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Changes in Oxalic Acid Content During Leaf Senescence in Sericultural Crop *Morus Alba* Linn

KEYWORDS: Oxalic acid content, *Morus alba* Linn.

S. K. Khade

Department of Botany, Dattajirao Kadam Arts Science and Commerce College, Ichalkaranji, Dist: Kolhapur. (Maharashtra) – 416 115.

ABSTRACT *Attempt has been made to study changes in the Oxalic acid content during leaf senescence in mulberry (Morus alba Linn.). The leaf senescence was accompanied with the changes in Oxalic acid content of the young, mature and senescent leaves of mulberry cultivars namely M5 (K2), V1 and S36 are recorded in the Figure. It is evident from the figure that, the oxalic acid content in mature leaves is higher than that young leaves of all the three cultivars. In the senescent leaves a reduction in oxalic acid level is noticeable in all the three cultivars.*

INTRODUCTION-

Mulberry *(Morus alba* Linn.) leaves are used as food while rearing monophagous silkworm, *Bombyx mori* L. (Ullal and Narasimhanna,1981). Cacoon production depends mainly on nutrient composition of mulberry leaves. (Krishnaswami *et al.*, 1971; Bhuyian, 1981). Health and growth of the larvae, cacoon quality and raw silk quality are influenced by quality of leaf. Since, the physiological status of mulberry leaf is important in determining the nutritional quality; the age of leaf may influence silkworm feeding. Ganga (2003) suggested that, over mature and yellow leaves with low protein content should be discarded to other nutritious feed to the worms. During present study nutritional constituents of young, mature and senescent leaves from three cultivars of mulberry (viz- M5, V1 and S36) studied has been compared.

MATERIAL AND METHOD -

The oxalic acid content in young, mature and senescent leaves of mulberry were estimated following the method of Abaza *et al.,* (1968). One gram oven dried plant material, 10ml 3N HCl and 65ml double distilled water were taken in a volumetric flask for oxalic acid estimation. The flasks were kept for digesting the plant material for 1hr on boiling water bath. Then flasks were cooled and diluted to 100ml volume and filtered through Whatman No. 1 filter paper. Two aliquots of 50ml extract were placed in 150ml beakers and in each beaker 20ml 6N HCl were added to increase acidity and avoid pectin retention. Then the mixture was evaporated to half volume and filtered through Whatman No. 1 filter paper and precipitate was washed several times with warm double distilled water. To this filtrate 3-4 drops of methyl red indicator (1g methyl red in 100ml alcohol) and then concentrated ammonia solution was added until solution turned faint yellow. Then this solution was heated to 90-100^{O}C carefully on water bath, cooled and filtered to remove interfering ferrous ions containing precipitate. The filtrate was heated to 90-100^{O}C on water bath and then 10ml 5% $CaCl_2$ was immediately added with 20-25 drops of ammonia solution to restore yellow colour. This solution was allowed to settle overnight and on next day, filtered through Whatman Filter Paper No. 44 (ashless). The precipitate was washed several times with double distilled water to make free from Ca (to check whether the ppt if free from Ca^{++}, 3ml of washing filtrate was taken in test tube and it was added with few drops of 5% sodium oxalate. The turbidity indicated presence of Ca^{++} and demanded further washing of ppt). Then filter paper containing ppt was dissolved in hot 1:5 H_2SO_4 and this was diluted to 125ml with double distilled water and transferred to 250 ml conical flask. The content of the conical flask was heated to 90 – 100^{O}C and carefully titrated with 0.05N $KMnO_4$. The percentage of oxalate was calculated by using following formula,

$$\% \text{ of oxalate} = \frac{\text{ml } KMnO_4 \times 0.05 \times 45.02 \times 100}{1000 \times \text{dry weight} \times 50/100}$$

RESULT AND DISSCUSSION-

Oxalic acid content of the young, mature and senescent leaves of mulberry cultivars namely M5 (K2), V1 and S36 are recorded in the Fig.1. It is evident from the figure that, the oxalic acid content in mature leaves is higher than that young leaves of all the three cultivars. In the senescent leaves a reduction in oxalic acid level is noticeable in all the three cultivars. Zindler Frank (1974) stated that aspartate former C_4 monocots accumulate oxalate salt while, malate formers C_4 monocots do not accumulate this organic acid. On the other hand, malate former dicots accumulate oxalic acids. The level of oxalic acid in oxalic acid accumulating species is in the range 7.2 to 9.1 % (Mathams and Sutherland, 1952 and Vityakon and Standal, 1989). It is evident from our observations that, the total oxalic acid content in mulberry leaves is relatively low when compared with that of oxalic acid accumulating species. Oxalic acid is present in two dominant fractions i.e. soluble and insoluble forms. According to Vityakon and Standal (1989), the soluble fractions consist mainly of K- oxalate and Mg- oxalate, while, the dominant cation in insoluble fractions is Ca, suggesting that most of this fraction is Ca- oxalate with small amounts of Mg. Oxalic acid is reported to be synthesized from several compounds in plants and these include oxaloacetate, glycolate, glyoxylate and ascorbic acid (Zindler Frank, 1974; Raven *et al.,* 1982 and Franceschi, 1987). According to Wagner (1981), oxalate is confined to vacuoles and can be used as marker for vacuoles. Oxalic acid is recognized by many plant researchers as a metabolic end product that undergoes little further metabolism. But Franceschi, (1987) found that significant radioactive label from oxalic acid was incorporated into starch in the light. He gives possible explanation that oxalic acid can be decarboxylated and the CO_2 released can be refixed and enters the carbon pool. In some halophytes, oxalic acid represents the major organic anion balancing excess cation content (Waisel, 1972). High oxalate levels in leaves of *Bassia uniflora, Chenopodium auricomum, Kochia pyrimi-*

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data and Salsola kali were associated with high inorganic cation concentrations but there was no relation between calcium concentration and oxalate level (Osmond, 1967). Allan and Trewavas (1987) found that active calcium uptake in the vacuole through Ca ATPase is dependent on oxalate. Trinchant *et al.,* (1994) found a high level of oxalate in the nodule cytosol in case of broad bean and suggested that oxalate may play a role as carbon substitute to support acetylene reduction (nitrogen fixation) and respiration in symbiosomes and free bacteriods in presence of leghaemoglobin. There is another possible implication of the observations made in the present study indicating relatively low level of oxalic acid in the leaves. Oxalic acid has been recognized as an antinutritional factor in vegetable foliage by some workers (Marshall *et al.,* 1967; Prakash and Pal, 1991 and Saigusa, 1999). According to Carlsson (1995), several of the secondary plant substances and vacuolar stored nitrate and oxalic acid, have a negative influence on leaf nutrient concentrate (LNC) quality, as they reduce its nutritive value. Such substances are called antinutritive substances. He further suggested that if these and other toxic or antinutritive substances are mixed they can contaminate the final LNC and reduce its original high quality. Aaron *et al.,* (2006) noticed that, the Ca-oxalate in *Vitis labrusca* protects against herbivory and develops Ca-oxalate crystals in malphigian tubules of *B. mori* L. after feeding Further they hypothesized that in case of grape (*Vitis labrusca*) Ca- oxalate crystals serve a number of functional roles including protection against herbivory, detoxification of heavy metals and as a mechanism of remove excess calcium from the cytosol. Conversely, silkworms well known for their important economic role in silk production, display an interesting physiological process were up to they develop Ca-oxalate crystals within their malphigian tubules, which developmentally and functionally resemble mammalian kidneys. In *Setaria sphacelata*, it was observed that the leaves continued to accumulate oxalate after reaching maturity and it is speculated that anions other than nitrate must be responsible for the cation excess which promotes the synthesis of this oxalate (Grattan and Warrington, 1976). Frank (1969) studied the content of oxalate, nitrogen and dry weight during the development of leaf in *Canavalia ensiformis.*

The values presented in the part –'Results and Discussion' represent average of three independent determinations.

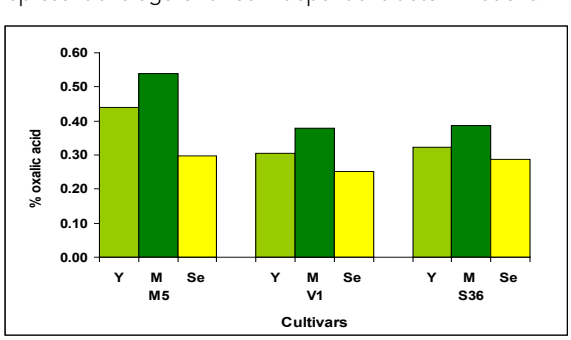

Fig. 1: Changes in oxalic acid content during leaf senescence in sericultural crop
Morus alba **Linn. (Y = Young, M = Mature and Se = Senescent)**

CONCLUSION-

In case of mulberry leaves there is increase in calcium level in the senescent leaves but this is not accompanied by increased synthesis of oxalic acid. In plants oxalic acid is oxidized by the enzyme oxalate: O_2 oxido reductase to CO_2 and H_2O_2. The reduction of oxalate level in the senescent leaves may probably take place through elevation of this enzyme activity which in turn can enhance H_2O_2 production there by increasing the oxidative stress.

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