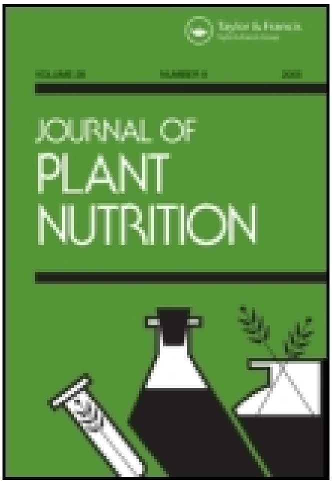

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Evaluation of Macro and Micro Nutritive Elemental Levels in the Mealy-Bug (Maconellicoccus Hirsutus)- Infested Leaves of Mulberry (Morus Sp.)

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EVALUATION OF MACRO AND MICRO NUTRITIVE ELEMENTAL LEVELS IN THE MEALY-BUG (*MACONELLICOCCUS HIRSUTUS*)-INFESTED LEAVES OF MULBERRY (*MORUS* SP.)

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□ *The quality of mulberry leaf supports good growth and development of silkworm larvae. Mulberry leaves are hampered by the various detrimental diseases and pests. The mealy bug is one of the important insect pests of mulberry; its sap sucking nature may alter nutritive levels. An attempt was made to evaluate the macro and micro nutritive elemental levels in the infested leaves. There was a large variation of nitrogen, phosphorus, potassium, magnesium, manganese and molybdenum in almost all the varieties. There was a small difference in calcium, sulphur, iron, copper, boron and chloride. However, there was no change in zinc content in all the varieties of infested leaves. Disparity shown in majority of the macro nutrients in almost all the mulberry cultivars leads to variation in their quality. This feature of the leaves may hinder the good growth and development of silkworm, in turns producing low quality and poor yield of silk.*

Keywords: macro- and micronutrients, mealy-bugs, mulberry, silkworm, tukra

INTRODUCTION

In sericulture industry, production of quality mulberry leaves, the sole food for silkworm, *Bombyx mori* L. plays an important role in silkworm crop success. By way of producing healthy cocoons of desired norms, the final end product of lustrous, quality silk filament is acquired. In nature, mulberry encounters lot of natural peril, which is major hurdle to produce nutritious leaves. One of the most important hazards is the infestation by different kinds of pests, of which *Maconellicoccus hirsutus* commonly known tukra disease

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or "mealy-bug" causing severe damage and recurring loss in leaf yield of about 3000–6000 kg hectare^{-1} year^{-1} (Chatterjee and Sarkar, 1993). Its infestation leads to changes in morphological and anatomical characters like curling and crinkling of leaves of apical shoots, swelling, and twisting of apical internodes. As a result, the shoots become brittle and the leaves become dark green in color and deformed (Handique and Baruah, 2000). Immature and mature mealy bugs are found in clusters on the stalks under overlapping leaf-sheath, below the node and spread up and down to the other internodes and buds. The damage mainly occurs by sucking cell sap, depriving plants of essential nutrients, which may lead to stunting, yellowing, and thin canes. In addition, mealy bugs play a vital role in virus transmission and the growth of black sooty-mould fungus due to large amount of honeydew secreted by the insect (Eid et al., 2011). The sucking nature of pest infestation is known to adversely alter the nutritive level of the leaves and they are not suitable for silkworm rearing as they are identified to affect the commercial characters of cocoon (Umesh Kumar et al., 1989; Kumar et al., 1992). An effort was made to identify the impact of mealy-bug infestation on the macro and micro nutritive elemental level in the leaves of some popular indigenous mulberry varieties.

MATERIALS AND METHODS

The healthy and mealy-bug-infested leaves of six popular indigenous mulberry varieties M_5, MR_2, Mysore local, S_{36}, S_{54}, and V_1 were collected in butter paper bags from plantations in and around Tumkur district and Kanakapura taluk, Ramanagara district (Karnataka state, India). Five leaves samples (for replication) were collected from the same tree to maintain same maturity level and to avoid difference among the trees. They were washed thoroughly with distilled water and blotted to dry. Later, they were dried in hot air oven for 48 hr at 60–65°C. The dried leaf materials were ground to fine powder separately and later used for analysing the total nitrogen and other mineral elements. The total nitrogen estimated by Micro-Kjeldahl flask by using the procedure of Piper (1966). For mineral analysis one gram of dried mulberry leaf powder was initially digested in 15 mL of nitric acid and then 10 mL of perchloric acid was added. This was digested over sand bath until a clear solution was obtained. It was cooled and the volume was made up to 100 mL with double distilled water. It was filtered through Whatman's No. 1 filter paper (GE Healthcare, Little Chalfont, UK). Aliquots of 25 mL were taken from this solution and the mineral nutrients of phosphorus and potassium were estimated by using Elicol CL 360 Flame Photometer while calcium, magnesium, sulphur, zinc, iron, manganese, copper, boron, molybdenum and chloride were estimated by using Atomic Absorption Spectrophotometer (Martin et al., 1987). Data obtained were analyzed by Student's t - test according to the equation of Dixon and Massey (1957). Significant differences were established at $p < 0.05$ and $p < 0.01$ levels. The information was also subjected to percentage of changes (decrease/increase) in the infested and healthy leaves and was calculated as:

$$\%\text{decrease/increase} = \frac{(\text{Values of healthy leaves} - \text{values of infested leaves})}{(\text{Values of healthy leaves})} \times 100$$

RESULTS AND DISCUSSION

The nutrients of six macro (nitrogen, phosphorus, potassium, calcium, magnesium, and sulphur) and seven micro (zinc, iron, manganese, copper, boron, molybdenum, and chloride) elements showed variation in the mealybug-infested leaves of six popular indigenous mulberry varieties compared to healthy ones.

Macronutrients

Nitrogen (N) is vitally associated with the activity of all living cells of mulberry. It is involved in the synthesis of low molecular weight organic nitrogenous compounds such as amino acids, amides, peptides, amines and urides, which are participate in the synthesis of proteins, nucleic acids and co-enzymes, and energy metabolism. The quality of mulberry foliage is dependent on their N content (Shankar, 1997). In this study, the nitrogen content was reduced in M_5, S_{36}, and S_{54} varieties (Table 1). The maximum (0.99%) and minimum (0.23%) reduction was observed in the leaves of S_{36} and M_5 varieties, respectively. It was increased in MR_2 (0.24%) variety. The nitrogen was not altered in the leaves of Mysore local and V_1 varieties due to tukra disease.

Satya Prasad et al. (2002) observed decrease in the N content in the thrips-infested tender and medium leaves of K_2, S_{13}, S_{34}, and S_{36} mulberry varieties. Narayanaswamy et al. (1999) recorded 35.22% decrease in the N content due to spiralling whitefly attack on mulberry leaves (M_5 var.). The decrease in the N content may be attributed to damage caused by the insect through sucking of the leaf sap, thus altering the metabolic functions leading to either decline in protein synthesis or mobilization of proteins for repair of the damaged tissues in order to develop resistance to insect bite (Satya Prasad et al., 2002). Proteins are the most important constituents in living tissues and account for a large proportion of the total N content. Reduced N level leads to stunted growth of mulberry shoot and root, reduced leaf area, prolonged bud dormancy, and delayed flowering (Sanchez, 2006). Leaf

TABLE 1 Alteration of macro-nutrients in the mealy-bug-infested foliage of mulberry varieties

	Nitrogen (%)		Phosphorous (%)		Potassium (%)		Calcium (%)		Magnesium (%)		Sulphur (%)	
Mulberry variety	Healthy	Infested	Healthy	Infested	Healthy	Infested	Healthy	Infested	Healthy	Infested	Healthy	Infested
M_5	4.30	4.29	0.28	0.25	3.90	3.89	2.70	2.70	0.82	0.80*	0.26	0.24*
	(−0.23)		(−0.71)		(−0.26)		(—)		(−2.44)		(−7.69)	
MR_2	4.15	4.16	0.20	0.18**	2.92	2.92	2.82	2.82	0.63	0.64	0.39	0.39
	(+0.24)		(−10.00)		(—)		(—)		(+1.51)		(—)	
Mysore local	3.89	3.89	0.17	0.15**	3.79	3.75*	2.37	2.35	0.76	0.76	0.37	0.37
	(—)		(−11.76)		(−1.06)		(−0.84)		(—)		(—)	
S_{36}	4.06	4.02	0.18	0.16**	2.69	2.68	2.68	2.68	0.53	0.52*	0.25	0.25
	(−0.99)		(−11.11)		(−0.37)		(—)		(−1.89)		(—)	
S_{54}	2.15	2.13	0.29	0.28*	2.89	2.88	1.95	1.93	0.49	0.49	0.49	0.46*
	(−0.93)		(−3.45)		(−0.35)		(−1.03)		(—)		(−6.12)	
V_1	4.10	4.10	0.39	0.39	4.08	4.06	3.19	3.19	0.89	0.87*	0.32	0.32
	(—)		(—)		(−0.49)		(—)		(−3.57)		(—)	

**Significant at 1% level; *Significant at 5% level; values in the parenthesis () indicate% difference over healthy (+ = more than; − = less than; — = not altered).

proteins form an important source for silkworms to biosynthesize silk, which comprises two proteins, i.e., fibroin and sericin (Rangaswami et al., 1976). A highly significant correlation was found between the N content of leaf and silkworm body weight, cocoon, and shell (Subbarayappa and Bongale, 1997). Derangement in the N level of mulberry leaves due to pest injury obviously causes reduction in the amino acids and protein content. Such leaves when fed to silkworms adversely affect their growth and development.

Phosphorus (P) is an important major nutrient in mulberry plant. It is a component of nucleotides, nucleic acids, phosphatides, and number of co-enzymes. In addition, it has a close relationship with the synthesis of proteins, metabolism of fat and carbohydrates, respiration, photosynthesis, and other metabolic activities (Shree et al., 2005). In the present work, the phosphorous were decreased significantly in the mealy-bug-infested leaves of MR_2, Mysore local, S_{36} and S_{54} and non-significantly in the M_5 variety. The maximum (11.76%) and minimum (0.71%) reduction was observed in the leaves of Mysore local and M_5 respectively. The phosphorus content was not altered in the tukra-infested leaves of V_1 variety. Narayanaswamy et al., (1999) observed a decrease (26.53%) in the P content in the spiralling whitefly infested mulberry leaves (M_5 var.) compared to healthy ones. The P content was decreased (43.14%) in the leaf roller infested mulberry (M_5 var.) foliage (Narayanaswamy, 2003). Variation in the P level affected the uptake of other elements in mulberry leaves, which in turn hampered the growth, and economic characters of silkworm (Ito and Nimura, 1966; Chakrabarti et al., 1997).

The potassium (K) reduced in the leaves of M_5 (0.26%), Mysore local (1.06%), S_{36}, S_{54} and V_1 varieties. It was significant only in Mysore local. No changes were observed in the leaves of MR_2 variety due to *M. hirsutus* infestation. Narayanaswamy et al., (1999) noticed the increased (19.41%) K content in the spiralling whitefly infested mulberry (M_5 var.) leaves. Narayanaswamy (2003) observed reduction (5.61%) in the K content of leaf roller infested mulberry (M_5 var.) foliage. Potassium has a role in carbon assimilation and N metabolism; it activates several kinds of enzymes and controls respiration. It also plays a significant role in high yield (productivity) and quality of leaf (Shree et al., 2005). It is involved in the translocation of carbohydrates and protein metabolism. K improves the thickness and colour of leaves and also disease tolerance particularly to a fungal disease, powdery mildew in mulberry. It has a relationship with the occurrence of viral diseases in mulberry. The deficiency leads to accumulation of hydrogen peroxide in plants, which is toxic, and results in abnormal respiration and catalase activity (Sanchez, 2006). In the silkworm body, strong alkalinity of the gastric juice originates from potassium and sodium compounds present in the heamolymph. The high alkaline condition of digestive fluid has a strong germicidal power against pathogens. K is a unique element that contributes to the growth of silkworms to the maximum extent. In addition, K has a stimulating effect on protein synthesis including silk protein in the silk glands and on the function of ovary (Shankar et al., 1990).

Calcium (Ca) plays an important role in the synthesis of pectin in the middle lamella, which provides firmness and rigidity to cell walls. Its deficiency causes incomplete cell division or mitosis, without formation of new cell wall resulting in multi-nucleatic cells (Bidwell, 1979). It is considered essential for the growth of meristematic tissues and for the functioning of root tips. It acts as detoxifying agent by neutralizing organic acids such as oxalic acid, which helps in membrane stability and maintenance of chromosome structure, activity of enzymes, and translocation of carbohydrates. It is also involved in the differential permeability of membranes (Shankar, 1997). In the present investigation, the calcium was decreased in the leaves of Mysore local (0.84%) and S_{54} (1.03%) varieties. There were no changes in mealy-bug-attacked leaves of M_5, MR_2, S_{36}, and V_1 varieties. Narayanaswamy et al. (1999) noticed a decrease (35.31%) in the Ca content of spiralling whitefly infested mulberry (M_5 var.) leaves. There was decrease (39.52%) in the leaf roller infested mulberry (M_5 var.) foliage over healthy with respect to Ca content (Narayanaswamy, 2003).

Magnesium (Mg), the central atom of chlorophyll, with its specific electron resonance properties to which the organic components of chlorophyll is responsible for photo-reduction and photochemical breakdown of water are attuned, is vital for the process of photosynthesis (Bergmann, 1992). Apart from this, Mg is of importance mainly as a co-factor and activator for many enzymes and substrate transfer reactions (Gunther, 1981). In the present investigation, the magnesium was reduced significantly in the tukra-affected leaves of M_5, S_{36}, and V_1 varieties. The reduction was maximum (3.57%) and minimum (1.89%) in the leaves of V_1 and S_{36} varieties, respectively. It was increased in the leaves of MR_2 (1.51%) and remains unaltered in the leaves of Mysore local and S_{54} varieties even after mealy bug infestation. The Mg content was reduced (23.59%) in the spiralling whitefly infested mulberry (M_5 var.) leaves (Narayanaswamy et al., 1999). Narayanaswamy (2003) observed reduced (27.11%) Mg content in the leaf roller infested mulberry (M_5 var.) foliage. When Mg is passed on to the silkworms, it accelerates their growth and increases the oviposition rate in the adult (Thangavelu and Bania, 1990). Calcium and Mg accelerated the growth of silkworms and reduced the larval duration; decrease in the intake of these elements reduced the body weight of silkworms (Chakrabarti et al., 1997).

Sulphur (S) is known to have an important role in the synthesis of proteins, oils, and vitamins (Epstein, 1972). It plays a vital role in the N metabolism and proper development of mulberry plant tissues (Munirathnam Reddy et al., 1990). It is a constituent of amino acids, cysteine (contains 27% of S), and methionine (contains 21% of S). In the present study, the sulphur was reduced significantly in the leaves of M_5 (7.69%) and S_{54} (6.12%) varieties. No changes were observed in the leaves of MR_2, Mysore local, S_{36}, and V_1 varieties due to *M. hirsutus* infestation. The S content was increased (11.11%) in the spiralling whitefly infested mulberry (M_5 var.) leaves (Narayanaswamy et al., 1999) and it decreased (47.76%) in the leaf roller - infested mulberry (M_5 var.) leaves (Narayanaswamy, 2003). This is because of the association of sulphur amino acids in methionine and cysteine. Methionine forms one of the ten essential amino acids for silk formation in silkworms. Cystine and cysteine are among the non-essential amino acids, the quantitative presence of which influences the formation of fibroin over sericin (Mahadevappa et al., 2001). Deficiency of S level leads to low level of S - containing amino acids, thus reducing protein synthesis. As a result, amino acids without S and amides of nitrate ions accumulate in the plant tissue and lead to decrease in sugar as well as insoluble N (protein) in plants (Munirathnam Reddy et al., 1990).

Micronutrients

Mulberry needs micronutrients like zinc, iron, manganese, copper, boron, molybdenum, and chloride in very small quantities. Their adequate amount and in proper proportion is one of the main factors which govern the growth, development and yield of mulberry, wherein they play an important role in enzymatic reactions. The metal activators in enzymes are nothing but micronutrients (Shankar, 1997). Mealy-bug-attacked mulberry leaves also showed variation in their micronutrient level (Table 2).

The zinc (Zn) content remains same in all the varieties considered for the experiment. In contrasting, Shree and Ravi Kumar (2002) observed a significant increase in the Zn content in tender and medium maturity leaves and decrease in the coarse leaves compared to healthy ones in mulberry plants infested by giant African snails. The assimilation of Zn in V instar silkworm is about 50% of total nutrients among the several elements and is the only micronutrient element that is passed on to the silkworm seed. In addition, Zn is known to increase the pupal weight and filament length (Chakrabarti et al., 1997). Lokanath et al. (1986) have reported that excess of Zn content in mulberry leaf leads to reduction in cocoon yield.

The iron (Fe) is present in the chloroplast proteins and several enzymes. It plays a dominant role in protein metabolism and N fixation (Shankar, 1997). The Fe content was increased (0.44%) in the leaves of S_{36} variety. In the remaining varieties M_5, MR_2, Mysore local, S_{54}, and V_1, the iron level remains same even after mealy bug attacks. In contrast, Shree and Ravi Kumar (2002) noticed a decrease in the Fe content of mulberry (M_5 var.) leaves of all the three maturity levels (tender, medium, and coarse) due to giant African snail attacks. The increase may be due to failure in its translocation to the physiologically active site (Nagaraja, 1987). The altered Fe content in mulberry foliage resulted in the reduced larval weight, cocoon weight, and silk filament length (Shankar, 1997).

TABLE 2 Alteration of micronutrients in the mealy-bug-infested foliage of mulberry varieties

	Zinc (ppm)		Iron (ppm)		Manganese (ppm)		Copper (ppm)		Boron (ppm)		Molybdenum (ppm)		Chloride (%)	
Mulberry variety	Healthy	Infested	Healthy	Infested	Healthy	Infested	Healthy	Infested	Healthy	Infested	Healthy	Infested	Healthy	Infested
M_5	211	211	186	186	213	214	10.12	10.12	26	26	0.39	0.39	1.16	1.12*
	(—)		(—)		(+0.47)		(—)		(—)		(—)		(−3.45)	
MR_2	184	184	324	324	249	249	12.43	12.43	33	33	0.60	0.57*	0.86	0.86
	(—)		(—)		(—)		(—)		(—)		(−5.00)		(—)	
Mysore local	219	219	340	340	253	254	12.30	12.29	31	31	0.57	0.56*	1.45	1.45
	(—)		(—)		(+0.40)		(−0.08)		(—)		(−1.75)		(—)	
S_{36}	243	243	227	228	268	268	10.76	10.76	38	38	0.53	0.51*	1.12	1.12
	(—)		(+0.44)		(—)		(—)		(—)		(−3.77)		(—)	
S_{54}	252	252	346	346	221	219	10.11	10.11	37	36*	0.29	0.29	1.43	1.43
	(—)		(—)		(−0.90)		(—)		(−2.70)		(—)		(—)	
V_1	257	257	230	231	230	230	12.13	12.10	41	41	0.37	0.36*	1.42	1.42
	(—)		(—)		(—)		(−0.25)		(—)		(−2.70)		(—)	

*Significant at 5% level; values in the brackets () indicate% difference over healthy (+ = more than; − = less than; —= not altered).

Manganese (Mn) is essential for the synthesis of chlorophyll. It is not mobile and its principal function is to activate some of the enzyme systems in plant physiology and to some extent regulation of Fe metabolism. In addition, it has a close relation with N metabolism (protein), assimilation of carbohydrates and formation of vitamin C. It is involved in oxidation - reduction processes and electron transport system (Shankar, 1997). In the current study, the manganese content was reduced negligible (0.90%) in the leaves of S_{54} variety. The leaves of M_5 (0.47%) and Mysore local (0.40%) varieties showed increased manganese content. In the leaves of MR_2, S_{36}, and V_1 varieties the manganese remains unaltered due to *M. hirsutus* infestation. The Mn content increased as well as decreased in the giant African snails infested mulberry (M_5 var.) leaves. In the infested medium and coarse leaves, the increase was significant while in the tender leaves it was significantly decreased (Shree and Ravi Kumar, 2002). Iron and Mn have potentiality to enhance the larval (silkworm) development, filament length of single cocoon, cocoon weight and yield (Lokanath et al., 1986).

A number of enzymes with diverse properties and functions are dependent on copper (Cu) and the metal is strongly found in many proteins especially chloroplast proteins (Shankar, 1997). In the present investigation, The copper content remained the same in almost all the samples of the mealybug-infested mulberry leaves except leaves of Mysore local (0.08%) and V_1 (0.25%) varieties, where it was reduced. Shree and Ravi Kumar (2002) noticed a decrease in the copper content of tender, medium, and coarse leaves of giant African snails infested leaves of M_5 mulberry cultivar.

The boron (B) content was reduced significantly in the leaves of S_{54} (2.70%) variety. It was not changed in the leaves of M_5, MR_2, Mysore local, S_{36}, and V_1 varieties. Boron (B) plays an essential role in the growth and development of new cells in plant meristems. This element bears a close relation with the translocation of carbohydrates and protein synthesis. In addition, the phenol metabolism and auxin activity is also regulated by B. It is associated with the uptake of Ca and its utilization. It also regulates the K and Ca ratio in plants (Shankar, 1997).

Molybdenum (Mo) has a close association with N utilization and metabolism in plants by regulating two important enzymes, nitrate reductase and nitrogenase. In addition, it also reduces protein metabolism in combination with other micronutrients especially Fe (Shankar, 1997). In the present investigation, the molybdenum reduced significantly in tukra-affected leaves of MR_2, Mysore local, S_{36}, and V_1 varieties. The maximum (5.00%) and minimum (1.75%) reduction was observed in the tukra infested leaves of MR_2 and Mysore local varieties, respectively. It was not altered in the leaves of M_5 and S_{54} varieties.

The chloride (Cl) content was remains normal even after *M. hirsutus* infestation in the leaves MR_2, Mysore local, S_{36}, S_{54}, and V_1 varieties. But it was decreased significantly in the tukra-infested leaves of M_5 (3.45%) variety. Chloride is involved in photosynthesis, synthesis of starch, cellulose and lignin. It influences water holding capacity of plant tissues. It stimulates the activities of some enzymes. It is not readily mobile in plants (Shankar, 1997).

From the present investigation it is clear that mineral nutrition of the host is known to be impaired by pest infestation. This may by due to direct plundering by the pest or indirect effects of the pest on absorption, mobilization, etc., (Vamseedhar et al. 1999). Most sap sucking insects use a specialized mouth part, the stylet, to locate, penetrate, and drain sap from the phloem sieve elements of the plants vascular tissue. Heavy infestation by sap sucking insects cause chronic shortages of photosynthates and thus severely reduces the growth potential of the plant (Kim Hammond-Kosack et al., 2000). The spiralling whitefly is a phloem sap feeder and its direct consumption of transportable carbohydrate and other nutrients carried in phloem reduces productivity of host plants by competing for available nutrients and causing premature leaf shedding (Bryne et al., 1990). Similar results of variation in mineral nutrition were observed in other cases when the leaves were infested by mealy bugs (Mahadeva and Shree, 2006) and jassids (Mahadeva et al., 2006).

If the mineral content is increased due to infestation/infection, it induces toxicity symptoms not only in mulberry plants, but also in silkworms when they are fed on such leaves. Similarly, if an element is decreased due to infestation/infection, it causes deficiency or physiological disorders in leaves and they become malformed, deformed, chlorotic and nutritionally inferior. Obviously, the increase or decrease in the mineral content(s), affects the growth and development of silkworms, which consequently alters the quality of silk produced (Ito and Nimura, 1966; Shree et al., 2005). Thus, mineral nutrition of mulberry foliage has a decisive role in the production of good quality cocoons. Growth and development of silkworms depend on the nutritive status of leaves. If there is an imbalance in elemental contents (mineral nutrition), the leaf quality is severely deteriorated. This could be detrimental to silkworms. Mahadeva and Shree (2005) fed the silkworm with spiralling whitefly infested mulberry leaves and observed its negative effects on them in respect of growth and cocoon parameters. Therefore, farmers must reject them as silkworm "feed" and try to protect mulberry plants from the mealy bug attacks by following suitable IPM methods.

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