DISCUSSION
DISCUSSION

Mulberry is grown extensively for its foliage. It is the chief source of food for silkworm *Bombyx mori* L. The nutritive quality of mulberry leaf together with the quantity of herbage produced per unit area largely varies depending upon the genotypes and agro-tech followed in cultivation. These aspects decide the profitability of sericulture industry. The physicochemical characteristic features of mulberry leaf affect the amount of food ingested by the silkworm larvae (Legay, 1958). Therefore, it is needless to stress the importance of using desired quality of mulberry leaf in rearing silkworms for the production of good quality cocoons.

In recent decades, efforts have been made to improve the quality of mulberry leaf by the use of optimum agricultural inputs (manures, fertilizers and irrigation), growing / cultivating newly evolved high yielding varieties, adopting scientific agro-techniques and better management practices. In addition, biofertilizers (nitrogen fixers and phosphate solubilizing microbes), vermicompost, plant growth regulators (PGR's), foliar formations, etc., were widely employed to boost up the yield and improve the quality of mulberry.

Several investigators have recorded the favourable response such as increased yield, improved quality, better larval performance and good cocoon as well as fibre characteristic features by using farm yard manure (Pain, 1961), extra dose of nitrogenous fertilizer (Sengupta *et al.*, 1972; Kasiviswanathan *et al.*, 1979), biofertilizers (Nagarajan *et al.*, 1986), topical application of proteins, carbohydrates and amino acids (Sharada *et al.*, 1956; Sengupta *et al.*, 1972; Kumararaj *et al.*, 1972; Bhaskar *et al.*, 1982; Sridhar and Radha, 1986), hormones like thyroxine and insulin (Kamada and Ito, 1984; Thyagaraja *et al.*, 1986).
1988; Magadum and Hooli, 1989), vitamins like Vit. C (El-Karaksy and Idriss, 1990; Madhu Babu et al., 1992), antibiotics like tetracyclin, chloromycetin, amoxyllin, compicilin, etc. (Murthy and Sreenivasaya, 1953; Shyamala et al., 1960; Verma and Atwal, 1963; Verma and Kushwaha, 1971; Radha et al., 1981; Tayade et al., 1988) and plant growth regulators (PGRs) such as GA3 (Kamada and Ito, 1984; Shantakumari et al., 1989).

Further, spectacular results have also been achieved by using foliar nutrition technique in mulberry fields. By using this technique PGRs, vitamins, foliar nutrients (micro and macro) are supplemented effectively in to the plant system at critical growth period to ensure luxurious growth and development of mulberry. The leaves obtained from these treated plots supported better larval growth and resulted in increased cocoon production (Viswanath and Krishnamurthy, 1982; Lokanath and Shivashankar, 1986; Lokanath et al., 1986; Ankalgi and Ansari, 1992; Bose et al., 1995a; Bose et al., 1995b).

Several multinational companies (MNCs) have released foliar formulations for various crop plants under different trade names viz., Vipul, Trinol, Simazine, Fasal, Cytozyme crop, Biozyme crop, etc. These products are known to contain growth factors and plant food elements such as growth regulators, vitamins, essential amino acids, micro and macro nutrients, hormones, steroids, proteins, sugars, wettable agents and surfactants. In the present study, two new foliar formulations viz., phytone and green leaf were utilized to evaluate their effect on growth and quality parameters of mulberry as well as nutrition, enzyme activity and commercial characteristic features of silkworms.

1 GROWTH PERFORMANCE OF MULBERRY VARIETY M₁ TREATED WITH PHYTONE AND GREEN LEAF.

Foliar spray of phytone and green leaf showed a positive effect on general growth pattern of mulberry. The shoot length, number of branches and leaf
per plant are found to be significantly higher in treated mulberry plants when compared to control. Similarly, leaf yield per unit area is increased significantly in green leaf and phytone treated plots. The increase in the shoot length is dependent on water uptake, cell elongation and cell division which are primary mechanism responsible for extensive growth of plants. The increase in the number of shoot initials and leaf primordia are due to the fact that kinetin and indole precursors present in the growth formulation always favour the formation of new shoot initials and leaf primordia (Figs. 23 & 24).

The reduction in the growth and yield attributes at low concentration of phytone and green leaf may probably be due to the dilution effect as higher the ratio of solvents, the lower will be concentration of chemicals and it might not be able to express it full potential on the physiological activity of crop plants which ultimately is responsible for the yield (Lokanath and Shivashankar, 1986). The high concentration may be toxic and might have affected the translocation of the nutrients. Hence, it is advisable to use the foliar formulations at optimum dose i.e., at 3% level. These results are in strong agreement with the works of Lokanath and Shivashankar (1986), Ankalgi and Ansari (1992) and Misra et. al., (1995). Lokanath and Shivashankar (1986) used micronutrients such as zinc, manganese, iron and boron along with magnesium on mulberry var. M_3. The results revealed that, these micronutrients along with magnesium significantly improves the growth and yield attributes of mulberry var. M_3. Ankalgi and Ansari (1992) used commercial foliar formulation Fasal, on mulberry var. M_3. They have reported the increased shoot length, number of leaves per plants and leaf weight. Similarly, Misra et. al., (1995) used Biozyme crop on mulberry var. BC_{25}. They have also reported the increased leaf yield and improved growth parameters.
QUALITATIVE PERFORMANCE OF MULBERRY VAR. M₄
SPRAYED WITH PHYTONE AND GREEN LEAF

A  MOISTURE CONTENT.

Moisture content of mulberry leaf determines the nutritive quality of the leaves and plays an important role in generation quality and quantity of cocoons (Dandin and Kumar, 1989). The water content in the leaves also serve as one of the criteria in estimating their quality and evaluation of its feed quality (Narayana Praksah et al., 1985). In the present study, tender leaves showed highest moisture percentage followed by medium and coarse leaves in all treatments and control. However, among treatments, 3% green leaf sprayed plants showed comparatively high moisture content when compared to plants treated with 3% phytone. The other two treatment doses (1% and 5%) of both green leaf and phytone showed marginal difference but definitely superior to control leaves (Tables 1, 2, 3 & 4; fig. 1). Further, the moisture content in mulberry leaf was found to be highest during rainy season when compared to winter and summer seasons. Analysis of variance for moisture percentage of mulberry leaf revealed that type of leaves and concentrations are highly significant in all the seasons (Tables 2D, 3D and 4D).

B  PROTEIN CONTENT.

Protein is the main constituent of mulberry leaf which plays a vital role in the development of silk gland (Anfinsen et al., 1958; Fukuda et al., 1959; Qader, 1987). Further, it has been proved that silkworm derives over 70% of the proteins from the mulberry leaves for the biosynthesis of silk (Fukuda et al., 1959; Kawase, 1975). Tender leaves sprayed with 3% green leaf showed an increased trend in total protein content during all the seasons when compared to medium and coarse leaves (Table 5). Overall proteins percentage in treated leaves was high during winter seasons. Leaves treated with green leaf formulation
showed comparatively high protein percentage than in phytone treated leaves. In general, green leaf and phytone at 3% concentration were found to be effective when compared to 1% and 5% treatment. Protein content in treated leaves were significantly higher when compared to protein content of control leaves (Tables 5, 6, 7 & 8; Fig. 2). Analysis of variance for protein content (haemolymph and midgut) of silkworms showed that all variables and interactions are highly significant in all the seasons studied (Tables 111D, 112D, 113D, 117D, 118D & 119D).

C TOTAL SUGAR

Higher carbohydrate content of the mulberry leaves is favourable for the healthy growth of the silkworm larvae (Anonymous, 1975; Kishi, 1954). The maximum increase in the content of total sugar was observed with the application of phytone foliar formulation at 3% concentration. Tender leaves showed highest concentration of total sugar followed by medium and coarse leaves. Leaves of control plants revealed lowest total sugar content when compared to the leaves of treated plants (Tables 9, 10, 11 & 12; fig. 3). Analysis of variance for total sugar content of mulberry leaf revealed that type of leaves, chemicals and concentrations are highly significant. Similarly, interaction between types of leaves x chemicals, type of leaves x concentration, type of leaves x chemicals x concentrations are highly significant (Tables 10D, 11D & 12D).

D REDUCING SUGAR CONTENT

Similarly, even in this parameter also, tender leaves contain high reducing sugar percentage when compared to medium and coarse leaves. However, leaves of plants treated with green leaf and phytone at 3% level showed comparatively high content of reducing sugar followed by leaves of plants with 1% and 5%
treatments as well as control (Tables 13, 14, 15 & 16; fig. 4). Among the seasons, plants grown during rainy season revealed highest reducing sugar content when compared to winter and summer seasons. Analysis of variance for reducing sugar of mulberry leaf revealed that type of leaves and concentrations are significant in all the seasons while, chemicals except in winter season are also highly significant. Similarly, interactions between type of leaf x chemical, chemical x concentration, type of leaf x concentration and type of leaf x chemicals x concentration are also significant (Table 14D, 15D & 16D).

E  CHLOROPHYLL CONTENT

The leaf chlorophyll content is indicative of the photosynthetic efficacy of a plant system (Bongale and Chaluvachari, 1995). High chlorophyll content was observed in tender leaves followed by medium and coarse leaves in all the seasons (Tables 17, 18, 19 & 20; fig. 5). However, leaves of plants treated with either green leaf or phytone at 3% concentration showed high chlorophyll content when compared to the leaves treated with 1% and 5% phytone and green leaf as well as control plants. Analysis of variance for total chlorophyll of mulberry leaf shows that, significant difference exists among type of leaf, chemicals and concentrations. Interactions between type of leaf x concentration, chemical x concentration, type of leaf x concentration and type of leaf x chemical x concentration were also found to be significant (Tables 18D, 19D & 20D).

The significantly increased moisture content was due to stimulation of xylem by growth regulators which results in increased water up take (Devlin and Witham, 1986b). Application of copper, zinc and iron on mulberry leaves trigger the various oxidases process, resulting in an increased photosynthesis, protein synthesis and metabolism. Apart from these, gibberellic acid is known to increase nucleic acids as well as protein content in plant tissues (Key, 1969; Glasziou, 1969). Further, Devlin and Witham (1986) reported that, auxins
increases the invertase activity suggesting that, sucrose which initially builds up from the lipid conversion is hydrolysed to the osmotically active sugars. Bose et al., (1995) opined that association of Boron with phosphogluconates and its involvement in the metabolism of carbohydrates is responsible for increase in sugar concentration. Similarly, the increased chlorophyll content may be attributed to the effect of mineral elements such as iron and manganese. Manganese increases the nutrient content of mulberry leaves which may be due to its association with many enzyme systems and its involvement in production of oxygen in chloroplast there by increasing the photosynthetic activity resulting in high metabolic activities (Pendias and Pendias, 1984).

Lokanath and Shivashankar (1986) reported micro nutrients and magnesium will enhance the yield and improves the quality parameter of the mulberry. Further, Ankalgi and Ansari (1992) used Triacontanol and Fasal on mulberry var. M, and recorded improved biochemical parameters of mulberry leaf. Bose et al., (1995) reported increase in nutrient content of mulberry leaf due to the application of micronutrients as foliar spray. These reports are further confirmed by Misra et al., (1995) by using Biozyme crop on mulberry var. BC<sub>259</sub> and recorded increased bio chemical parameters. The results recorded in the present investigation also fall in line with these research findings.

Since both phytone and green leaf are known to contain growth regulators, micronutrients and vitamins, they influence increased moisture percentage and other nutritive constituents of mulberry. High moisture content of mulberry leaf favoured the palatability of feed and also the assimilation of the nutrients. Moreover, higher values of protein and sugar in the feed are known to favour better performance of silkworm larval growth and their cocoon crop (Legay, 1958; Ito, 1960; Horie, 1980). Since these treated leaves are highly nutritious, larvae consumed more mulberry leaves and attained good growth and
development. Hence, larvae were healthy and no incidence of disease was observed. Because of rapid growth and development, larvae attain maturity at a faster rate than the larvae reared on leaves procured from control plots. Thereby reducing the larval duration and resulting in better economy (Figs. 25 & 26). Further, these larvae spun the cocoons which are heavier and qualitatively good when compared to cocoons spun by larvae fed with untreated leaves (Figs. 25 & 26).

3 PHYSIOLOGICAL PERFORMANCE OF SILKWORMS FED WITH PHYTONE AND GREEN LEAF ENRICHED LEAF

Insects feed upon a remarkably diverse organic substances. At the same time, most species including *Bombyx mori* L. show a high degree of specificity in their choice of food. The dietary constituents in the form of carbohydrates, proteins, fats and nitrogen are essential nutrients required for the growth during development. Insects utilize these nutrients from their food differently depending upon the nature of the food, mode of life and abiotic environmental factors (Poonia, 1985). Nutritional studies in silkworm with respect to cocoon productivity (Takano and Arai, 1978), silk production (Muthukrishnan *et. al.*, 1979), economic characters (Sumioka *et. al.*, 1982 a, b), silk protein (Fukuda, 1960) and body weight gain (Ueda and Suzuki, 1967) have elucidated their dependency on qualitative and quantitative variations of the feed, mulberry leaf. The food ingested and digested by the insects must fulfil their nutritional requirements for normal growth and development.

In the present findings, physiological parameters such as rates in feeding, defaecation, assimilation, conversion and growth were studied to evaluate the effect of treated leaves on nutritional parameters of silkworm larvae.

The feeding rate was high in larval batches fed with leaves treated with 3% green leaf or phytone. In control batches feeding rate was minimum when
compared to treated batches. These results are similar during summer, winter and rainy seasons (Tables 21, 22, 23, 54, 55 & 56; figs. 6D, E & F). Among the silkworm varieties, pure mysore showed better feeding rate followed by NB₄D₂ and PM x NB₄D₂ silkworms. The feeding rate remains maximum on 1st day of V instar and minimum on last day of V instar i.e., spinning day in all the treatments. Analysis of variance for feeding rate suggests that, there exists significantly high difference among varieties, chemicals and concentrations in all seasons. Interactions between varieties x chemicals, chemicals x concentration, varieties x concentration and varieties x chemicals x concentration were highly significantly (Table 57D, 58D & 59D).

The rate of defaecation was high in NB₄D₂ silkworms followed by PM x NB₄D₂ and PM throughout the V instar in all treatments when compared to control (Tables 24, 25, 26, 60, 61 & 62; figs. 7A, B & C). The defaecation rate was maximum in larvae fed with green leaf treated leaves than phytone treated leaves. More over, highest defaecation rate was recorded in larvae fed with leaves recovered from 3% green leaf sprayed plants when compared to other treated batches and control. The analysis of variance for defecation rate showed that all variables and interactions are highly significant in all the three seasons studied (Table 63D, 64D & 65 D).

The rate of assimilation was high in pure mysore silkworms when compared to NB₄D₂ and PM x NB₄D₂ silkworms. The rate of assimilation was high on 1st day and low on last day of V instar in all the silkworm varieties and in all treatments. However, assimilation rate was comparatively high in larvae fed with leaves recovered from 3% green leaf treated plants when compared to larvae fed on leaves recovered from 3% phytone treated plants. But the rate of assimilation remains low in larvae of all the varieties reared on untreated leaves (Tables 27, 28,29, 66, 67 & 68; fig. 8). The analysis of variance for assimilation
rate showed significantly high differences among varieties, chemicals and concentrations. Interactions between varieties x chemical, varieties x concentration, chemicals x concentrations and varieties x chemicals x concentrations were also significant (Tables 69D, 70D & 71D).

The conversion efficiency was maximum in NB4D2 larvae when compared to PM x NB4D2 and PM larvae (Tables 30, 31, 32, 72, 73 & 74; fig. 9). The conversion efficiency was maximum on 3rd day and minimum on last day of V instar in NB4D2 and PM x NB4D2 larvae while, it was maximum on 4th day and minimum on 8th day of V instar PM larvae in all treatments and control. However, larvae showed maximum conversion efficiency when fed with leaves recovered from green leaf treated plants than phytone treated plants. More precisely, green leaf sprayed at 3% level on plants found effective than at 1% and 5% treatments. Similar trend was also noticed with phytone treatments. The analysis of variance for conversion efficiency showed highly significant difference among varieties x chemical, varieties x concentration, chemicals x concentrations and varieties x chemicals x concentrations were also significant (Tables 75D, 76D & 77D).

The growth rate was high on 4th day in NB4D2 larvae and on 1st day in PM x NB4D2 and PM larvae. However, the growth rate was minimum on the last day of V instar in all the silkworm varieties (Tables 33, 34, 35, 78, 79 & 80). Larvae fed with leaves treated with green leaf revealed comparatively high growth rate when compared to larvae fed on phytone treated leaves. But larvae fed with untreated leaves showed low growth rate in all the silkworm varieties. The larvae fed on leaves treated with 3% green leaf or phytone showed maximum growth rate than larvae fed on either 1% or 5% treated leaves and control. Analysis of variance for growth rate showed highly significant difference among varieties, chemicals and concentrations, chemicals x concentrations and
varieties x chemicals x concentrations were highly significant (Tables 81D, 82D & 83D). These results are in agreement with the works of Naik and Delvi (1987), on PM, NB₄D₂, KA and a Japanese strain. The high feeding rate and food consumption in larvae fed with treated leaves confirmed that the chemicals are not phytoretardants. The variations observed with regard to rate of defaecation were due to more water content and other biochemical constituents in treated leaves because of foliar formulations.

It is an established fact that about 85% of the total amount of food is consumed during final instar of lepidopteran insects (Waldbauer, 1968). Nayak et. al., (1985) showed that in Antheraea mylitta, the food consumption is directly proportional to age and shows extraordinary increased during V instar period. The maximum feeding rate during V instar is attributed to the active feeding stage, because the feeding cycle keeps an increasing with the age of the larvae and it declines towards spinning (Krishnaswamy et al., 1978). Thus it is needless to stress the importance of quality of food provided to silkworms during V instar period.

High assimilation rate in pure mysore is because of high feeding rate in pure mysore silkworm larvae (Naik and Delvi, 1987). Thus the feeding rate gives an idea about the rate of food consumed, while assimilation rate indicate the metabolic level and productive rate of insects (Delvi and Pandian, 1972).

In any lepidopteran insect the degree of utilization of food depends upon the digestibility of food and efficiency with which the ingested food is converted in to body substance (EC1) and it varies significantly among the different breeds.

In the past, to judge the utilization of leaves by insects three criteria have been drawn viz., 1) digestibility of food 2) conversion of ingested or digested
food and 3) rate of food consumption (Soo Hoo and Fraenkel, 1966). The conversion efficiency of the silkworm increased with the increase in age and is directly correlated with the body weight of the larvae. It is reported that in NB$_4$D$_2$ variety, the food intake is as twice as in case of pure mysore silkworm. Hence the conversion efficiency is more in NB$_4$D$_2$ larvae when compared to hybrid and pure mysore silkworms (Naik and Delvi, 1987). Further, Delvi (1972) reported that the conversion efficiency depends upon the type of food. In the present study also larvae showed high conversion efficiency when fed with treated mulberry leaves. Further, better or faster assimilation and conversion rate in larval period helps to accumulate sufficient food energy in order to tide over the subsequent non feeding stages.

The efficiency of conversion of ingested food is low during summer season in NB$_4$D$_2$ larvae. This factor may be attributed to high temperature observed during summer season. Similar results were reported by Poonia (1985) in Philosamia ricini and Bhat and Bhattacharya (1978) in Spodoptera litura fed on soybean leaves. The conversion efficiency of the silkworm Bombyx mori L. increases with an increase in age and is correlated with the body weight of the larvae. Kinne (1960) considered the conversion of food into body substances and into biologically useful energy as additional in many cases more suitable and sensitive parameter for assessing rates and efficiency of metabolic process.

Growth rate explains how much of dry matter increased in the body of the animal per day per gram of body weight. It affects the speed of development of insects which directly depends on food and abiotic factors as well. Waldbauer (1968), Delvi and Pandian (1971) reported that the higher feeding rates, better food assimilation, conversion efficiency and growth rate for lepidopteran can be taken as adaptive features to meet the energy requirements. These values will influence the pupation and further on cocoon production. In the present
Investigation high growth rate was observed in NB₄D₂ larvae and it may perhaps be due to high food consumption. Thus, the foliar nutrients used in the present study were found to be effective and useful in influencing the growth and development of silkworms in general and bivoltine silkworm (NB₄D₂) in particular.

4 BIOCHEMICAL PARAMETERS OF SILKWORMS FED WITH PHYTONE AND GREEN LEAF ENRICHED LEAF

Silkworm larvae during V instar consume high amount of food in order to accumulate sufficient energy resources to support its metabolic activities during its non-feeding stage (pupa and adult). Hiratsuka (1920) estimated that leaf consumption of V instar Bombyx mori accounts to 85% of the total instars. The leaves consumed by the larvae is converted in to body substances by various metabolic activities for the growth and development of silkworms. Thus, digestive enzymes may play a vital role in converting complex food material in to body substances in order to provide energy and metabolites for growth, development and other important functions such as spinning of cocoons (Waterhouse, 1957; House, 1965; Wigglesworth, 1972). So it may be assumed that, feeding stimulates the production of digestive enzymes in the body of silkworm Bombyx mori. Isshaaya et. al., (1971) while working on the larvae of Spodoptera littoralis shown that certain protein factors present in the food can stimulate digestive enzymes probably through a hormonal mechanism. The activity of enzyme may increase or decrease depending upon the nature of the enzyme, source of the enzyme, type and quantum of food ingested and finally abiotic factors.

In the present investigation, treated leaves are utilized for feeding silkworms (NB₄D₂, PM x NB₄D₂ and PM larvae) and it is observed that the larvae consumed more leaves. So there is an increased rate of metabolism in the silkworm body suggesting that high activities of digestive enzymes. Further,
the activity of amylase was high in haemolymph in all the silkworm varieties, reared by supplementing 3% green leaf or phytone treated leaves when compared to the quantum as well as activity of enzyme in larvae, reared on 1% and 5% green leaf or phytone treated leaves and also on untreated leaves. The amylase activity in larvae irrespective of variety, reared on untreated leaves, showed minimum enzyme activity both in haemolymph and midgut tissue when compared to larvae reared on green leaf or phytone treated leaves. The low enzyme activity in those larvae clearly depicts that, the intake of food is very less and thus showed low metabolic rate and enzyme activities. Further, the enzyme activity is comparatively high in haemolymph than midgut tissue in larvae of all treatments and control (Tables 87, 88, 89, 93, 94 & 95; figs. 11 & 12). High enzyme activity was noticed during summer season when compared to winter and rainy seasons. Analysis of variance for amylase activity in haemolymph and midgut tissues of silkworm showed that varieties, chemicals and concentrations are significant in all seasons (Tables 87D, 88D, 89D, 91D, 92D & 93D).

Similarly, the activity of protease was found to be high in haemolymph than in midgut tissue in all the silkworm varieties studied. Further, in different seasons and in different treatments including control the haemolymph of silkworm larvae showed high enzyme activity. The activity of protease was maximum on 3rd day in haemolymph and 4th day in midgut tissue of NB₄D₂ larvae. In hybrid PM x NB₄D₂ larvae, the activity of enzyme is maximum on 3rd and 5th day in haemolymph and midgut tissue respectively. In pure mysore larvae, the protease activity was maximum on 4th and 5th day in haemolymph and midgut tissue respectively. The enzyme activity was high in tissue of larvae fed with leaves recovered from 3% green leaf sprayed plants than 3% phytone treated plants. However, there is not much difference in enzyme activities in tissues of larvae fed on leaves obtained from 1% and 5% either green leaf or
phytone treated plants. But in larvae fed on untreated leaves the enzyme activities remains low. Moreover, the effect of these treated leaves has got more response in NB$_4$D$_2$ larvae when compared to PM x NB$_4$D$_2$ larvae (Tables 99, 100, 101, 105, 106 & 107; figs. 13 & 14). Analysis of variance for protease activity in haemolymph and midgut tissues of silkworms showed that varieties, chemicals and concentrations are highly significant. Similarly, interactions between varieties x chemicals and varieties x concentrations are highly significant (Tables 99D, 100D, 101D, 105D, 106D & 107D).

The high protein concentration is an indication of a greater metabolic activity of the tissue. The concentration of proteins was found to be high in haemolymph than midgut tissue, in all the varieties of silkworms studied in all seasons. Further, concentration of proteins in silkworm showed an increased trend with the development of silkworm during V instar. But there exists a difference in protein concentration among the silkworm varieties studied being highest in NB$_4$D$_2$ followed by PM x NB$_4$D$_2$ and PM larvae. Among the treatments, the concentration of protein was high in larvae reared on leaves procured from 3% green leaf or 3% phytone sprayed plants when compared to protein concentration in larvae fed with leaves recovered from 1% and 5% green leaf or phytone treated plants. However, the protein concentration was lowest in larvae fed with leaves of control plots. This difference exists in all the varieties of silkworms and among all the seasons studied. The protein concentration was found to be high in larval tissue during winter season when compared to rainy and summer seasons (Tables 111, 112, 113, 117, 118 & 119; figs. 15 & 16). Analysis of variance for protein content of mulberry leaf revealed that type of leaves, chemicals and concentrations are highly significant (Tables 6D, 7D & 8D).
Since, feeding stimulates the secretion of enzymes in the body of the silkworm, the enzyme activity is dependent on the quantum of food ingested into the body of silkworm larvae. Thus, the activity of enzymes (amylase and protease) was high during the middle period of larvae because of highest amount of food consumed in that period (active feeding stage). On the other hand, when the larvae attains spinning stage, the food intake will be naturally low, results in low metabolic activities like low enzyme activity. Further, the peak / maximum activity of different enzymes is on different days may be because of the difference in the quantum of food intake depending upon the variation in genotypes. However, the enzyme activity was low, when larvae attains spinning stage because of low food intake during that period. High enzyme activity in larvae (all strains) fed with leaves harvested from 3% green leaf or phytone treated leaves, is an indication of the high food intake of treated leaves by silkworm larvae. Enzyme activity was slightly high during summer season. It may be due to sensitiveness of enzymes to high temperature. These results are in confirmation with the works of Yokoyama (1959) and Matsumura (1951). They reported that the enzyme activity is high in silkworms reared in tropical conditions than in larvae reared in temperate climate condition. Further, the enzyme activity was comparatively high in haemolymph than in midgut tissue in all the silkworm varieties studied. Since, haemolymph is the carrier of food material, the enzyme activity may be high in haemolymph. These results are in agreement with the findings of Eguchi and Iwamoto (1975). They clearly showed that enzyme activity in midgut tissue is lower than that in the digestive fluid.

Difference in the enzyme activity among the silkworm varieties studied is because of genetic diversity. Further, Hirata (1974) analysed the relation between amylase activity in the larval digestive juice and several quantitative characters using bivoltine strains with high and low digestive amylase activity. He concluded that larval survival rate as well as economic characters correlated
with the high amylase activity. The results of the present investigation are in agreement with these research findings.

The increased protein concentration in the body of silkworm is due to supplementation of enriched leaves to silkworms. The observed influence of dietary protein on the increase of haemolymph has also been reported by Horie et al., (1971). Further, the variation in the protein concentration in the haemolymph and midgut tissue on particular days was a clear indication of a circadian rhythm like phenomenon in soluble tissue proteins. It is believed that, the high concentration of protein in haemolymph is converted into silk protein before spinning stage. Finally, high protein concentration in NB₄D₂ larvae than PM x NB₄D₂ and PM larvae may be attributed to genetic differences among them.

5 COMMERCIAL PARAMETERS OF COCOONS (NB₄D₂, PM x NB₄D₂ & PM)

The cocoons obtained by rearing silkworms (NB₄D₂, PM x NB₄D₂ and PM) were subjected to critical analysis of important economic parameters such as cocoon weight, shell weight, shell weight percentage, filament length, raw silk percentage, silk waste percentage, denier and renditta (Figs. 27, 28, 29, 30, 31, 32 & 33).

Mean cocoon weight was more in NB₄D₂ variety followed by PM x NB₄D₂ and PM cocoons. Larvae spun heavier cocoons when fed with 3% either green leaf or Phytone treated leaves. The cocoons spun by larvae reared on leaves obtained from 1% and 5% green leaf or phytone treated plants, were comparatively less in weight. However, larvae fed with untreated leaves produced cocoons which are inferior in quality. Cocoons obtained during winter season were quite superior in quality, in all the varieties (Tables 121, 122 & 123; Fig. 17). The analysis of variance for cocoon weight suggests that, there
exits a significant difference among varieties x chemicals and concentrations. Interactions between races x concentration, chemical x concentration and races x chemicals x concentrations were also significant (Tables 121, 122 & 123).

The shell weight and shell weight percentage were highest in NB₄D₂ cocoons and PM cocoons. Both parameters were superior in all varieties of cocoons obtained by rearing silkworms feeding the leaves procured from 3% green leaf or phytone treated plots. Between phytone and green leaf, shell weight and shell weight percentage of cocoons produced by silkworm fed on green leaf enriched leaf were found to be superior. The shell weight and shell weight percentage of cocoons procured from batches of larvae fed on either 1% or 5% green leaf or phytone enriched leaves were more or less similar. But shell weight and shell weight percentage was low in cocoons spun by larvae fed on untreated leaves. Further, shell weight and shell weight percentage was high in cocoons of all varieties obtained during winter season when compared to cocoons produced during rainy and summer seasons (Table 125, 126, 127, 129, 130 & 131; figs. 18 & 19). Analysis of variance for shell weight and shell weight percentage suggests that varieties, chemicals and concentrations are highly significant. Interaction between varieties x concentration, chemicals x concentration, varieties x chemicals x concentrations were also significant (Tables 125, 126, 127, 129, 130 & 131).

Similarly, filament length was more in NB₄D₂ cocoons when compared to PM x NB₄D₂ and PM cocoons. Further, filament length was more in cocoons obtained from silkworm batches fed on leaves procured from 3% green leaf or phytone treated plots. Difference in filament length of cocoons spun by silkworm larvae reared on leaves harvested from 1% and 5% either green leaf or phytone treated mulberry plots is not significant. But filament length was low in cocoons spun by larvae fed on leaves procured from untreated plots.
Even in this parameter also, cocoons obtained during winter rearing were superior and yielded more silk filament (Tables 133, 134 & 135). The analysis of variance for filament length showed that all the variables, varieties, chemicals and concentrations were highly significant. Similarly, interactions between varieties x chemicals, varieties x concentrations, chemicals x concentrations and varieties x chemicals x concentrations were also highly significant (Tables 133D, 134D & 135D).

The raw silk percentage was much high in bivoltine NB₄D₂ cocoons when compared to PM x NB₄D₂ and PM cocoons. The raw silk percentage of cocoons of all the varieties, was high when the larvae were fed with leaves harvested from either 3% green leaf or 3% phytone treated plots. There was no difference with regard to raw silk percentage of cocoons obtained by rearing silkworms using leaves of 1% and 5% green leaf as well as phytone treated plants. However, low raw silk percentage was recorded in cocoons produced by larvae fed on untreated leaves.

Similarly, silk waste percentage was low in NB₄D₂ cocoons when compared to PM x NB₄D₂ and PM cocoons. Further, silk waste percentage was much reduced in cocoons produced by silkworms reared on leaves harvested from 1% and 5% green leaf or phytone treated plots including control.

Denier value was very low in NB₄D₂ cocoons when compared to PM x NB₄D₂ and PM cocoons (Tables 139, 140 & 141; fig. 21). In addition, improved denier value was noticed in cocoons produced by larvae reared on leaves of 3% green leaf or phytone treated plants. The denier value was high in cocoons spun by larvae fed on control leaves. The analysis of variance for filament denier showed that, varieties, chemicals and concentrations are highly significant. Interactions between varieties x concentrations, chemicals x concentrations and

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Finally, the renditta was low in NB₄D₂ cocoons followed by PM x NB₄D₂ and PM cocoons. In addition, the renditta value was improved in cocoons produced by larvae fed on leaves harvested from 3% green leaf or phytone treated plants. However, the improvement in renditta value was marginal in cocoons spun by larvae reared by using leaves of either 1% or 5% green leaf or phytone treated leaves. But the renditta value was comparatively low in cocoons of silkworm, fed with untreated leaves (Table 143, 144 & 145). The analysis of variance for renditta showed that, there exists significant differences among varieties, chemicals and concentrations. Interactions, between varieties x chemicals, chemicals x concentration, varieties x concentrations and varieties x chemicals x concentrations were also highly significant (Table 143D, 144D and 145D).

The results of the present study suggest that the repeated applications of either green leaf or phytone improves the economic parameters of cocoons. Improvement in quality and quantity of silk by using enriched mulberry leaves has also been showed by many workers (Ito and Niminura, 1966; Horie et. al., 1967; Sengupta et. al., 1972; Viswanath and Krishnamurthy, 1982; Lokanath et. al., 1986; Verma and Kushwaha, 1971; Shantakumari et. al., 1989; El-Karakxy and Idriss, 1990; Magadum and Hooli, 1991; Ankalgi and Ansari, 1992; Krishnan et. al., 1995; Rahaman Khan and Saha, 1995; Misra et. al., 1995; Sarker and Absar, 1995).

In the present investigations, larvae consumed more mulberry leaves with less wastage. High intake of foliar nutrient treated mulberry leaves by the silkworm larvae results in high metabolic activity as well as enzyme activity. Thus, silkworm accumulates more proteins and other nutrients which helps in
producing better quality cocoons. These aspects have been reported by Hirata (1974), while analysing relation between amylase activity in the larval digestive juice and several quantitative characters, showed that higher cocoon weight, cocoon shell weight, shell percentage, cocoon shell productivity per day in V instar and survival rate for larvae with a high amylase activity. Probably high amylase activity helps in converting complex food material into body substance resulting in high rate of silk synthesis and production of heavier cocoons.

It is evident that the elevation of nutrient level in the food/diet of silkworm at an optimal level resulted in the acceleration of larval and cocoon weight. Above the optimal concentration, there was a reduction in larval weight and a gradual increase in the mortality rate. This may be attributed to nature of chemicals including phagostimulants and nutrients which acts as deterrents when applied in excess concentration. On the other hand, foliar formulation applied at low concentration may not yield the expected results because of ineffectiveness. Hence, it is absolute necessary to supplement optimal level of foliar nutrients to mulberry in order to enrich nutritional status, thereby promoting the better growth and development of silkworms in order to produce good quality and quantity cocoons.

The foregoing discussion clearly unravel the beneficial effect of phytone and green leaf foliar formulations. Out of the 3 concentrations (1%, 3% and 5%) of the formulations used in the present investigation, 3% green leaf and phytone were found to be optimum since, various growth parameters of mulberry like, plant height and branching pattern, number of leaves per plant, leaf area and herbage productivity exhibited improved trend. Similarly, nutritional status of mulberry has largely improved in treated leaves when compared to control. Further, when green leaf and phytone treated leaves were used for silk worm rearing (NB₄D₂, PM x NB₄D₂ and PM), larvae showed good
growth and development. In addition, nutritional parameters such as feeding rate, defaecation rate, assimilation rate, conversion efficiency and growth rate were improved to a great extent. Besides, the activity of digestive enzymes (amylase and protease) and protein content in the body of silkworm were markedly increased. Finally, all these beneficial aspects were reflected in good quality cocoon production.

The improvement in the yield and quality of mulberry leaf due to the influence of foliar nutrients (green leaf and phytone) may be due to the effect of plant growth regulators, vitamins and micronutrients present in these formulations. Further, when larvae fed on these enriched mulberry leaves, the silkworm larvae consumed more quantum of food because the treated leaves are highly nutritious and good in quality. So larvae fed on the enriched mulberry leaves continuously there by accumulated large quantity of nutrients in their body for good growth and development and produced superior quality cocoons. Mortality rate is greatly decreased and resulted in high effective rate of rearing percentage.

Therefore, the present investigation strongly recommends the use of green leaf and phytone at 3% concentration on juvenile mulberry plants at initial growth phase in order to increase foliage production per unit area and to improve the nutritional status of mulberry. There by, more layings can be brushed per unit area besides producing good and increased cocoon yield coupled with superior quality of silk yarn.
SUMMARY

The present study deals with improvement in nutritive status of mulberry to increase silk production in three different varieties of silkworms (NB₄D₂, PM x NB₄D₂ and PM). In order to improve the nutrient content of mulberry leaf, foliar nutrition technique was adopted. Two foliar nutrients viz., phytone and green leaf were used in the experiment. These commercial formulations are widely used to increase the yield and improve the quality of several other agricultural, horticultural and vegetable crop plants. In the present investigation, three different concentration (1%, 3% and 5%) of the phytone and green leaf were sprayed separately on the juvenile plants of mulberry var. M₃. Repeated sprays were given at an interval of 10 -15 days. These experiments were done during summer, winter and rainy seasons. Some plants were also maintained as control with water sprays. Growth and development of the plants sprayed with leaf nutrients were observed periodically to score the data. Similarly, evaluation was done on the plants maintained for the purpose of comparison as control.

Plants treated with either 3% green leaf or phytone exhibited luxuriant growth when compared to 1% and 5% phytone or green leaf treated plants. However 1% and 5% green leaf or phytone treated plants showed profuse growth when compared to control.

Further, growth parameters such as plant height, branching pattern, number of leaves per plant and leaf area were highly improved in 3% green leaf or phytone treated plants. Plant treated with 1% or 5% either phytone or
green leaf showed marginal increase in growth when compared to control. Increased growth and development of mulberry were recorded during winter season than in summer and rainy seasons.

Leaves harvested from the treated plants were analysed for qualitative parameters such as moisture percentage, protein, sugars (total and reducing) and chlorophyll content. The percentage of moisture, proteins, total sugar, reducing sugar and chlorophyll content were quite high in leaves harvested from the plants treated with 3% green leaf or phytone when compared to control. Leaves recovered from the plants sprayed with 1% green leaf or phytone showed comparatively high moisture and protein percentage when compared to 5% phytone or green leaf treated plants and control. On the other hand, high percentage of sugars and chlorophyll content were recorded in leaves procured from 5% green leaf or phytone treated plants when compared to 1% green leaf or phytone sprayed plants. All these parameters show comparatively a low trend in leaves of control when compared to treated plants.

Bio-assay tests were conducted on NB₄D₂, PM x NB₄D₂ and PM silkworm varieties by feeding enriched mulberry leaves of variety M₅ using phytone / green leaf. Larvae fed with leaves of 3% green leaf or phytone treated plants showed better growth and development. The larvae behaved normally during moulting and spinning time. Obviously, mortality rate was highly reduced. Similarly, silkworm larvae fed with leaves of 1% or 5% green leaf or phytone treated plants, also revealed better growth and development when compared to control. However, in control batches, as larvae were poorly nourished, their growth and development was retarded and hence quite high mortality rate was noticed. In general, larval growth and development were quite good during winter season than rainy and summer seasons.
During silkworm rearing, important nutritional parameters such as feeding rate, defaecation rate, assimilation rate, conversion efficiency and growth rate were studied. In general, all these physiological parameters showed an improvement in larvae fed with leaves harvested from the plots treated with 3% green leaf or phytone compared to larvae fed with leaves of 1% or 5% either greenleaf or phytone treatments and control. The feeding rate and assimilation rate were high in pure mysore larvae when compared to NB₄D₂ and cross breed (PM X NB₄D₂) silkworms, irrespective of treatments. Similarly, defaecation rate, conversion efficiency and growth rate were high in NB₄D₂ larvae followed by PM x NB₄D₂ and PM silkworm larvae irrespective of treatments. These nutritional parameters were high in larvae reared during winter season irrespective of silkworm varieties and treatments.

Biochemical studies indicated that two digestive enzymes namely, amylase and protease as well as protein content were high in the silkworm lots reared by using 3% green leaf or phytone enriched leaves. In general, the enzyme activity and protein content were high in haemolymph than midgut tissues of silkworm. The enzyme activity and protein content were high in NB₄D₂ larvae followed by PM x NB₄D₂ and pure mysore silkworm larvae. The activity of enzymes and protein content more or less similar in larvae reared with either 1% or 5% green leaf and phytone treated leaves. Silkworm showed higher enzyme activity during summer season when compared to winter and rainy seasons. However, high protein content was observed in tissues of silkworm larvae reared during winter season when compared to rainy and summer seasons. But in all the silkworm varieties the activity of enzymes and protein content were low when larvae fed with leaves harvested from control plants.

Economic parameters of cocoons were good in 3% green leaf or phytone treated batches. Cocoons were well built, sturdy, bigger in size, heavier in