

SINGLE AND MULTIPLE SECONDARY NUTRIENT DEFICIENCY EFFECTS ON FLUE-CURED TOBACCO

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With the introduction of high yielding varieties of crops, nutrient removal from the soil is increasing day by day. Though the primary nutrients (N, P and K) are applied liberally to offset their removals from soils, the secondary nutrients (Ca, Mg and S) are seldom used in adequate quantities in crop production to replenish their reserves in soils. This situation often results in occurrence of single and/or multiple secondary nutrient deficiencies that may affect the nutrition of crops. Against this backdrop, the role of single and multiple secondary nutrient deficiencies were studied on growth, development and nutrient uptake of FCV tobacco in sand culture experiment. Uniform tobacco seedlings of variety Kanchan were planted in pots filled with sand and were supplied with different combinations of nutrients using Hoagland solution. The treatments included six types of secondary nutrient stresses (Ca, Mg, S, Ca + Mg, Ca + S, Mg + S and Ca + Mg + S). At the age of 45 days, observations on morphological, physiological and biochemical characters were recorded. The leaf and stem samples were processed and analyzed for N, P, K, Ca, Mg and S. Results revealed that secondary nutrient stress caused marked adverse effects on the plant in terms of visual deficiency symptoms, reduced plant growth and nutrient uptake. Single and multiple nutrient deficiencies reduced total plant weight, leaf area, and total chlorophyll content. Secondary nutrient stress was associated with the elevated concentration of anti-oxidative enzymes. The reduction in growth and nutrient uptake due to single and multiple stresses are in the order of control <Ca < Mg <S < Ca + S<Ca + Mg < Mg + S <Ca + Mg + S. Secondary nutrient deficiencies influenced the nutrient ratios which affect the quality of leaf. Hence work on secondary nutrients in field conditions will help to understand the role of secondary nutrients on tobacco leaf yield, quality and also facilitates the demand driven fertilizer usage that in turn saves input expenditure to some extent and also protects environment.

INTRODUCTION

The intensification of agriculture in India has led to the increasing removal of secondary and micronutrients and multiple nutrient deficiencies are becoming major constraint to further increase in production. This is due to nutrient removals far in excess of nutrient additions. The number of elements deficient in Indian soils increased (Sammi Reddy *et al.*, 2007) from one in 1950 to 9 in 2005 which might also increase by the year 2025 if the imbalanced fertilization continues. Usually primary nutrients (N, P, and K) are recommended and managed in the cultivation of all crops but secondary nutrients are not given consideration while making fertilizer recommendations and are not managed which are important in getting good yield with desirable leaf quality. As the tobacco being grown for its leaf, it is important to understand the influence of single and multiple secondary nutrient deficiencies to provide balanced nutrition to the crop. With this background, the present study has been taken up to find out the influence of single and multiple secondary nutrient deficiencies on plant growth, development metabolism and nutrient composition in tobacco.

MATERIALS AND METHODS

Uniform tobacco seedlings of variety Kanchan were planted in pots containing Godavari river sand and treated with one dose of modified Hoagland solution (Jhonson *et al.*, 1957) containing all the nutrients. The plants were allowed to grow for a week. Later treatments viz., Ca, Mg, S, Ca & Mg, Ca & S, Mg & S and Ca, Mg & S stresses were imposed with different nutrient combinations using Hoagland solution. The plants were supplied with

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treatment solutions at weekly intervals and allowed to grow. At the age of 45 days green leaf samples were analyzed for chlorophylls using Dimethyl sulphoxide (Hiscox and Israelstam, 1979) *in vivo* nitrate reductase activity (Kapoor and Paul, 1984) activity of enzymes viz., Poly Phenol, Acid phosphatase, Super-oxide dismutase, Thiobarbuteric acid and phenol content (Sadasivam and Manikam, 1991). Later the plants were uprooted and leaf area was recorded. Then plant parts were dried in hot air oven and weights were recorded. The leaf and stem samples were processed and analysed for total nitrogen (AOAC, 1950,) Phosphorus, potassium (Jackson, 1967) calcium and magnesium using Atomic Absorption Spectrometer (Jackson, 1967) and Sulphur (Tandon, 1993). From the data shoot nutrient uptake and leaf nutrient ratios were computed. The statistical analysis of data was carried using MSTATC software.

RESULTS AND DISCUSSION

Secondary nutrients viz., calcium, magnesium and sulphur play important role in growth and development of tobacco plant. Calcium, a structural component of plant cell walls, is the most abundant in plant leaves. It is involved in cell growth, both at the plant terminal and at the root tips and apparently enhances uptake of nitrate-N. Magnesium plays critical role in nearly all parts of plant metabolism. It is a constituent of chlorophyll. Sulphur is essential for plant growth and is a structural component of protoplasm. Short supply of any of these nutrients alone or in combination leads to adverse effects on

cellular metabolism, growth and development of plants.

Plant growth and metabolism

Single and multiple nutrient stresses showed visual deficiency symptoms, reduction in leaf area and biomass production (Table.1). The reduction in leaf area is 15-29% due to single secondary nutrient stress and 31-62% due to multiple nutrient stresses (Ca + Mg, Ca + S, Mg + S and Ca + Mg + S). Single secondary nutrient stress (Ca, Mg and S) individually reduced total plant weight by 26-32% and in combination with other secondary nutrients (Ca + Mg, Ca + S, Mg + S and Ca + Mg + S) caused 34-68% reduction in total plant weight. The reduction in growth is caused as the secondary nutrient deficiencies prevent plants from utilizing primary nutrients to their fullest extent which ultimately stunts growth (Bekele and Beehan, 2021 and Chaudhry *et al.*, 2021) in turn productivity.

Secondary nutrient stress influenced the total chlorophyll content (Fig 1), activity of *in vivo* nitrate reductase, poly phenol oxidase, acid phosphatase, super oxide dismutase and thiobarbuteric acid and phenol content (Table 2). Total chlorophyll content is reduced due to Magnesium, Sulphur, and all the treatments of multiple secondary nutrient stresses. *In vivo* nitrate reductase activity and acid phosphatase were reduced due to single and multiple stresses whereas, poly phenol oxide activity and super oxide dismutase activity were increased with the single and multiple secondary nutrients stresses. Thiobarbuteric acid content and phenol content also increased with single and multiple

Table 1: Influence of single and multiple secondary nutrient deficiencies on biomass production

Nutrient deficiency	Total plant weight (g plant ⁻¹)	Leaf area (m ² plant ⁻¹)
Control	82.00	0.7335
Ca	60.50	0.6208
Mg	58.50	0.6210
S	55.50	0.5215
Ca & Mg	54.00	0.5049
Ca & S	54.50	0.5154
Mg & S	42.00	0.3928
Ca, Mg & S	26.50	0.2781
LSD (0.05)	4.84	0.0441

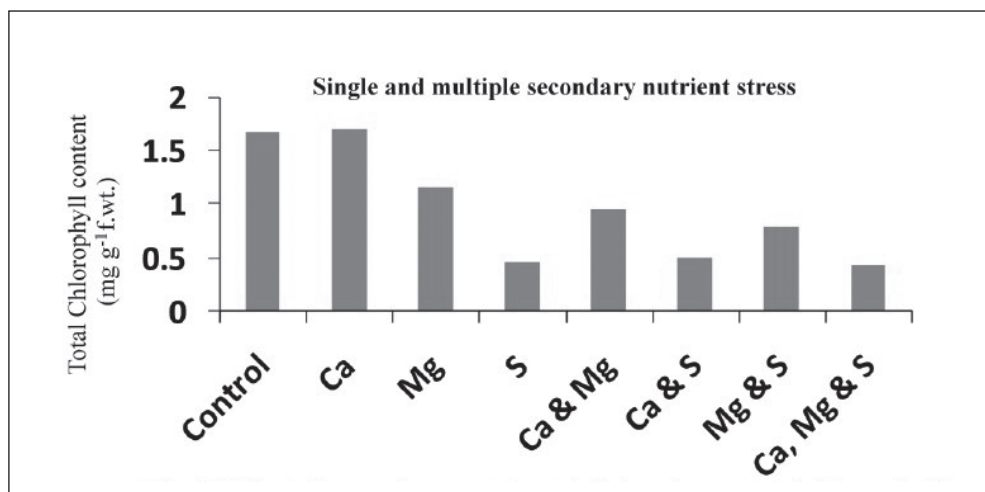


Fig.1: Effect of secondary nutrient deficiencies on total chlorophyll content (mg g⁻¹.wt.)

Table 2: Effect of Secondary nutrient deficiencies on the activity of enzymes

Nutrient deficiency	NRase	Poly phenol Oxidase	Acid Phosphatase	Super oxide dismutase	Thiobarbuteric acid	Phenols
Control	698	0.20	67.30	3.27	152	4.49
Ca	375	0.18	51.81	5.74	248	6.61
Mg	983	0.17	40.39	4.27	205	5.40
S	624	0.24	42.79	11.07	207	7.81
Ca & Mg	564	0.21	28.45	8.94	219	6.77
Ca & S	505	0.32	60.11	16.33	210	6.59
Mg & S	460	0.76	49.31	9.91	207	6.34
Ca, Mg & S	334	0.37	49.43	12.81	208	7.35
L.S.D. (0.05)	47	0.05	2.41	0.71	14.56	0.39

(Nitrate reductase activity ((μ moles g⁻¹ f.wt.min⁻¹), Poly Phenol Oxidase (OD/mg protein/min; Acid phosphatase (g p-nitrophenol/mg protein/min; Super-oxide dismutase (Units) 1 unit = 50% inhibition of NBT/mg protein/10 min), Thiobarbuteric acid (μ moleg⁻¹ f.wt.), Phenols (mg/g)).

nutrient stresses. Reports also showed that Secondary nutrient deficiencies disturb the nitrogen balance in plants (Witold *et al.*, 2023). Super oxide dismutase is known as the first line of defense against oxidative stresses and play vital role in scavenging the reactive oxygen species produced during metabolic processes as well as under abiotic stress conditions (Berwal and Chetram, 2018). Decreased acid phosphatase activity was recorded under N, P, Ca, Mg and S deficient conditions (Anuradha *et al.*, 2007). A

change in phenolic compounds might sometimes result from a change in the activity of key enzymes in phenolic biosynthesis such as phenylalanine ammonia lyase or from a change in the supply of substrates. Higher level phenolic compounds are the indication of nutrient disorders (Chishaki and Horiguchi, 1997). The enhanced activity of anti-oxidative enzymes was associated with secondary nutrient stress. The enhancement of enzymatic activity was more under multiple secondary nutrient stresses.

Nutrient Uptake and Ratios

Single (Ca, Mg and S) and multiple (Ca + Mg, Ca + S, Mg + S and Ca + Mg + S) secondary nutrient deficiencies reduced uptake of nutrients (Fig. 2). The reduction in uptake of N, P, K, Ca, Mg and S is to an extent of 13-50%, 1-69%, 19-61%, 36 to 81%, 14-78% and 16-73%, respectively due to different combinations of secondary nutrient stress. Single and multiple nutrient deficiencies also showed marked influence on ratios of different nutrients. Ratios of N/Ca, N/Mg, N/S, K/Ca, K/Mg, K/S were presented in Fig. 3. There is lot of variation in nutrient ratios due to secondary

nutrient stresses. The variation was wider under multiple secondary nutrient deficiencies which affects the metabolic activities in turn yield and quality. Reports revealed that Calcium regulates growth and nutrient absorption in plants and N/P ratio in leaves showed significant positive correlation with Calcium concentration (Weng *et al.*, 2022). Under Mg deficient conditions, Ca, K have strong antagonistic uptake behavior which results in excess calcium and ammonical form of N under Mg deficiency (Bergamann, 1992). Under Mg deficiency no effect on K concentration was observed (Farhat *et al.*, 2013). The availability of Mg can modify the uptake of Ca and K while Ca

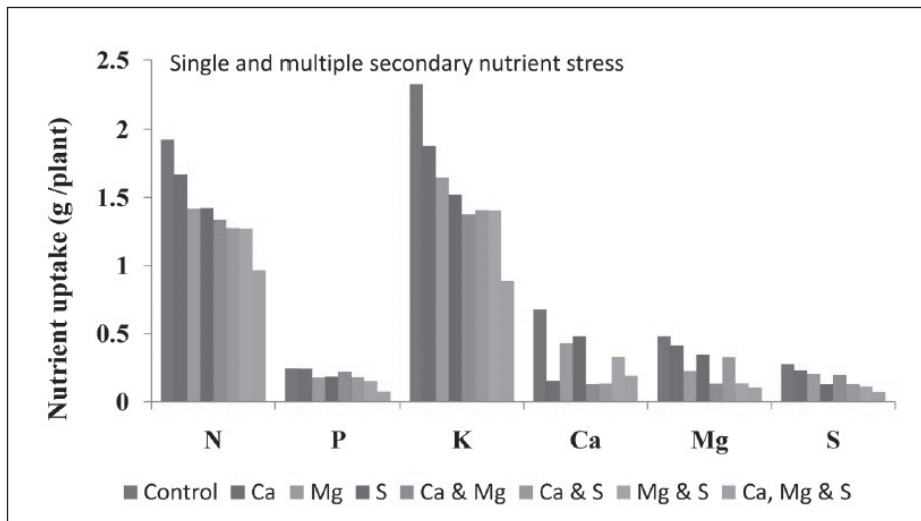


Fig.2: Influence of single and multiple secondary nutrient deficiencies on total plant nutrient uptake

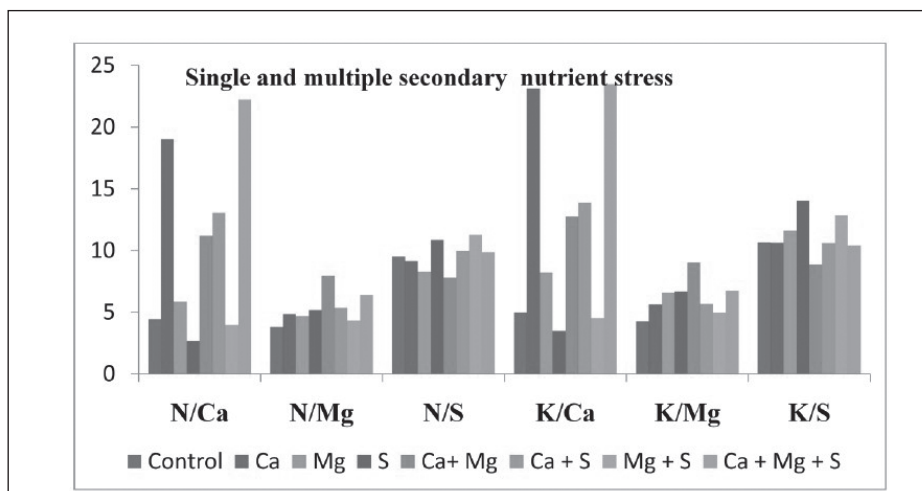


Fig.3: Effect of single and multiple secondary nutrient deficiency on nutrient ratios

and K can restrict the translocation of Magnesium from roots to shoots under insufficient Mg supply (Schimansky, 1981). Under sulphur deficiency, a characteristic feature found across different plant species is the marked accumulation of nitrate in root tissue combined with nitrate reductase activity showing that S deficient plants are unable to utilize N in their metabolism and consequently becomes N deficient (Kaur *et al.*, 2011). The results of the present study and reports on secondary nutrients clearly show that secondary nutrient stress caused marked differences in nutrient uptake and nutrient ratios. The effect is more under multiple secondary nutrient stresses.

It is concluded that single and multiple nutrient stresses reduced the plant growth, nutrient uptake and enhanced the anti oxidative enzymatic activity to counteract the ill effects of nutrient stresses. The effect of single and multiple nutrient stresses caused the nutrient imbalances which will influence the yield and quality of tobacco. Further studies on influence of single and multiple nutrients on yield and quality of tobacco leaf will help to recommend demand driven fertilization which will help in saving the input expenditure and environment compared to normal practice.

REFERENCES

- Anuradha, M., K. Nageswararao. K. Sivaraju, K. Deo Singh and V. Krishnamurthy. 2007. Influence of deficiency and toxicity of essential plant nutrients on nutrient composition and biochemical changes in flue-cured tobacco. **Tob. Res.** 33(1&2):71-78.
- AOAC, 1950 Official methods of Analysis USDA, 7thedn pp 12-14.
- Bekele, D. and M. Berhan 2021. The impact of secondary macro nutrients on crop production. **Int. J. Res. Stud. Agric. Sci.** 7(5): 37-51. DOI:10.20431/2454-6224.0705005
- Berwel, M.K. and Chet Ram. 2018. Superoxide Dismutase: A stable Biochemical Marker for Abiotic Stress Tolerance in Higher Plants In Abiotic and Biotic Stress in Plants Ed Alexandre Bosco de Oliveira. DOI: 10.5772/intechopen.82079
- Bergmann W. 1992. Colour Atlas: Nutritional Disorders of Plants Development, Visual and Analytical Diagnosis. Fischer Verlag; Jena, Germany
- Chaudhry, A.H., S. Nayab, S. B. Hussain, M. Ali , Z. Pan 2021 Current Understandings on Magnesium Deficiency and Future Outlooks for Sustainable Agriculture. **Int J Mol Sci.** 22(4):1819 doi: 10.3390/ijms22041819
- Jackson, M.L. 1967. Soil chemical Analysis, halls of India Private limited. New Delhi pp498.
- Johnson,C,M., P.R. Stout, T.C. Broyer and A.B. Carlton 1957 Comparative Chlorine requirements of different plant species. **Plant and Soil** 8:337-357
- Kapoor, A.C. and H.L. Paul. 1984. Effects of age and variety on nitrate reductase and nitrogen fractions in potato plants. **J. Sci. Food Agric.** 33: 401-6
- Kaur, G., R. Chandna, R. Pande, Y.P. Abrol. 2011. Sulfur starvation and restoration affect nitrate uptake and assimilation in rapeseed. **Protoplasma** DOI 10.1007/s00709-010-0171-3
- Hiscox, J.D and G.F. Israelstam. 1979. A Method for Extraction of Chlorophyll from Leaf Tissue without Maceration. **Canad. J Bot.** 57:1332-1334. <http://dx.doi.org/10.1139/b79-163>
- Chishaki, N and T. Horiguchi, 1997. Responses of secondary metabolism in plants to nutrient deficiency. **Soil Sci. Plant nutr.** 43: 987-991.
- Farhat N., M. Rabhi and H.Falleh .2013. Interactive effects of excessive potassium and Mg deficiency on safflower. **Acta Physiol. Plant.**35:2737-2745.
- Sadasivam, S and A. Manikam, 1991. Biochemical Methods for Agricultural Sciences. New Age International Publishers. New Delhi pp246.

- Sammi Reddy, K., S. Srivastava, and A.K. Sharma, (2007). IISS Perspective Plan: Vision- 2025. Indian Institute of Soil Science, Bhopal. Pp.1-64.
- Sammi Reddy, K., S. Srivastava, and A.K. Sharma, (2007). IISS Perspective Plan: Vision- 2025. Indian Institute of Soil Science, Bhopal. Pp.1-64.
- New Delhi India pp 71.
- Weng, X., H. Li, C. Ren, Y. Zhou, W. Zhu, S. Zhang and L. Liu 2022. Calcium regulates growth and nutrient absorption in Poplar seedlings. **Front. Plant Sci .** 13:8870-98 <https://doi.org/10.3389/fpls.2022.887098>
- Schimansky C. 1981. The influence of certain experimental parameters on the flux characteristics of Mg-28 in the case of barley seedlings in hydroculture experiments. *Landwirtsch. Forsch.* 34:154–163.
- Tandon, H.L.S. 1993 Methods of Analysis of Soils, plants, waters and fertilizers. Fertiliser development and consultation organization
- Witold, G, Waldemar, Z, Zielewicz, K. and Przygocka, C. 2023. Deficiencies of Secondary Nutrients in Crop Plants—A Real Challenge to Improve Nitrogen Management. **Agronomy**, 13 (1), 66; <https://doi.org/10.3390/agronomy13010066>