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Research Article

Nutrient Dynamics in Cotton Leaf Tissues as Affected by Zinc Fertilization and Ontogeny

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Abstract: Plant-tissue analyses are performed to assess plant nutrient status, and to determine fertilizer requirements of the current and the future crop grown in the field. Application of zinc (Zn) fertilizer may increase or decrease levels of other nutrients in the crop plants. Field experiments were conducted during 2004 and 2005 to study the effect of Zn fertilization on nutrient concentrations in leaf-tissues of cotton plants at different growth stages. Five rates of Zn (i.e., 0.0, 5.0, 7.5, 10.0 and 12.5 kg Zn ha^{-1}) were applied as $ZnSO_4 \cdot 7H_2O$, in a randomized complete block design, with four replications. All plots were uniformy supplied (in kg ha^{-1}) with 150 nitrogen (N) as urea, 60 phosphorus (P) as triple superphosphate, 50 potassium (K) as potassium sulfate and 1.0 B as borax. Most recent fully expanded leaves (without petioles) from the main stems were sampled 30, 45, 60, 90, 120 days after planting of the crop and the plant tissues were analyzed for N, P, K, calcium (Ca), magnesium (Mg), Zn, iron (Fe), manganese (Mn), and copper (Cu). Concentrations of Fe, Mn, Cu and P in leaves decreased significantly with Zn application indicating that optimum levels of these nutrients must also be maintained along with Zn application especially under marginal levels of these nutrients in soils. Leaf concentrations of the studied macro- and micro-nutrients, except K, increased with advancement of plant growth up to 60 days after sowing and then decreased gradualy. Observed concentrations of nutrients in cotton leaves at different critical stages will help in focusing future research aimed at defining critical nutrient concentrations at different stages of cotton.

Keywords: Cotton, leaf, micronutrients, macronutrients, nutrient dynamics

1. INTRODUCTION

The alkaline-calcareous soils of arid and semi-arid regions of the world, including Pakistan, are highly conducive to deficiency of zinc (Zn). The low Zn content in plant biomass (i.e., food and fodder) is believed to be a major cause of Zn malnutrition in humans and livestock. The low availability of Zn in soils is attributed to its precipitation in alkaline soil solution, binding with free $CaCO_3$ particles, low contents soil organic matter (generally <1%) and there by inadequate replenishment of soil solution with Zn [1]. There are estimates that two-thirds of agricultural soils in Pakistan are deficient in plant available Zn [2].

The plants, being immobile in nature, are dependent on soils and environmental variations for their growth, development and reproduction. Drossopoulos et al. [3] reported that quantum of absorption of nutrients is a function of growth stage, genotypes, and farm management practices. In most of the cases, nutrients are absorbed by the plant organs in higher quantities during their early stages of growth; and then their absorption is slowed down with advancement in plant age. The declining trend in the nutrients concentration with advancement of plant age has been elucidated by a number of researchers [4, 5, 6, 7].The reason being that degree of mutual sharing among leaves of plants is increased due to enhancement in index with advancement in age. Thereby, compound effects are reduction in net photosynthesis per unit leaf area and relatively lowered growth rate [3, 8].

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Clark [9] reported that rate of photosynthesis decreased due to reduction in chlorophyll contents occurring with advancement in age. Other researchers [10, 11, 12] also found that decline in the absorption of nutrients by plant organs is a function of genetic make-up, stage of physiological processes and environmental factors prevalent in the ecology. The quantum of nitrogen (N) content is decreased due to greater production of carbon-rich compounds relative to the accumulation of nutrients, viz., N, potassium (K), calcium (Ca) and magnesium (Mg) by the plant. The requirement of nutrients is much higher during early growth stages, because of greater assimilatory and accumulation capacity of roots and shoots to accommodate the future needs. Fageria [7] observed a reduction in phosphorus (P) concentration in rice and bean with successive increase in plant age. Moreover, Mullins and Burmester [12] reported lowering concentration of K in cotton plants with advancement in age. In another study, Mullins and Burmester [13] observed a decreasing trend in the absorption of Ca by cotton plants with increase in their age.

Over the years, inadequate research has been conducted to quantify the effects of Zn fertilizer on the absorption of macro- and other micro-nutrients in consonance of age of cotton plant. Therefore, field experiments were conducted to determine the impact of Zn fertilization on concentrations of other nutrients in leaf tissues of cotton plants in relation to stage of crop growth.

2. MATERIALS AND METHODS

Field experiments on cotton were conducted at the Agricultural Research Farm, Bahauddin Zakariya University, Multan, Pakistan (longitude: 71°, 30.79′ E; latitude: 31 °, 16.4′N; altitude: 128 m) during 2004 and 2005. Composite soil samples (0-15 cm depth), collected prior to imposition of fertilizer treatments to cotton crop of 2004,were analyzed for physical and chemical characteristics following standard methods [14]. Soil of the experimental area was non–saline (EC_e 2.1 dSm^{-1}), alkaline (pH 8.0), moderately calcareous (4% free $CaCO_3$), low in organic matter (0.76%) and silt loam in texture. The soil had 10 mg $NaHCO_3$ extractable P kg^{-1}, 175 mg NH_4OAc extractable K kg^{-1}, 0.54 mg DTPA extractable Zn kg^{-1} and 0.47 mg dilute HCl-extractable B kg^{-1} soil. The soil belongs to Sultanpur series (coarse silty, hyperthermic, Typic Haplocambid) [15].

The experiment comprised of five levels of Zn fertilizer (i.e., 0.0, 5.0, 7.5, 10.0, and 12.5 kg Zn ha^{-1}) applied in a randomized complete block design, with four replications. Zinc as zinc sulfate heptahydrate ($ZnSO_4 \cdot 7H_2O$) was broadcasted and incorporated in the soil prior to sowing. Cotton (*Gossypium hirsutum* L.), cv. CIM-473, was grown as a test crop. The planting geometry was 75 cm between rows and 30 cm between plants, and maintained 45,000 plants per hectare. Plots were also supplied with 150 kg N ha^{-1}as urea, 60 kg P ha^{-1} as triple superphosphate, 50 kg K ha^{-1}as potassium sulfate and 1.0 kg B ha^{-1}as borax. Whole quantities of P, K, and one-third dose of N were broadcast and incorporated before sowing. The remaining N was top-dressed in equal splits at full bloom and peak flowering. The weedicide Stomp-330E was sprayed (2.5 L ha^{-1}) to control weeds. The infestation of insects was controlled at economic threshold levels by spraying Zn-free insecticides. Recommended agronomic practices were adopted throughout the crop growth season. Weather data (air temperature and precipitation) are depicted in Fig. 1.

From each experimental plot, fully expanded leaves of 20 plants at fourth nodal position of the main stems were sampled 30, 45, 60, 90 and 150 days after planting. Petioles were removed from leaf blades, and leaf samples were washed with distilled water and blotted dry with tissue papers before oven drying at 70±1°C for 72 h. Concentration of N was measured after wet digestion in sulfuric acid and hydrogen peroxide [14]. For a better recovery, concentrations of P, K, Ca, Mg, Cu, Fe, and Zn were measured after wet digestion in di-acid mixture (2:1) of nitric and percholeric acid [14] and that of B after dry ashing [17, 18].

Data were subjected to statistical analysis by employing ANOVA on a PC based software “MSTAT C” and using least significant differences among mean values [19]. As leaf nutrient concentrations varied non-significantly between 2004 and 2005, average data of two years are presented in this paper. Moreover, interactive

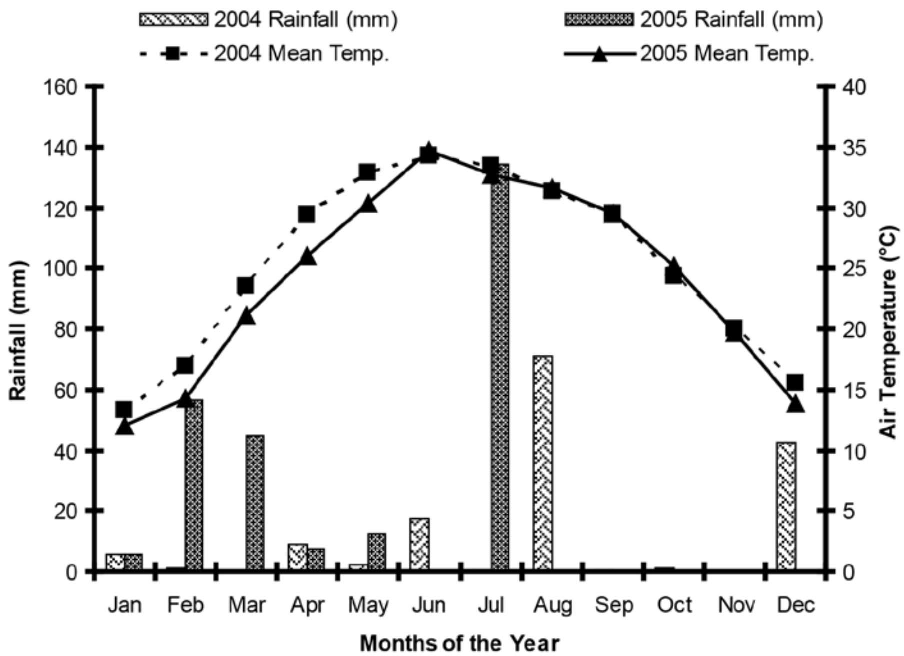

Fig. 1. Mean monthly rainfall and temperature during 2004 and 2005 [16].

effect of growth stages × Zn application was non-significant on concentrations of all nutrients. Therefore, only main effects of Zn application and growth stages are presented.

3. RESULTS

Concentrations of various nutrients in leaves were influenced significantly ($P \leq 0.05$) by advancement of age of the crop and Zn application. However, interactive effect of growth stages × Zn application was non-significant on concentrations of all nutrients, indicating that concentrations of nutrients in cotton leaves at different crop growth stages were independent of Zn supply from the soil. Therefore, main effects of Zn application and growth stages are presented.

3.1 Concentrations of Macronutrients

Zinc application increased leaf N and K concentrations ($P \leq 0.05$; Table 1). Comparing control with maximum applied rate of Zn, however, the decreases were only as high as 4% for N and 6% for K. On the contrary, application of Zn reduced leaf P, Ca and Mg concentrations ($P \leq 0.05$; Table 1). However, leaf P concentrations were statistically similar at higher rates of Zn fertilization (i.e., 7.5 to 12.5 kg Zn ha^{-1}). As compared with 0 kg Zn ha^{-1}, maximum decrease of 16% for P, 5% for Ca and 10% for Mg was observed with 12.5 kg Zn ha^{-1}.

Leaf N concentration was influenced primarily by advancement of crop age. The concentration of N started to increase from that at day-30 and

Table 1. Effect of zinc application on concentration of macronutrients in cotton leaves[1].

Zn applied (kg ha^{-1})	N (%)	P (%)	K (%)	Ca (%)	Mg (%)
0.0	2.67d	0.25a	2.94e	2.60a	0.78a
5.0	2.72c	0.23b	2.98d	2.54b	0.74b
7.5	2.74b	0.22c	304c	2.51bc	0.72c
10.0	2.76a	0.21c	3.09b	2.49cd	0.71cd
12.5	2.76a	0.21c	3.12a	2.47d	0.70d

[1]Values are means of nutrient concentrations at 30, 45, 60, 90 and 150 days after planting

reached to maximum at day-60, i.e., 3.1% (Fig. 2A). Thereafter, N concentration started to decline progressively till crop maturity. Nitrogen concentration was decreased by 6 and 32% respectively at 90 and 150 days after planting compared to the concentration at day-30 after planting.

Phosphorus concentration was 0.24% at day 30 after planting and reached its maximum 0.29% at day-60 after planting; showing an increase by 21% (Fig. 2B). Thereafter, concentration of P started to decline till crop maturity. The minimum P concentration of 0.10% was observed at 150 days after planting that was 57% lesser than that at 30 days after planting.

Concentration of K was 3.6% at 30 days after sowing and decreased to 3.2% at 60 days after sowing, showing a decrease by 13% (Fig. 2C). Thereafter, there was an abrupt decrease with advancement of crop growing period. Potassium concentration was decreased by 24 and 38%, respectively, at 90 and 150 days after planting compared to the concentration at day-30 after planting.

Calcium concentrations increased from 2.6% at day-30 to 2.8%at day-60 after sowing; thereafter, it decreased with advancement of age of plants (Fig. 2D). Calcium concentration was decreased to 2.0% at 150 days after sowing, showing a decrease of 25% with increase in age of cotton plants.

Concentration of Mg started increasing at 30 days after sowing and reached its maximum at 60 days after sowing; thereafter, it decreased up to 90 days after sowing ($P \leq 0.05$; Fig. 2E). Magnesium was 0.85% at 60 days after sowing and 0.73%at 90 days after sowing, showing a decrease of 14%. Concentration of Mg was 0.45% at 150 days after sowing and decrease in Mg was 38% from 90 days after sowing to 150 days after sowing. Concentration of Mg was 0.79% at 30 days after sowing, which decreased to 0.45% at 150 days after sowing, showing a decrease of 43% with age of plant.

3.2 Concentrations of Micronutrients

Similar to macronutrients, concentrations of various micronutrients were influenced significantly ($P \leq 0.05$) by advancement of crop age as well as with Zn application (Table 2, Fig. 3). Concentrations of Fe, Mn and Cu in leaves decreased significantly with Zn application ($P \leq 0.05$; Table 2). As compared to control, the maximum decreases of 6% for Fe, 7% for Mn and 8% for Cu were observed with 12.5 kg Zn ha^{-1}. As expected, however, incremental rates of Zn application progressively increased leaf Zn concentration from 17 mg kg^{-1} at control level of Zn to 40 mg kg^{-1} at 12.5 kg Zn ha^{-1}. Leaf B concentration also significantly increased with Zn application ($P \leq 0.05$).

Similar to macronutrients, concentrations of various micronutrients were mainly influenced by advancement of crop age (Fig. 3). Concentration of all micronutrients increased from day-30 after sowing to a maximum at day-60 after sowing. Thereafter, it decreased to a minimum at day-150 after sowing. Iron was 206 mg kg^{-1} at 30 days after sowing and was 219 mg kg^{-1} at 60 days after sowing, exhibiting an increase of 6% (Fig. 3A). Iron concentration was 170 mg kg^{-1} at 150 days after sowing, exhibiting a decrease of 17% compared to at day-30 after sowing.

At day-30 after sowing, cotton leaves had 138

Table 2. Effect of zinc application on concentration of micronutrient in cotton leaves[1].

Zn applied ($kgha^{-1}$)	Fe (mg kg^{-1})	Mn (mg kg^{-1})	B (mg kg^{-1})	Zn (mg kg^{-1})	Cu (mg kg^{-1})
0.0	209 a	133 a	38 c	17e	9.1a
5.0	202 b	130 b	41 b	27 d	8.8 b
7.5	200 bc	127 c	42 a	33c	8.6 c
10.0	198 c	125 cd	42 a	36 b	8.4 d
12.5	197 c	124 d	42 a	40 a	8.4d

[1]Values are means of nutrient concentrations at 30, 45, 60, 90 and 150 days after planting

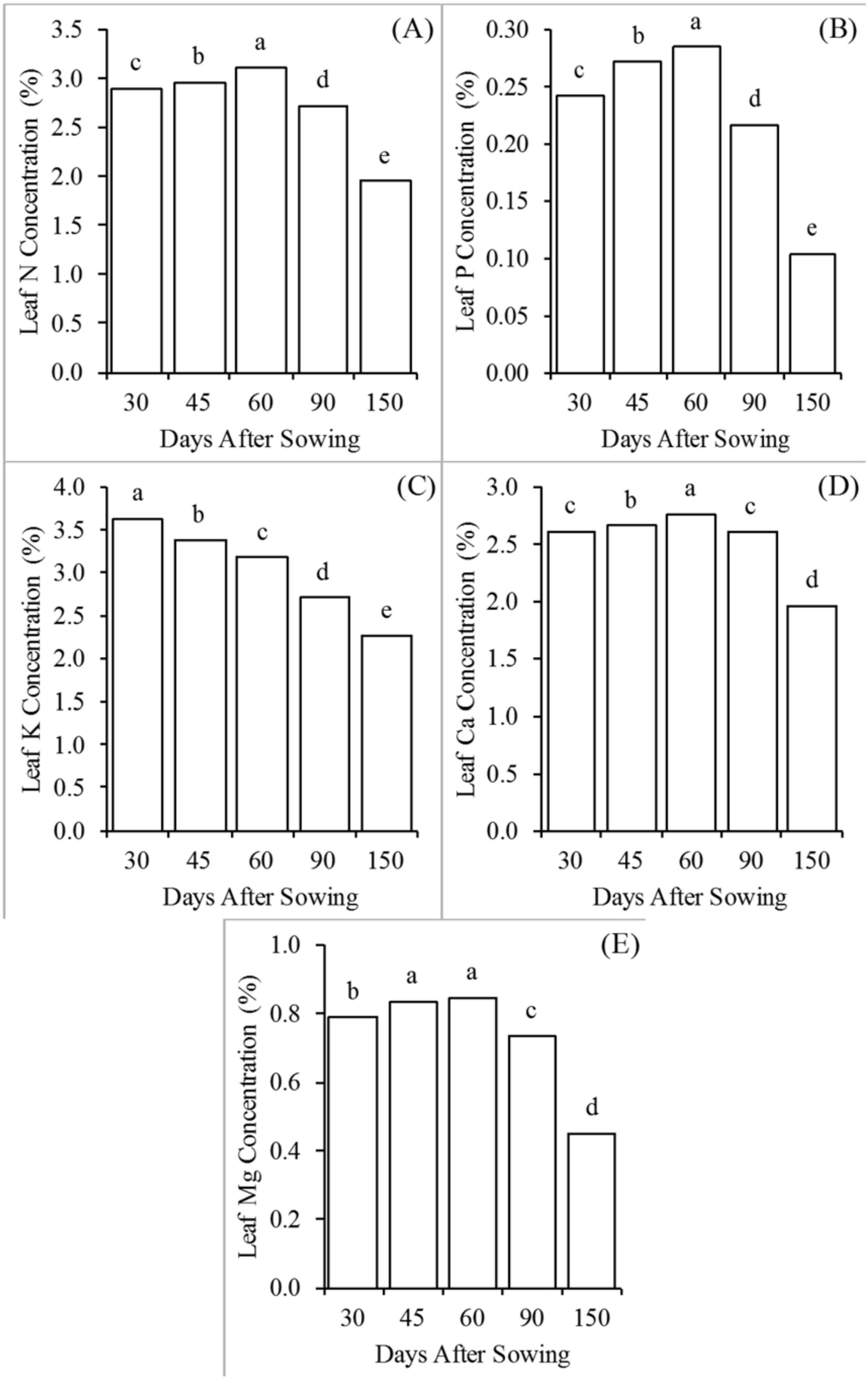

Fig. 2. Concentrations of macronutrients in cotton leaves at various crop growth stages.

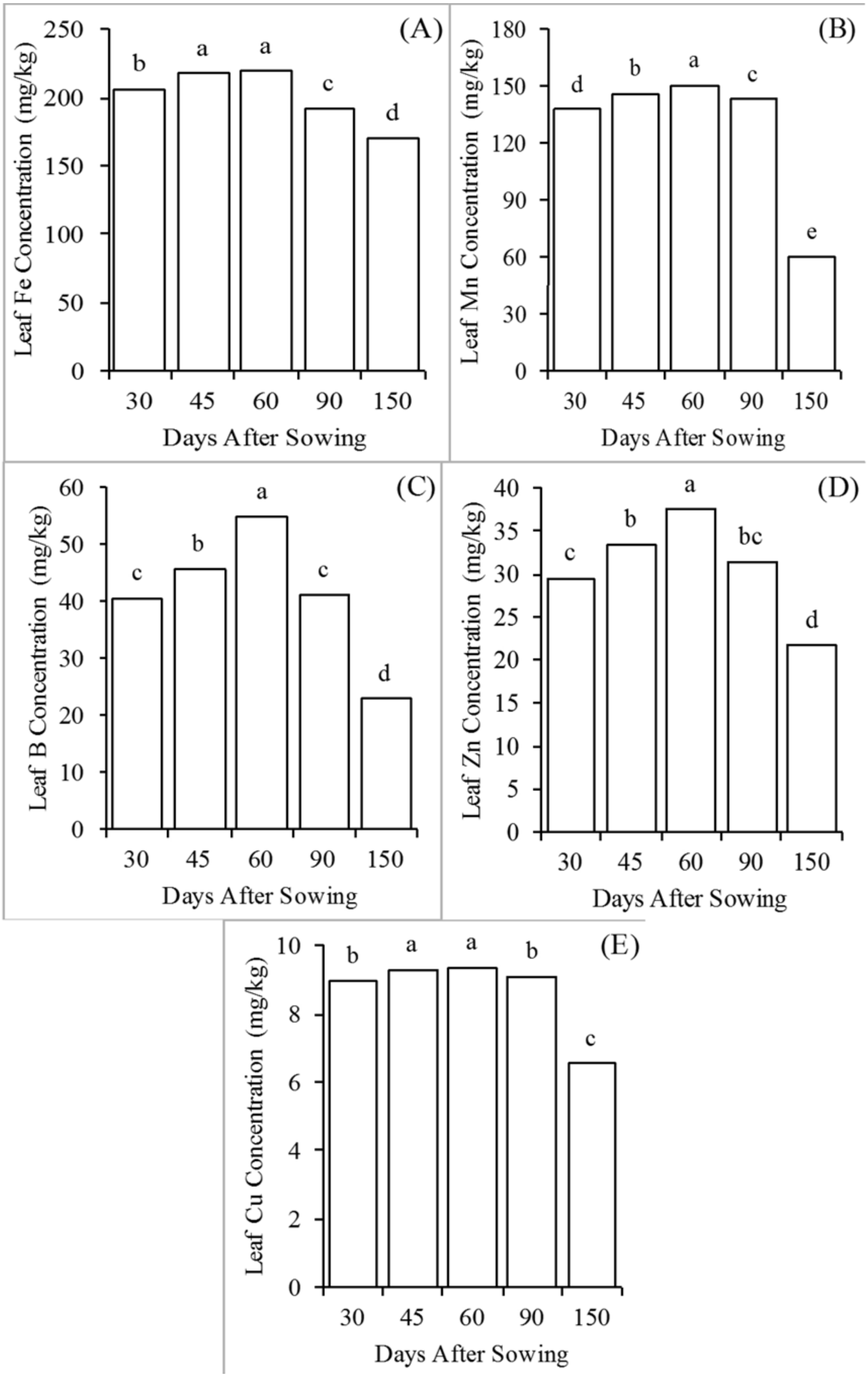

Fig. 3. Concentrations of micronutrient in cotton leaves at various crop growth stages.

mg Mn kg^{-1} (Fig. 3B). As compared to day-30 after sowing, concentration of Mn was increased by 9% at day-60 after sowing and decreased by 57% at day-150 after sowing.

Boron concentration was 41 mg kg^{-1} at 30 days after sowing, which decreased to 23 mg kg^{-1} at 150 days after sowing, showing a decrease of 43% (Fig. 3C). Maximum B concentration of 55 mg kg^{-1} was observed at 60 days after sowing.

Concentration of Zn leaves was 30 mg kg^{-1} at 30 days after sowing, which reached to a maximum of 38 mg kg^{-1} at 60 days after sowing, showing an increase of 27% (Fig. 3D). Zinc concentration was decreased to 22 mg kg^{-1} at 150 days after sowing, indicating a decrease of 42% over Zn concentration at 30 days after sowing.

Copper in leaves started increasing steadily from 30 days after sowing and reached its maximum of 9.1 mg kg^{-1} at 60 days after sowing (Fig. 3E). Copper concentration in leaves was 8.9 mg kg^{-1} at 30 days after sowing and decreased to 6.6 mg kg^{-1} at 150 days after sowing, showing a decrease of 26%.

4. DISCUSSION

Application of one nutrient can influence mobility of other nutrient in soils, its uptake by plant roots and its metabolism in plant tissues [20]. Therefore, Zn application significantly influenced leaf concentrations of other nutrients in cotton (Table 1, 2). Rochester [21] has reviewed several sources and has given critical concentration of 2.0% N, 0.3% P, 4.0% K, 2.3% Ca, 0.5% Mg, 20 mg Zn kg^{-1}, 30 mg Fe kg^{-1}, 5 mg Cu kg^{-1}, 20 mg B kg^{-1} and 25 mg Mn kg^{-1} at first flower of cotton. Data presented in tables are means of nutrient concentrations at 30, 45, 60, 90 and 150 days after planting (Table 1, 2). Data of nutrient concentrations at first flower (data not sown) and data presented in tables (Table 1, 2) suggest all nutrients were in sufficient concentrations in cotton leaves at all Zn levels except Zn concentration at 0 mg Zn kg^{-1}. This means that Zn application did not induced deficiency of any nutrient; although it decreased concentration of many nutrients in cotton leaves. It is hypothesized that excessive Zn application may induce deficiency of other nutrient if soil level of the other nutrient is marginal.

Drossopoulos [3] explained that amount of mineral elements absorbed by plants is a function of genotype, cultural practices, growth stage and environmental conditions. Changes in N concentration and N accumulation by the developing cotton leaves are well documented in literature [22, 23]. In cotton plant, N concentration in leaves and whole plants decreased with advancement of plant age in field studies [24]. Similarly, Fritschi et al. [25] noted a reduction in N concentration in the range of 29 to 54% in leaves of corn crop with advancement of its age. In alfalfa 57% reduction in N concentration from vegetative to blooming stages was observed in a field [26]. Nitrogen concentration in shoots and grains of rice and dry bean decreased significantly in a quadratic form with advancing in plant age [7]. It is reported that N concentration in cotton leaves decreased with plant age; being greater at the bloom stage [24, 25].

Fritschi et al. [25] reported that P concentration in corn leaves decreased by 24 to 32% with age. Similarly, Terman [27] observed 65% reduction in P concentration from 0.58% P at vegetative to 0.20% P blooming stage in alfalfa on Miami silt loam soil. Data of this study (Fig. 2B) are in accord with those of Welch [11] and Fageria [7] who reported that P concentration decreased in cotton, upland rice and dry bean with advancement of age of these crops.

Rominger et al. [26] reported 28 to 78% decreases in K concentration in corn leaves at 28 and 36 days after grown in a Mount view silt loam soil. Decrease in K concentration in leaves of cotton with advancement of crop age was also reported by Welch [11] and Sabbe [28].

Oosterhuis et al. [29] suggested 2.0 to 2.5% Ca in cotton leaves as adequate levels at flowering and peak flowering stages in newly mature leaves. In another study, Brar and Sekhon [30] reported 2.2 to 3.8% of Ca in mature cotton leaves as sufficient levels from vegetative to flowering stages. Magnesium concentration in cotton decreased with advancement of crop age [12]. Thus, results of the present study (Fig. 2C) were in accord with those of Mullins and Burmester [13]. Oosterhuis et al. [289] suggested 0.40 to 0.76% and 0.30 to 0.45% Mg as sufficient levels in recently matured leaves of cotton at pre-flowering and flowering stages, respectively. Brar and Sekhon [30] suggested 0.02 to 0.2% and 0.30 to 0.9% Mg as marginal and adequate levels in youngest mature leaf blades of cotton plants from vegetative to flowering stages.

Boron concentration tended to decrease with advancement of cotton plant age (Fig. 3C). Weir and Cresswell [31] proposed 20 to 50 and 20 to 25 mg B kg^{-1} in recently mature cotton leaves as sufficient levels at pre-flowering and flowering stages. However, Rashid and Rafique [17] in Pakistan established 53.0 mg kg^{-1} as critical level of B for cotton leaves at flower initiation stage. Reduction in Cu concentration in cotton leaves was observed with advancement of crop age. Mullins and Burmester [32] reported reduction in Cu concentration in cotton leaves with advancement of crop age. Sabbe et al. [33] reported 5.0 to 15.0 mg Cu kg^{-1} at pre-flowering and 3.0 to 10.0 mg Cu kg^{-1} at flowering stage of recently mature cotton leaves. Likewise, there was also reduction in Mn concentration with plant age. Oosterhuis et al. [29] proposed Mn sufficiency range of 35.0 to 100 mg kg^{-1} at pre-flowering and 30 to 90 mg kg^{-1} at flowering stages of cotton plant. Brar and Sekhon [30] reported various Mn concentrations as 8.0; 15 to 20; 25 to 500; 1000-2000; > 4000 mg kg^{-1} as diagnostic criteria for deficient, marginal, adequate, high and toxic levels from vegetative to flowering stages for cotton crop. The result of present studies also showed that crop had sufficient Mn in the leaves to maximize seed cotton yield. Iron concentration decreased with age in cotton plants [32]. Fageria et al. [34] suggested 30 to 300 mg Fe kg^{-1} as an adequate level in cotton leaf blades for seed cotton yield. Weir and Cresswell [31] proposed 50 to 300 mg Fe $kg^{-1}$and 40 to 100 mg Fe kg^{-1} in recently matured cotton leaves as sufficient levels at pre-flowering and flowering growth stages, respectively, for maximum relative seed cotton yield. Zinc concentration in corn shoot at 18 days after sowing was 36 mg kg^{-1} and decreased to 15 mg kg^{-1} at harvest stage. Decrease in Zn concentration in annual crops is associated with increase in shoot dry weight with increase in plant age (dilution effect) up to certain growth stage. Similarly, Rominger et al. [26] observed 62.0% reductions in Zn concentration from vegetative (46 mg kg^{-1}) to blooming stage (17.5 mg kg^{-1}) in alfalfa. For cotton crop, Weir and Cresswell [31] reported 7.0 to 13; 16 to 20; 25 to 60 mg Zn kg^{-1} as diagnostic criteria for deficient, marginal and adequate from vegetative to flowering stages. In present studies, average across Zn treatments, Zn concentration was 29.5, 33.4, 37.5, 31.4 and 21.6 mg kg^{-1} at 30, 45, 60, 90 and 150 days after sowing (Fig. 3D), which was sufficient to maximize seed cotton yield.

The reduction in nutrient concentration from vegetative to reproductive stage is attributed to leaf abscission, immobilization in organic compound and/or translocation to other parts of plants [28, 34]. Actually, many nutrients are translocated from leaves to fruiting bodies such as cotton bolls and wheat grains [29, 30, 35]. Sufficient concentrations of all nutrients should be present in all plant tissues during all critical growth stages and interpretation of tissues analysis should accordingly be made. Present work will provide a good base for such future investigations on cotton plants.

5. CONCLUSION

Nutrient concentrations in cotton leaves generally increased from day-30 after sowing to day-60 after sowing and thereafter decreased with plant age. Concentrations of Fe, Mn, Cu and P in leaves were decreased significantly with Zn application indicating that optimum levels of these nutrients must also be ensured along with Zn fertilization especially under marginal levels of these nutrients in soils. Under agro-ecological conditions of Pakistan, present work provided general concentrations of nutrients in cotton leaves at different critical stages. Maintenance of optimum concentrations of nutrients in leaf tissues at all growth stages could result in optimum production.

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