect of enrichment of mulberry leaves with soya flour and test chemicals on shell weight of silkworm, Bombyx mori L.

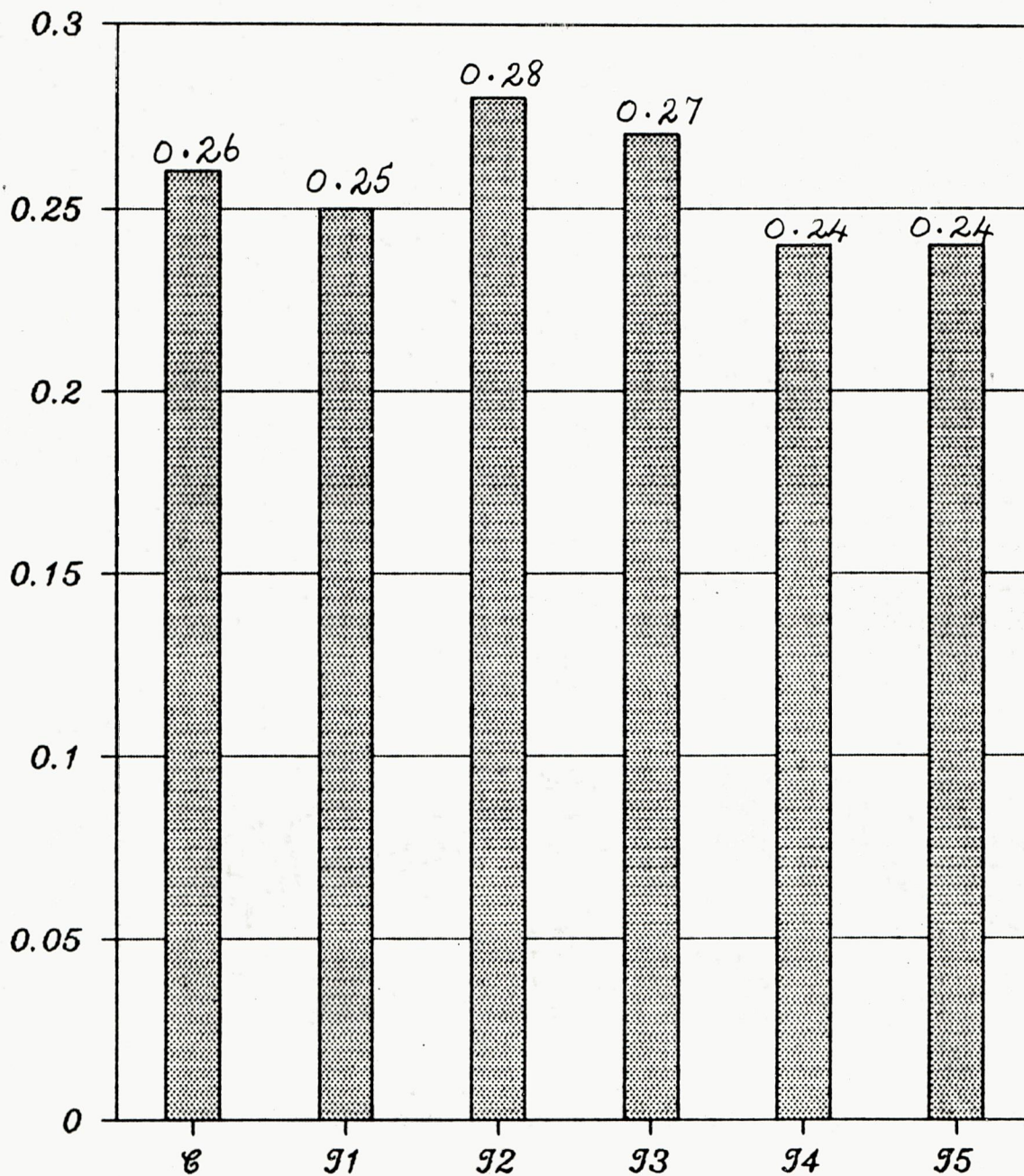
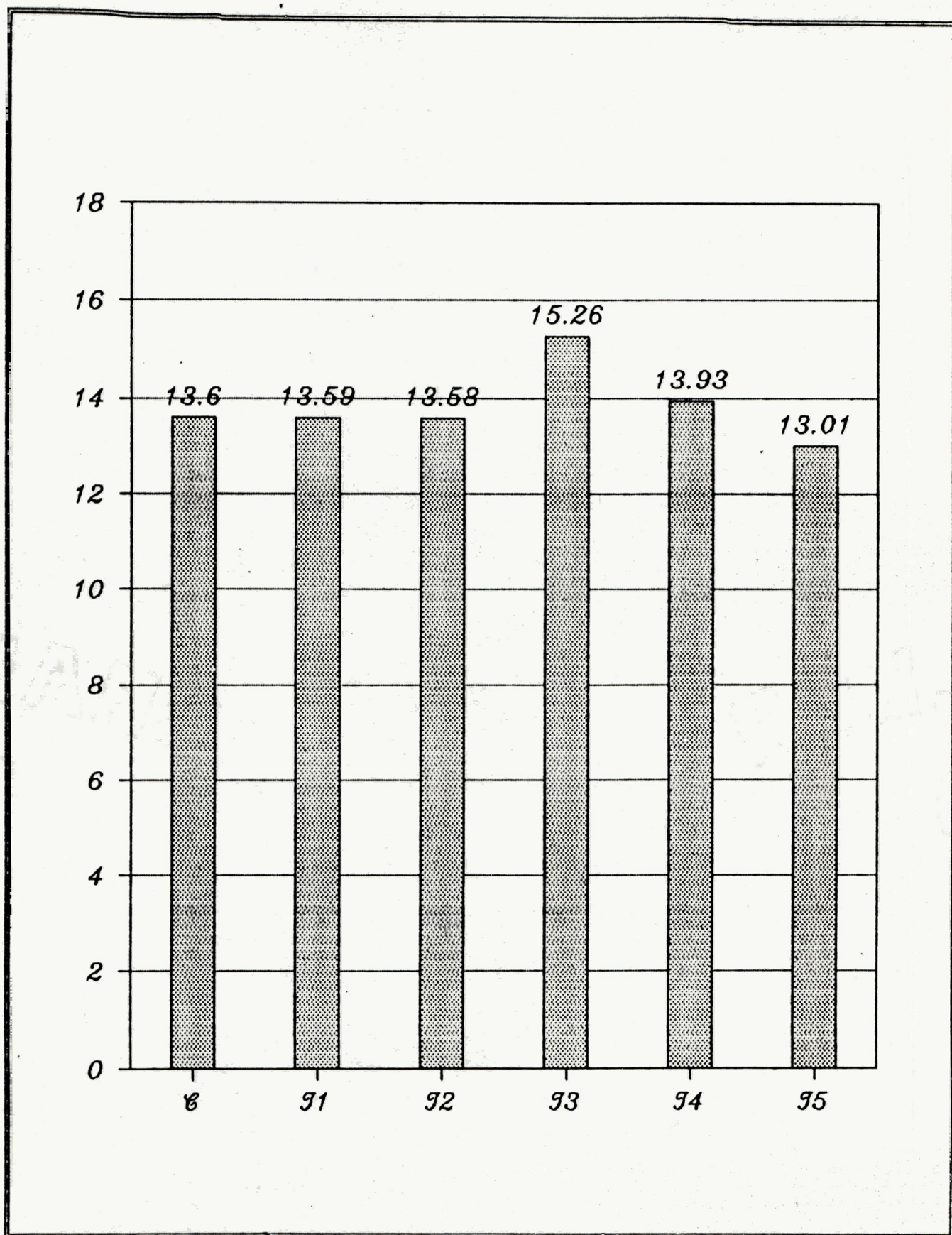


Fig. 21. Effect of enrichment of mulberry leaves with soya flour and test chemicals on silk ratio of silkworm, Bombyx mori L.



IV.6. Effect on pupal weight:

Table II and figure 19 indicate maximum pupal weight of 1.63 gm in treatment with soya flour +20 ppm orabolin, followed by treatment with soya flour +60 ppm orabolin +B complex and control (1.48 gm). The minimum weight (1.41 gm) recorded was in treatment with soya flour +40 ppm orabolin. The results were found to be non-significant.

IV.7. Effect on shell weight:

Statistical analysis of data on shell weight was found to be highly significant. It is evident from table II and figure 20. that shell weight was maximum (0.28gm) in treatment with soya flour +20 ppm orabolin, followed by treatment with soya flour +40 ppm orabolin (0.27 gm). Minimum shell weight was recorded in treatment with soya flour +60 ppm orabolin and in treatment with soya flour +60 ppm orabolin +B complex (0.24 gm).

IV.8. Effect on silk ratio:

The results obtained in respect of silk ratio was found to be statistically significant. The silk ratio was found to be maximum (15.26%) in treatment with soya flour +40 ppm orabolin, followed by treatment with soya flour +60 ppm orabolin (13.93%). The minimum (13.01%) silk ratio was found in treatment with soya flour +60 ppm orabolin +B complex (Table II and figure 21).

Fig. 22. Effect of enrichment of mulberry leaves with soya flour and test chemicals on fecundity of silkworm, *Bombyx mori* L.

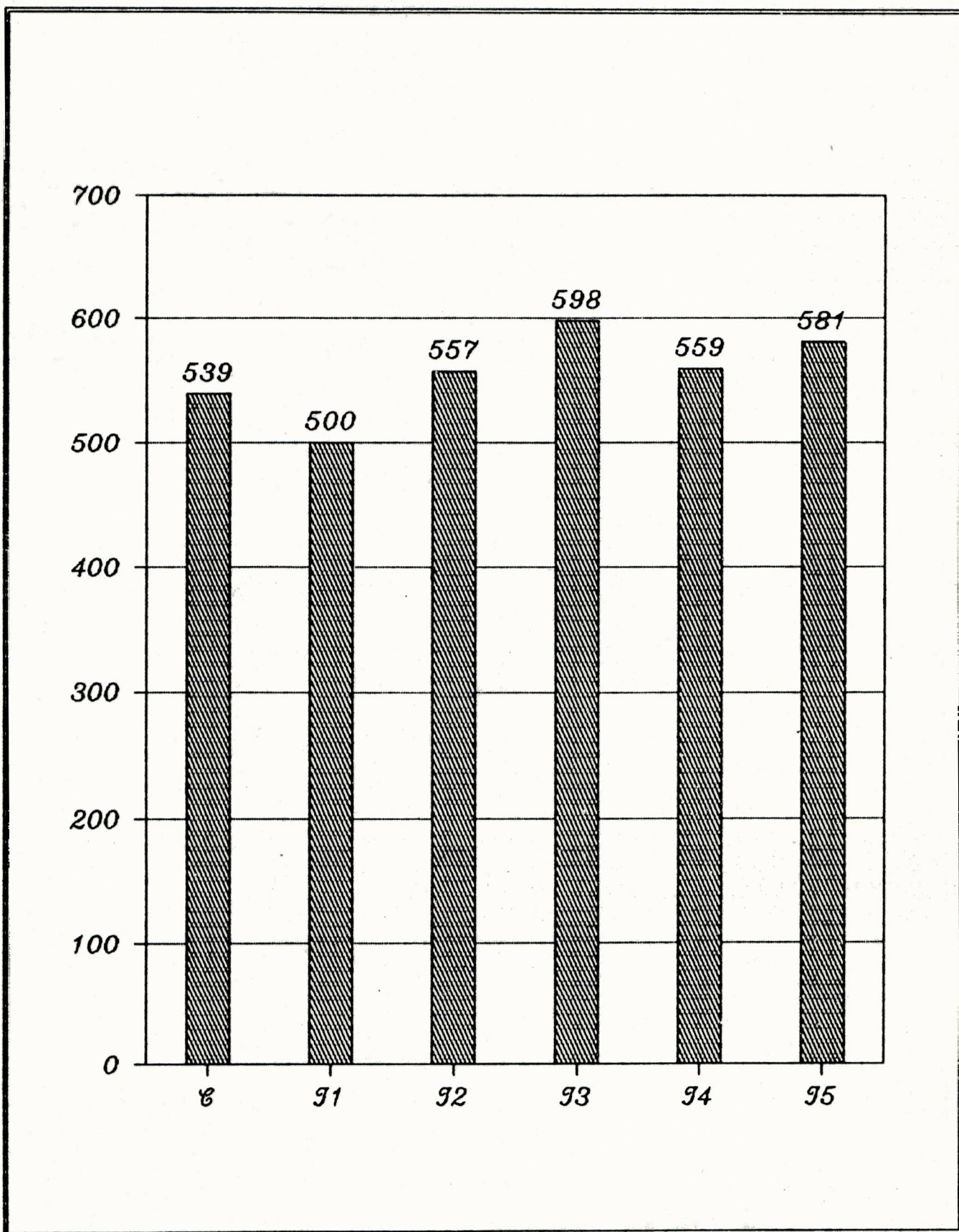


TABLE III

EFFECT OF ENRICHMENT OF MULBERRY LEAVES WITH SOYA FLOUR AND TEST CHEMICALS ON APPROXIMATE DIGESTIBILITY AND REFERENCE RATIO OF SILKWORM, Bombyx moriL.

Treatment	WEIGHT OF THE FOOD GIVEN (gm)	WEIGHT OF THE LEFT OVER FOOD (gm)	WEIGHT OF THE FOOD INGESTED (gm)	WEIGHT OF THE EXCRETA (gm)	WEIGHT OF THE FOOD DIGESTED (gm)	APPROXIMATE DIGESTIBILITY (%)	REFERENCE RATIO
C	4.805 ±0.568	0.927 ±0.340	3.88 ±0.860	2.66 ±0.253	1.22 ±0.954	31.44	1.46
T ₁	5.508 ±0.995	2.142 ±0.333	3.37 ±0.740	2.23 ±0.559	1.17 ±0.365	33.83	1.51
T ₂	5.723 ±1.495	3.518 ±1.556	2.21 ±1.971	1.77 ±0.710	0.43 ±1.625	19.91	1.25
T ₃	6.185 ±0.992	4.098 ±0.610	2.09 ±1.625	1.67 ±0.354	0.42 ±1.313	20.10	1.25
T ₄	5.695 ±1.155	3.853 ±1.265	1.84 ±0.937	1.72 ±0.715	0.12 ±0.192	6.52	1.07
T ₅	6.036 ±1.010	3.876 ±2.310	2.16 ±1.965	1.74 ±0.351	0.42 ±1.844	19.44	1.24

IV.9. Effect on fecundity:

The data on fecundity as given in table II and figure 22. showed maximum number of eggs (598) in treatment with soya flour +40 ppm orabolin, followed by treatment with soya flour +60 ppm orabolin +B complex (581). The minimum number of eggs (500) was recorded in treatment with soya flour alone. The results were found to be statistically non-significant among the treatments.

IV.10. Effect on approximate digestibility and reference ratio:

Table III indicates the effect of different treatments on approximate digestibility and reference ratio. Approximate digestibility was high in treatment with soya flour alone (33.83 %) in comparison with the control (31.44%). Correspondingly, the reference ratio was also high (1.51) in soya flour treatment compare to control (1.46). Approximate digestibility and reference ratio were minimum in treatment with soya flour +60 ppm (6.52% and 1.07 respectively). In the other three treatments, the approximate digestibility and reference ratio were almost similar ranging from 19.44% to 20.10% and reference ratio ranging from 1.24 - 1.25.

The overall results indicated a significant improvement in cocoon width, cocoon weight and shell weight in treatments with soya flour +20 ppm orabolin. Treatment with soya flour +40 ppm orabolin showed improvement in larval weight, shell weight and fecundity. However, approximate digestibility and reference ratio were maximum in treatment with soya flour alone.

V. DISCUSSION

Nutritional studies concerning silkworm, Bombyx mori have gained considerable importance recently and are considered to be one of the major areas of research in sericulture. As mulberry leaves are the only source of food for the mulberry silkworm, the mulberry silk industry mainly relies on the production of quality mulberry leaves.

Legay (1958) opined that the chief reason for the economic difficulties in sericulture is the fact that the silkworm, B.mori feeds primarily on mulberry leaves and this selective food preference limits silkworm rearing in time, specific seasons and places and prevents real industrialization. Therefore, during the past four decades, active research has been undertaken by sericulture scientists all over the world, to tackle this problem from different angles. New hybrid varieties of mulberry plants are developed and released into the fields to promote the yield and the nutritional quality of the leaves. Studies have also been undertaken to develop semi-artificial and artificial diet in order to overcome the shortage of leaves in autumn and winter seasons in temperate countries. Sengupta et al.(1972) remarked that in India growth of mulberry is continuous and

good quantity of leaves is obtained in most part of the country. As such, stress is given to enrich mulberry leaves with different substances like glucose, protein rich flours, aminoacids and vitamins with a view of improving silk yield.

Venkataramana and Bhat (1960), Sidhu et al. (1969) and Krishnaswamy et al. (1971) have shown that feeding with nutritionally superior leaves promoted better growth and development of silkworm larvae as well as economic characters of cocoons.

The present investigation was undertaken to assess the effect of enrichment of mulberry leaves with soya flour, orabolin (Ethylesterenol), an anabolic steroid, and vitamin B complex on the larval and cocoon characters and fecundity of silkworm B.mori.

Soya flour is used as an important ingredient in the artificial diet (Fukuda et al., 1960 and Magadum et al. 1994). Miyoshi (1975) attempted supplemental method by painting leaves with fortified food based mainly on soyabean powder and sugar on the autumn leaves and found it to be effective and resulted in high quality cocoons.

Sengupta et al. (1972) observed improvement in larval weight, effective rate of rearing, total weight of cocoon harvested, shell weight and shell ratio in treatments with casein and milk powder.

Subba Rao et al. (1989) reported that 1 to 3% concentrations of soyabean meal enriched leaves fed to the 5th instar larvae improved the qualitative and quantitative characters of cocoons and fecundity.

Ganga and Gowri (1990) reported maximum cocoon weight in wheat supplementation and maximal shell ratio and larval weight in rice flour supplementation.

Subburathinam et al. (1992) also reported positive effects of the use of soya flour in B.mori. In the present study, the treatment with soya flour alone did not bring about any significant improvement in the larval and cocoon characters. However, soya flour in combination with graded doses of orabolin was found to promote significant improvement in larval and cocoon characters.

The anabolic steroids are reported to promote protein metabolism and to increase muscle mass, strength and endurance (Schwartz and Miller, 1990 and Satovskar et al., 1995). In order to assess the effect of orabolin and soya flour on larval and cocoon development and fecundity, graded doses of this steroid with soya flour were applied to the mulberry leaves and the leaves were fed to the 5th instar larvae.

Anabolic steroid, durabolin was reported to evoke dose dependent effects on the development of Dysdercus similis and this effect was similar to the effect produced by juvenoids. (Sita et al., 1981). The vertebrate steroid, cortisol was found to increase the growth rate of Oncopeltus fasciatus (Gawienowsik et al., 1987) and progesterone was found to stimulate vitellogenesis in panaeid prawn, Panopenaeopsis hardwickii (Nagabushanam et al., 1980). Reddy et al. (1994) reported the presence of estradiol 17 β , testosterone and progesterone RIA positive materials in the mulberry leaf and also in the haemolymph of silkworm and suggested that these steroids may play a role in the biosynthetic activities.

Of the five treatments, in the present investigation a combination of soya flour + 40 ppm orabolin was observed to improve larval weight, silk ratio and fecundity. While soya flour +20 ppm orabolin was found to improve cocoon parameters such as cocoon weight, cocoon length, pupal weight and shell weight.

Hamano (1989) reported improvement in the larval growth in diet with 30% protein +10 μ g of B₆ and improvement in reelable cocoon, average weight of reelable cocoon layer with improved mineral mixture and B complex mixture (Hamano and Okano, 1989). The work of Bajpeyi et al. (1991) on Bombyx mori stressed the importance of vitamin B supplementation in

silkworm feed. In the present study, a combination of soya flour +60 ppm orabolin + vitamin B complex was found to produce improvement in fecundity.

Study on digestibility and reference ratio corroborates with the findings of Remadevi et al. (1993) who observed an increase in ingesta as well as approximate digestibility when ingesta increases. In the present study, in correlation with the increase in ingesta (weight of the food ingested), digesta, (weight of the food digested), approximate digestibility and reference ratio were found to be high in treatment with soya flour alone. In all the other four treatments a decrease in ingesta was noticed and correspondingly digesta, approximate digestibility and reference ratio were low.

Despite a reduction in the approximate digestibility (19.91%) and reference ratio (1.25), the treatment with soya flour +20 ppm orabolin was found to produce improvement in several cocoon parameters such as cocoon weight, cocoon length, pupal weight and shell weight. Similarly, treatment with soya flour +40 ppm orabolin brought about improvement in larval weight, silk ratio and fecundity inspite of low approximate digestibility (20.10%) and low reference ratio (1.25). These results are in accordance with the statement of Remadevi et al. (1992) that heavy feeders need not be high producers.

It is evident from the observations made in the present study, soya flour in combination with 20 ppm and 40 ppm orabolin produced beneficial effects. This may be ascribable to the stimulatory effect of anabolic steroid in protein metabolism which might have resulted in the improvement in larval and cocoon parameters. These two combinations can be considered as optimal doses for improving the larval and cocoon parameters of silkworm Bombyx mori.

In many districts of Tamilnadu especially in Coimbatore and Salem, farmers use soya flour as a supplement in mulberry leaves. Soya flour is dusted on the mulberry leaves once a day and the leaves are fed to the larvae to promote growth. A spray of 20 ppm or 40 ppm orabolin followed by dusting of soya flour can be recommended to farmers to improve the cocoon parameters and yield.

Summary and Conclusion

VI SUMMARY AND CONCLUSION

(1) Investigation was carried to find out the effect of enrichment of mulberry leaves on the growth and development of the silkworm, Bombyx mori L. belonging to PM X NB D 4 2 variety.

(2) The 5th instar larvae were fed with leaves enriched with soya flour alone and with leaves enriched with soya flour +20 ppm orabolin, soya flour +40 ppm orabolin, soya flour +60 ppm orabolin and soya flour +60 ppm orabolin + vitamin B complex.

(3) Five replications each with 30 larvae were maintained for control and other five treatments.

(4) Observations were made on the larval, cocoon characters, fecundity and on the approximate digestibility and reference ratio.

(5) The data pertaining to larval weight was found to be highly significant. Maximum larval weight (3.84 gm) was observed in treatment with soya flour +40 ppm orabolin as compared with that of control (3.69 gm) and minimum (3.41 gm) in treatment with soya flour alone.

(6) Statistically significant results were obtained with reference to cocoon weight. Maximum cocoon weight (2.03 gm) was recorded in treatment with soya flour +20 ppm orabolin as compared to that of control (1.94 gm).

(7) The mean cocoon length was statistically non-significant. With respect to this parameter, control and treatment with soya flour alone showed maximum length (3.67 and 3.66 cm respectively).

(8) With respect to cocoon width, control and treatment with soya flour +20 ppm orabolin were found to be on a par with each other (1.88 cm). Statistical analysis of this data was found to be non-significant.

(9) The mean floss weight showed only marginal increase in all the treatments and was not found to be statistically significant.

(10) Of the 6 treatments, maximum increase in pupal weight (1.63 gm) was observed in treatment with soya flour +20 ppm orabolin, while control recorded a pupal weight of 1.48 gm. However, the results were found to be non-significant.

(11) Statistical analysis of the data on shell weight showed significance at 1% level. Maximum shell weight (0.28 gm) was observed in treatment with soya flour +20 ppm orabolin. In comparison control recorded 0.26 gm.

(12) The results obtained in respect of silk ratio was found to be statistically significant. Maximum silk ratio (15.26%) was observed in treatment with soya flour +40 ppm orabolin as compared to the control (13.60%).

(13) Maximum number of eggs (598) could be obtained in treatment with soya flour +40 ppm orabolin as compared to that of the control (539).

(14) The results obtained with reference to approximate digestibility and reference ratio showed maximum value in treatment with soya flour alone (33.83% and 1.51 respectively) as compared to the control (31.44% and 1.46 respectively).

(15) In the other four treatments, approximate digestibility and reference ratio were found to be low, the minimum being observed in treatment with soya flour +60 ppm orabolin (6.52% and 1.07 respectively).

(16) Though, a reduction in approximate digestibility (19.91%) and reference ratio (1.25) was noticed, the treatment with soya flour +20 ppm orabolin recorded improvement in several cocoon parameters such as cocoon weight, cocoon length, pupal weight and shell weight.

(17) A combination of soya flour with 40 ppm orabolin was found to produce marked improvement in larval weight, shell ratio and fecundity, despite low approximate digestibility (20.10%) and reference ratio (1.25). This finding falls in line with the statement of Remadevi et al. (1992) that high consumers need not be good producers.

(18) The results obtained in the present investigations prove the efficacy of soya flour in combination with orabolin, an anabolic steroid.

(19) Soya flour +20 ppm orabolin and soya flour +40 ppm orabolin were found to produce beneficial effects and therefore can be considered as optimal doses for improving larval and cocoon characters in B.mori.

(20) A spray of 20 ppm orabolin or 40 ppm orabolin followed by dusting of mulberry leaves with soya flour can be recommended to the farmers to increase the yield.

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