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Impact of leaf roller (*Diaphania pulverulentalis*) infestation on the mineral nutrition of mulberry (*Morus* spp.) varieties

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Abstract

Mulberry (*Morus* spp.) foliage is the exclusive food for the silkworm, *Bombyx mori* L. The healthy growth of silkworm and its economic traits such as larval, cocoon and grainage parameters are known to be influenced largely by the nutritional status of the leaves fed to them. The mineral elements comprise major part of nutritional quality of mulberry leaves, which may alter due to injury caused by pests and diseases. Therefore, an attempt was made to know the variations in the mineral nutrition of mulberry foliage under leaf roller (*Diaphania pulverulentalis*) infestation. Six macro (nitrogen, phosphorus, potassium, calcium, magnesium and sulphur) and seven micro nutrients (zinc, iron, manganese, copper, boron, molybdenum and chloride) were analysed in six popular indigenous mulberry varieties (M_5, MR_2, Mysore local, S_{36}, S_{54} and V_1). The nutrients decreased in the pest - infested leaves of almost all the varieties except nitrogen and potassium in Mysore local and V_1, phosphorus in S_{36} and V_1, magnesium in M_5 and Mysore local and sulphur in V_1, zinc in S_{36} and S_{54}, boron in S_{36} and chloride in Mysore local varieties where they were increased. However, no changes were observed in the contents of nitrogen and potassium in MR_2, phosphorus in MR_2 and Mysore local, calcium in M_5, S_{36} and V_1, magnesium in S_{54} and V_1 and sulphur in M_5, S_{36} and S_{54}, zinc in V_1, iron in M_5, Mysore local and V_1, manganese in M_5, copper in S_{54} and V_1, boron in M_5, MR_2, Mysore local and V_1, molybdenum in M_5, MR_2, S_{54} and V_1 and chloride in M_5, S_{54} and V_1 varieties. These variations in the mineral nutrients results in the nutritional inferiority of mulberry leaves. Feeding such leaves will affect the growth and development of silkworms which in turn adversely brings down the quality of silk production.

Key words : leaf roller, mineral, mulberry, nutrition.

INTRODUCTION

Mulberry (*Morus* spp.) forms the exclusive food plant of silkworm, *Bombyx mori* L. Hence, production of an appreciable quantity of superior quality mulberry foliage assumes great significant in order to realise silkworm rearing a profitable venture. However, the process of mulberry leaf production is often hampered due to the interference caused by insect and non-insect pests. Leaf roller, *Diaphania pulverulentalis* (Hampson) (Lepidoptera: Pyralidae) is one such serious pest causes a foliage loss upto 12.18 % [1]. The target site of leaf roller - infestation is the apical portion, un-opened tender leaf of mulberry shoot. The infested leaves are brought together and bound through silky salivary secretion produced by the larva. Sometimes, a single leaf is rolled into folded shape with the web from the larva which remains inside. Hence, the pest is commonly called as 'leaf roller'. It devours the soft green tissues and later damages the apical shoot leading do drying up of either part and results in stunted growth of the plant [2]. The pest not only reduces the yield but also alters the nutritional level of the foliage. So, an attempt was made to know the changes in the macro and micro nutrients in the leaf roller - infested mulberry foliage.

MATERIALS AND METHODS

The healthy and leaf roller - infested leaves of six popular indigenous mulberry varieties *viz*., M_5, MR_2, Mysore local, S_{36}, S_{54} and V_1 were collected in butter paper bags from mulberry plantations. They were washed thoroughly in distilled water and blotted to dry. Later, they were dried in hot air oven at a temperature of 60^0 - 65^0C for 48 hrs. The dried leaf materials were grinded to fine powder and later used for analysing the total nitrogen and mineral elements. The total nitrogen estimated by Micro-Kjeldahl flask by using the standard procedure[3]. 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 deionised water. It was filtered through Whatman No. 1 filter paper. Aliquots of 25 ml were taken from this solution and the mineral nutrients 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 (GBC 932 plus)[4] . The results were analysed statistically by applying Student's t-test.

RESULTS

The six macro - nutrients (nitrogen, phosphorus, potassium, calcium, magnesium and sulphur) and seven micro - nutrients (zinc, iron, manganese, copper, boron, molybdenum and chloride) were altered in the leaf roller - infested leaves of six popular indigenous mulberry varieties (*M_5, MR_2, Mysore local, S_{36}, S_{54} and V_1)* compared to their healthy ones.

Macro - nutrients (Table 1)

Macronutrients play a very important role in plant growth and development. Their functions range from being structural units to redox-sensitive agents. In the present investigation, the Nitrogen (N) content was decreased in the leaf roller - infested foliage of M_5, S_{36} and S_{54} varieties. But it was significant only in the S_{54} var. The reduction was in the range 0.48 % to 1.49 % in M_5 and S_{54} varieties. It was increased in Mysore local and V_1 varieties. It was low (0.22 %) in V_1 and relatively high (0.40 %) in Mysore local

var. However, there was no change in MR_2 var. inspite of leaf roller - infestation. Phosphorus (P) content was decreased in the leaves of M_5 and S_{54} varieties in the range of 7.14 % to 7.41 % respectively. It was significantly only in the S_{54} var. It was increased with a minimum (5.26 %) in S_{36} and maximum (5.56 %) in V_1. But, P was not altered in the leaves of MR_2 and Mysore local varieties due to pest - infestation. The leaf roller infested leaves of M_5 and S_{36} varieties shows decreased potassium (K) content. The decrease was significant in the S_{54} var. It was in the range of 1.14 % to 1.65 % in S_{36} and M_5 varieties respectively. The K content was increased in Mysore local (0.66 %) and V_1 (0.26 %) varieties. Calcium (Ca) content was decreased in MR_2, Mysore local and S_{54} varieties. It was minimum (0.65 %) in MR_2 and maximum (1.47 %) in S_{54} varieties. Whereas, in the remaining varieties *viz*., M_5, S_{36} and V_1 there was no alteration in their Ca content inspite of leaf roller - infestation. The MR_2 (7.69 %) and S_{36} (2.17 %) varieties showed decrease in their Magnesium (Mg) content due to leaf roller - infestation. It was increased in the range of 3.57 % to 7.14 % in M_5 and Mysore local varieties respectively. However, there was no change in Mg content in S_{54} and V_1 varieties. Sulphur (S) content was decreased in MR_2 (10.34 %) and Mysore local (2.17 %) varieties. Whereas, it was increased (2.56 %) in V_1 var. But, there was no change in M_5, S_{36} and S_{54} varieties inspite of pest - infestation.

Micro - nutrients (Table 2)

Mulberry needs micronutrients like zinc, iron, manganese, copper, boron, molybdenum and chloride in very small quantities.

Table 1: Changes in the macro - nutrients (%) of leaf roller - infested mulberry foliage.

Mulberry Varieties	Nitrogen		Phosphorus		Potassium		Calcium		Magnesium		Sulphur	
	Healthy	Infested	Healthy	Infested	Healthy	Infested	Healthy	Infested	Healthy	Infested	Healthy	Infested
M_5	4.20	4.18	0.14	0.13	1.21	1.19	1.31	1.31	0.28	0.29	0.30	0.30
	(-0.48)		(-7.14)		(-1.65)		(----)		(+3.57)		(----)	
MR_2	3.90	3.90	0.26	0.26	1.29	1.29	3.08	3.06	0.39	0.36	0.29	0.26
	(----)		(----)		(----)		(-0.65)		(-7.69)		(-10.34)	
Mysore local	2.53	2.54	0.21	0.21	3.01	3.03	2.76	2.73	0.42	0.45	0.46	0.45
	(+0.40)		(----)		(+0.66)		(-1.09)		(+7.14)		(- 2.17)	
S_{36}	3.56	3.52	0.19	0.20	1.76	1.74	2.25	2.25	0.76	0.74	0.16	0.16
	(-1.12)		(+5.26)		(-1.14)		(----)		(-2.63)		(----)	
S_{54}	3.36	3.31**	0.27	0.25*	1.99	1.96*	1.36	1.34	0.82	0.82	0.22	0.22
	(-1.49)		(-7.41)		(-1.51)		(-1.47)		(----)		(----)	
V_1	4.60	4.61	0.18	0.19	3.81	3.82	2.11	2.11	0.69	069	0.39	0.40
	(+0.22)		(+5.56)		(+0.26)		(----)		(----)		(+2.56)	

** - Highly significant at 1% level ; * - Significant at 5 % level; Values in the parenthesis indicate % difference over healthy (+ = more than; - = less than; ---- = not altered).

Table 2: Changes in the micro - nutrients of leaf roller - infested mulberry foliage.

Mulberry Varieties	Zinc (ppm)		Iron (ppm)		Manganese (ppm)		Copper (ppm)		Boron (ppm)		Molybdenum (ppm)		Chloride (%)	
	Healthy	Infested	Healthy	Infested	Healthy	Infested	Healthy	Infested	Healthy	Infested	Healthy	Infested	Healthy	Infested
M_5	96	95	301	301	70	70	7.90	7.86	47	47	0.13	0.13	0.70	0.70
	(-1.04)		(----)		(----)		(-0.50)		(----)		(----)		(----)	
MR_2	210	209	296	294	210	208	8.12	8.10	32	32	0.60	0.60	0.96	0.95
	(-0.48)		(-0.68)		(-0.95)		(-0.25)		(----)		(----)		(-1.04)	
Mysore local	116	114	126	126	196	194	12.25	12.19	41	41	0.42	0.39	1.20	1.21
	(-1.72)		(----)		(-1.02)		(-0.49)		(----)		(-7.14)		(+0.83)	
S_{36}	171	173	210	208	141	138	10.06	10.02	26	28	0.26	0.25	1.70	1.69
	(+1.17)		(-0.95)		(-2.13)		(-0.40)		(+7.69)		(-3.85)		(-0.59)	
S_{54}	192	193	251	249	153	152	8.36	8.36	29	28	0.29	0.29	1.30	1.30
	(+0.52)		(-0.80)		(-0.65)		(----)		(-3.44)		(----)		(----)	
V_1	169	169	284	284	128	126	9.94	9.94	25	25	0.30	0.30	1.12	1.12
	(----)		(----)		(-1.56)		(----)		(----)		(----)		(----)	

Values in the parenthesis indicate % difference over healthy (+ = more than; - = less than; ---- = not altered).

The presences of micronutrients were varied in leaf roller infested mulberry leaves compare to healthy ones. The zinc content was decreased in M_5, MR_2 and Mysore local varieties. The reduction was in the range of 0.48 % to 1.72 % in MR_2 and Mysore local varieties respectively. It was increased in S_{36} and S_{54}, but not altered in V_1 var. Zinc increase was negligible (0.52 %) in S_{54} and maximum (1.17 %) in S_{36} var. due pest - infestation. The Iron (Fe) content was decreased in MR_2, S_{36} and $S_{54,}$ but not altered in M_5, Mysore local and V_1 varieties. The reduction of Fe was very less (0.68 %) in MR_2 and maximum (0.95 %) in S_{36} var. due to leaf roller - infestation. Manganese content was decreased in all the mulberry varieties except M_5 var. where it showed no change inspite of leaf roller - attack. The minimum (0.65 %) and maximum (2.13 %) decrease of manganese was noticed in S_{54} and S_{36} varieties respectively. The copper (Cu) content was reduced in the leaves of M_5, MR_2, Mysore local and S_{36}, but it was not changed in the leaves of S_{54} and V_1. The decrease was negligible (0.25 %) in MR_2 and relatively maximum (0.50 %) in M_5. The boron (B) content was decreased (3.44 %) in S_{54} and increased (7.69 %) in S_{36} var. due to leaf roller. It was not changed in the leaves of M_5, MR_2, Mysore local and V_1 varieties. Molybdenum (Mo) content remained unchanged in all the mulberry varieties studied except Mysore local and S_{36} which recorded a reduction. It was minimum (3.85 %) in S_{36} and maximum (7.14 %) in Mysore local due to leaf roller - infestation. The chloride (Cl) content was decreased in MR_2 and S_{36} but, slightly increased (0.83 %) in Mysore local var. The reduction was minimum (0.59 %) in S_{36} and maximum (1.04 %) in MR_2 var. It remained unchanged in M_5, S_{54} and V_1 inspite of leaf roller infestation.

DISCUSSION

Nitrogen (N) is vitally associated with the activity of all living cells of mulberry. The quality of mulberry foliage is dependent on their N content[5]. It was 35.22 % decrease in the N content due to spiralling whiteflies - attack on mulberry leaves *(M_5 var.)*[6]. It has been 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. But, it was decreased as well as increased in the tender and medium leaves of V_1 var. respectively[7]. It was noticed a reduction (39.90 %) in the N content of leaf roller - infested mulberry (M_5 var.) foliage[8]. The decrease in the N content may be attributed to damage caused by the insect through scratching the mesophyll of the leaf 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[7]. Reduced N level leads to stunted growth of mulberry shoot, leaf and root. Leaf area and shoot production is reduced, bud dormancy is prolonged and flowering is delayed[9]. A highly significant correlation was found between the N content of leaf and weight of silkworm body, cocoon and shell[10]. 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 a component of nucleotides, nucleic acids, phosphatides and number of co-enzymes. In addition, it has a close relation with the synthesis of proteins, the metabolism of fat and carbohydrates, respiration, photosynthesis and other metabolic activities[11]. There was a decrease (26.53 %) in the P content in the spiralling whiteflies - infested mulberry leaves (M_5 var.) compared to healthy ones[6]. The P content was decreased (43.14 %) in the leaf roller - infested mulberry (M_5 var.) foliage[8]. Variation in the P level affects the uptake of other elements in mulberry leaves, which in turn hampers the growth, and economic characters of silkworm[12][13].The K content was increased (19.41 %) in the spiralling whiteflies infested mulberry (M_5 var.) leaves[6]. It was observed a reduction (5.61 %) in the K content of leaf roller - infested mulberry (M_5 var.) foliage[8]. K has a role in carbon assimilation and N metabolism; activates several kinds of enzymes and controls respiration. It also plays a significant role in high yield (productivity) and quality of leaf[11]. 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 powdery mildew in mulberry. It has 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[9]. In the silkworm body, strong alkalinity of the gastric juice originates from K and sodium compounds present in the blood. The high alkaline condition of digestive fluid has a strong germicidal power against pathogens. K is a unique element which contributes for the growth of silkworms to 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[14]. 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[15]. It was noticed a decrease (35.31 %) in the Ca content of spiralling whiteflies - infested mulberry (M_5 var.) leaves[6]. There was a decrease (39.52 %) in the leaf roller - infested mulberry (M_5 var.) foliage over healthy with respect to Ca content[8]. 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[16]. The Mg content was reduced (23.59 %) in the spiralling whiteflies - infested mulberry (M_5 var.) leaves[6]. It has been observed a reduced (27.11 %) Mg content in the leaf roller - infested mulberry (M_5 var.) foliage[8]. When Mg is passed on to the silkworms, it accelerates their growth and increases the oviposition rate in the adult[17]. Ca 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[13]. Sulphur (S) is known to have an important role in the synthesis of proteins, oils and vitamins[18]. The S content was increased (11.11 %) in the spiralling whiteflies infested mulberry (M_5 var.) leaves[6] and it decreased (47.76 %) in the leaf roller - infested mulberry (M_5 var.) leaves[5]. This is because of the association of sulphur - amino acid *viz.,* methionine and cystine, 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[19]. 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[20].

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[5]. Zn content significantly increased in the tender and medium maturity leaves whereas significant decrease was noticed in the coarse leaves compared to healthy ones when mulberry plants were infested by giant African snails[21]. The assimilation of Zn in fifth instar silkworm is about 50 % of total nutrients among the several elements and is the only micronutrient element which is passed on to the silkworm seed. In addition, Zn is known to increase the pupal weight and filament length[13]. It was reported that excess of Zn content in mulberry leaf leads to reduction in cocoon yield[22]. Iron (Fe) is present in the chloroplast proteins and several enzymes. It plays a dominant role in protein metabolism and N fixation[5]. It was 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 snails attack[21]. The reduction in the Fe level may be due to its competition with other iron-binding compounds[23]. The increase may be due to failure in its translocation to the physiologically active site[24]. The altered Fe content in mulberry foliage resulted in the reduced larval weight, cocoon weight and silk filament length[5]. Manganese (Mn) is essential for the synthesis of chlorophyll. It is involved in oxidation reduction processes and electron transport system[5]. Both increase as well as decrease in the Mn content was observed in the mulberry (M_5 var.) leaves due to giant African snails infestation *i.e*., it caused significant increase in the Mn content of medium and coarse leaves, while it was significantly decreased in the tender leaves when compared to healthy ones[21]. Fe and Mn have potentiality to enhance the larval (silkworm) development, filament length of single cocoon, cocoon weight and yield[22]. 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[5]. It was noticed a decrease in the copper content of tender, medium and coarse leaves of giant African snails infested mulberry (M_5 var.) plants[21]. Boron (B) plays an essential role in the growth and development of new cells in plant meristems. This element bears 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 K and Ca ratio in plants[5]. Molybdenum (Mo) has a close association with N utilization and metabolism in plants by regulating two important enzymes viz., nitrate reductase and nitrogenase. In addition, it also reduces protein metabolism in combination with other micronutrients especially Fe[5]. Chloride (Cl) 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[5].

Mineral nutrition of the host is known to be impaired by pest - infestation. This may be due to direct plundering by the pest or indirect effects of the pest on absorption, mobilization, *etc*.,[25]. Similar results of variation in mineral nutrition were observed in other cases when the leaves were infested by spiralling whiteflies[26], mealy bugs[25][27] and jassids[28]. 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[11, 12, 13, 14, 22, 29].

CONCLUSION

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. 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. Thus, mineral nutrition of mulberry foliage has a decisive role in the production of good quality cocoons. Therefore, the farmers must protect mulberry plants from the "leaf roller" attack by following suitable eco-friendly Integrated Pest Management (IPM) practices.

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