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How Does N: P: K Fertilizer Applications in Different Levels Affect The Nutrition of Anatolian Chestnut (*Castanea sativa* Mill.) ?

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Abstract

The sweet chestnut has provided the fuel, shelter, and nutrition needs of mankind for centuries. However, it was mostly accepted as a forest tree. This study aims to determine the effect of chestnut leaves of fertilizers containing nitrogen, phosphorus, and potassium on nutrient elements and to create a macro fertilization program for the chestnut tree. In this context, nitrogen (0, 110, 220, 330, 440 lb N ha^{-1}) phosphorus (0, 55, 110, 165, 220 lb P ha^{-1}), and potassium (0, 110, 220, 330, 440 lb K ha^{-1}) fertilizers were applied to chestnut trees for two years in different levels. While nitrogen applications increase N, Zn, and Cu concentrations in chestnut leaves; It reduced Fe and Mn content, and no statistically significant effect was found on P, K, Ca, and Mg concentrations. However, increasing phosphorus applications increased N, P, and Cu concentrations in chestnut leaves, but decreased K, Ca, and Mg content, and no statistically significant effect was detected on Fe, Zn and Mn concentrations. This research, which is among the very limited number of studies related to the nutrition of chestnut, maybe a guide for future studies. Keywords: Chestnut (*Castanea sativa* Mill.), plant nutrition, interaction, macronutrients, micronutrients.
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1.Introduction

Chestnut (*Castanea sativa* Mill.) is one of the most important tree nuts in the world (Ertan et al., 2015). Many species of the genus Castanea are grown in several parts of the world for timber and/or edible nut production (Portela et al., 2007). However, some herdsmen also cut down wild fruit trees to enlarge their meadow area, leading to the disappearance of the primary wild chestnut forest (Ercişli et al., 2009). According to the Food and Agriculture Organization Statistical Database (2019), worldwide chestnut production is 2.327.500 tons. Chestnut fruits are highly regarded and widely consumed throughout Europe, America, and Asia. In addition, chestnuts are one of the most popular nuts in the oriental world. Chestnuts are mainly cultivated in China 1.879.000 ton, Bolivia 84.800 ton, Turkey 64.750 ton and the Republic of Korea 56.200 ton.

Since nutrient management is a critical aspect of crop production, nutrient recommendations have been developed for several fruit and nut species. Sufficiency or survey ranges for individual nutrients are routinely used by soil and plant testing facilities as a basis for providing fertilizer recommendations. The sufficiency range, used for foliar testing, indicates the values at which the tissue is at the optimal nutritional status, as determined by field testing (Bryson et al., 2014).

However, there is limited scientific data on chestnut management, in particular in the field of mineral nutrition and crop fertilization (Portela et al., 2007).

In the past, this species was not fertilized, the soil only being amended when farmyard manure was available. Regular application of mineral fertilizers is a recent introduction (Arrobas et al.,2017). However, studies done with young potted plants have shown that the crop responds to the application of mineral nutrients (El Kohen et al., 1992; Laroche et al., 1997), and therefore it is expected that a positive response to fertilization may also occur in the field. Pérez-Cruzado et al. (2011), used wood-bark ash, a product rich in Ca, K, Mg, and to a lesser extent P, as a fertilizer in a young chestnut orchard. They recorded an increase in the diameter and height of the trees and also an improvement in the nutritional status of the plants in terms of K, Ca, and Mg. In the world, there are very limited data and researches to determine the need for chestnut fertilization and plant nutrients.

This study was carried out to determine the change of plant nutrient elements in the chestnut plant with nitrogen, phosphorus, and potassium fertilizer applications.

2.Material and Methods

2.1. Climate Data of Research Area

The region is located in the Marmara and the Aegean climate transitional zone. In the chestnut vegetation period (from March to October), the total amount of rainfall was 333.8 mm in the first year and 396.3 mm in the second year. The average temperature in the period of the research is consistent with the average temperature long term years, and the total rainfall is consistent with the total rainfall long term years. Climate data for the experimental sites and periods are shown in Figure 1.

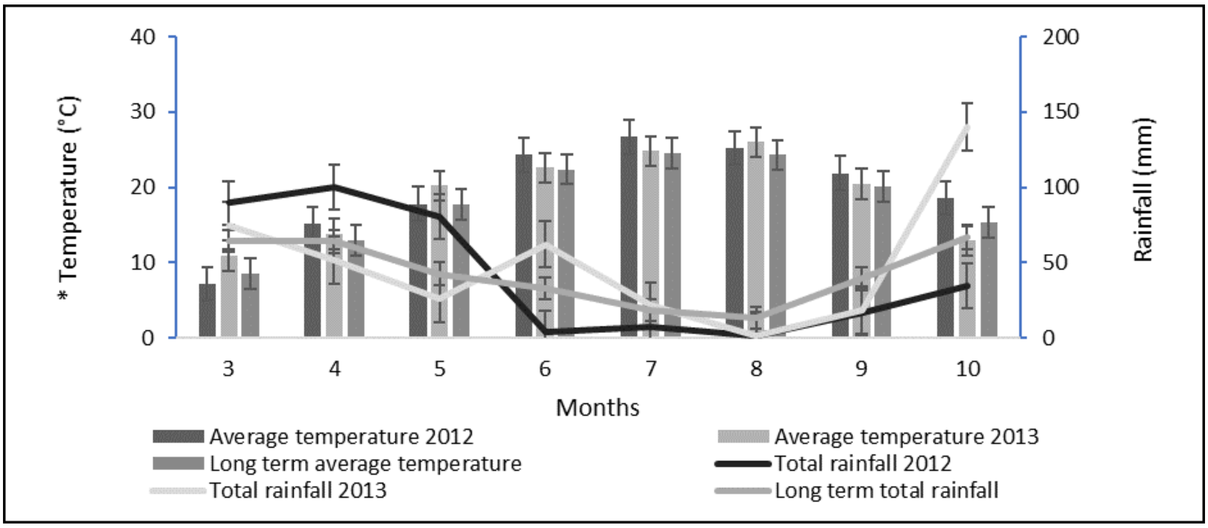

Figure 1. Climate data at the research area over two years (2012 and 2013). The values are shown (bars or symbols) are means ± standard deviation (SD).

2.2.Experimental Design

The study was conducted consecutive years (2012-2013) in İnegöl district of Bursa province in Southern Marmara section of Turkey. For this purpose, chestnut orchard (three zone for N, P, and K fertilizer applications) was chosen in commercial farms planted with Sarıaşılama sweet chestnut cultivars. The trees (three trees for each plot) were selected from different points of the orchard (three zones) each 1 ha fertilizer application zones had 100 chestnut trees (10 m x 10 m planting design).

The research was planned as a randomized plots design with three replications. In the study, nitrogen, phosphorus, and potassium fertilizers were applied in 2012 and 2013. Nitrogen fertilizer applications were adjusted to be N_0 (Control): 0, N_1: 110, N_2: 220, N_3: 330, N_4: 440 lbN ha-1, phosphorus fertilizer applications, P_0 (Control): 0, P_1: 55, P_2: 110, P_3: 165, P_4: 220 lbP ha^{-1} and potassium fertilizer applications, K_0 (Control): 0, K_1: 110, K_2: 220, K_3: 330, K_4: 440 lbK ha^{-1}.

However, support fertilizers were applied for nitrogen application treatments as 165 lb P ha^{-1} and 330 lb K ha^{-1} , for phosphorus application treatments as 330 lb N ha^{-1} and 330 lb K ha^{-1}, for potassium application treatments as 330 lb N ha-1 and 165 lb P ha^{-1}. All fertilizers (treatments and support) were applied to under the crown diameter of a chestnut tree in April mixed in 0-30 cm soil depth. In the experiment, urea (CH_4N_2O) was used as a nitrogen source, triple superphosphate $Ca(H_2PO_4)_2.H_2O$) as a phosphorus source, and potassium chloride (KCl) as a potassium source.

2.3. Soil Characteristics of Research Areas

The soil samples of chestnut orchard was taken in March. Soil samples were taken from four subsamples with stainless steel shovel at each plots and brought to the laboratory in plastic bags after mixing. Soil samples were air-dried and prepared by sieving 2 mm for analysis. The pH of the soil samples in a mixture of 1 / 2.5 of soil pure water with pH meter; $CaCO_3$ with calcimetric method; salt, conductometric method in saturation sludge; organic matter, according to the Walkley and Black titrimetric method; changeable K, Ca, Mg, Na photometric according to 1 N ammonium acetate method; availeble P, colorimetric according to Olsen method with 1.5 M sodium bicarbonate; Fe, Mn, Zn, Cu were spectrophotometrically analyzed by DTPA (Kacar 1995).

The classification and interpretation of soil analyzes were done according to Kellog (1952), Forth and Jacobs (1964), U.S. Soil Staff (1954), Evliya (1960), Schlincting and Blume (1966), Kovancı (1969), Pizer (1967), Loue (1968), Viets and Lindsay (1973).

2.4.Plant Analysis

Chestnut leaf samples were taken between 15 July and 15 August (Toprak and Seferoğlu, 2013). The samples were taken from the shoots on four sides of the tree and the leaves in the middle size. Leaf samples taken from the chestnut orchard was brought to the laboratory in paper bags, washed with sterile distilled water, and dried at 65 °C for 24 hours in the oven. The dried samples were grounded in the steel mill and had been made ready for the analysis (Kacar and İnal, 2008). Total N analysis was performed according to the Kjeldahl distillation method by using grounded leaf samples. Phosphorus, potassium, calcium, magnesium, iron, copper, zinc, and manganese were determined at leaf samples. The samples were weighed into 0.25 g microwave tubes. By adding 10 ml of the HNO_3 mixture containing 20% $HClO_4$, the weighted samples were digested for 30 min. The liquid resulting from digestion was completed to 50 ml and the elements were determined by ICP-OES (Optima 8000, Perkin Elmer, USA). Results were given as % dry matter for P, K, Ca, Mg, and mg kg^{-1} in dry matter for Fe, Cu, Zn, Mn.

2.5.Statistical Analysis

Data were subjected to analysis of variance (ANOVA). Data were analyzed by using SPSS (Ver. 25.0) statistical program and significant differences among means were compared with DUNCAN multiple comparison test. In the study, the Pearson method was used in correlation effect analysis to determine the interactions between nutrients and application treatments.

3.Results and Discussion

The physical and chemical soil properties of the chestnut experiment orchard is shown in Table 1. Soil was determined that the physical structure of the soil was loamy, the pH was slightly acidic, organic matter contents were insufficient. Also, the macro and micronutrient contents were found to be insufficient in the soil.

N, P and K fertilizers were found to synergistic and antagonistic effects on macro and micronutrient element concentrations in leaves. The effects of nitrogen, phosphorus, and potassium applications on macronutrient content of chestnut are shown in Figure 2.

N applications significantly increased the N concentrations of the leaves as expected. The highest foliar N concentration was determined for T_3 or / N_3 treatment (330 lb N ha^{-1}) in 2012 and 2013 as 2.78 % and 2.77 %, respectively (N application zone). Chestnut trees averaged 2.38% and 2.41% foliar N in 2013 to 2015 when an annual application of 308 or 370 lb ha-1 of soilapplied N was applied and a nut yield response was recorded (Warmund, 2018).

The leaf N concentrations were positively affected by P treatments but the difference between the P levels was not significant for the N concentration in the leaves. Although the foliar N concentrations in the leaves did not respond to K applications in the first year, significant differences were determined between the K doses in the second year. Synergistic interactions are well known for N x K and N x P interactions (Aulakh and Malhi, 2005). They are not only important for the yield but also help to explain their combined effect on root growth, and the relevance for synchronized applications of, for example, N and K, during the growing season (Aulakh and Malhi, 2005). Despite there were significant differences in the foliar P concentrations in the first year of N fertilization, these differences were not found the second year. In a study in apples, it was found that increasing N levels did not make a difference in the foliar P concentrations (Raese and Drake, 1997).

Table 1. Selected properties of the soil sampled shortly before the experiment started at a depth of 0–30 cm

Soil properties	**Values**	**Soil properties**	**Values**
Sand (%)	59.20	Available Fe (mg kg^{-1})	46.10
Clay (%)	12.20	Available Zn (mg kg^{-1})	0.43
Silt (%)	28.60	Available Cu (mg kg^{-1})	0.81
pH	5.79	Available Mn (mg kg^{-1})	52.10
$CaCO_3$ (%)	0.44	Exchangeable K (mEq 100 g^{-1})	0.35
Total salt (%)	0.02	Exchangeable Ca (mEq 100 g^{-1})	4.85
Organic matter (%)	1.46	Exchangeable Mg (mEq 100 g^{-1})	0.62
Total N (%)	0.07	Exchangeable Na (mEq 100 g^{-1})	0.08
Available P (mg kg^{-1})	6.96	CEC (mEq 100 g^{-1})	5.89

CEC: Cation Exchangable Capasity

K applications were found almost no contribution to the foliar P concentrations in this study (Table 2). On the other hand, P fertilizers applied increased the foliar P concentrations with significant statistical differences. The highest foliar P concentration was determined in T_3/ or P_3 treatment (165 lb P ha^{-1}) (first year: 0.38% and second year: %0.43) at the P application zone. These values were above the limit value (2.5 g kg^{-1}or/ 0.25%) for the chestnut leaf P concentration indicated by Arrobas et al. (2017). K fertilizers applied with increasing levels significantly increased the foliar K concentrations.

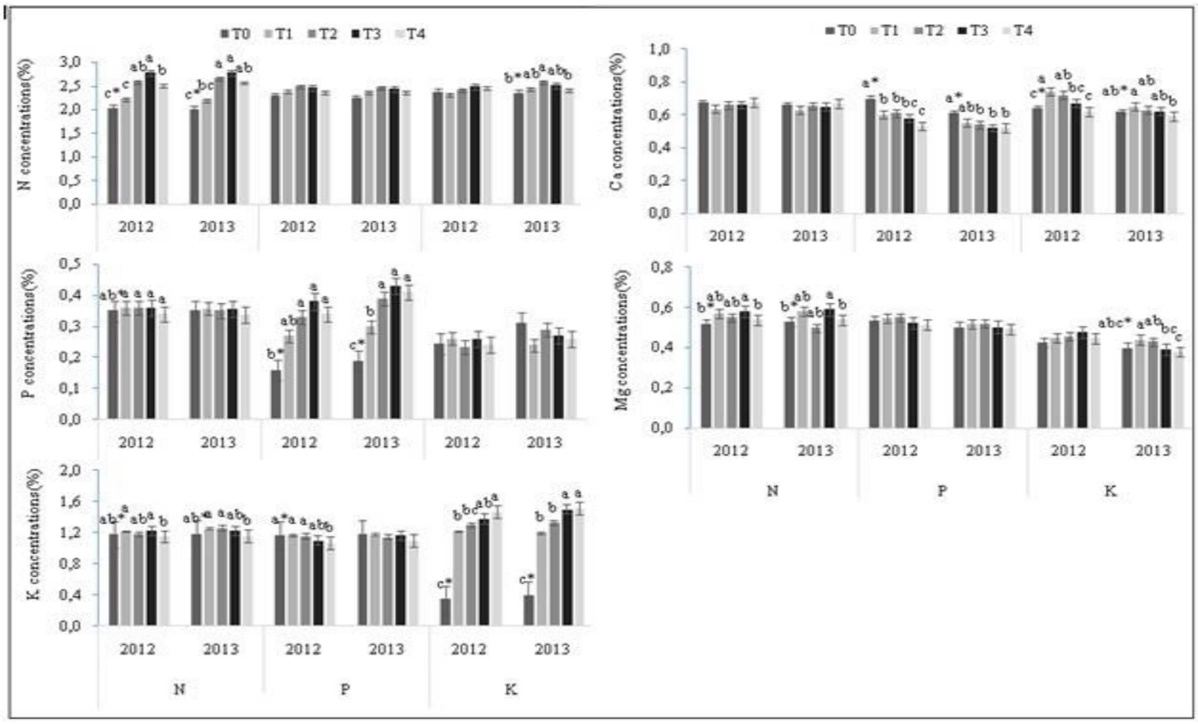

Figure 2. The effect of nitrogen, phosphorus, and potassium application on the foliar macronutrient in the chestnut. The values are shown (bars or symbols) are means ± standard deviation (SD). N, P, and K letters are fertilizer application zone in the chestnut research area (*: P≤0.05). There was no statistical difference between the treatments not shown with letters. T0: N_0+P_3K_3, T1: N_1+P_3K_3, T2: N_2+P_3K_3, T3: N_3+P_3K_3, T4: N_4+P_3K_3= for N application zone, T0: P_0+N_3K_3, T1: P_1+N_3K_3, T2: P_2+N_3K_3, T3: P_3+N3K3, T4: P_4+N_3K_3= for P application zone, T0: K_0+N_3P_3, T1: K_1+N_3P_3, T2: K_2+N_3P_3, T3: K_3+N_3P_3, T4: K_4+N_3P_3 = for K application zone.

The highest K content was recorded as 1.47% in the first year and 1.51% in the second year for T_4 or / K_4 treatment (440 lb K ha^{-1}). These values were above the limit value (11 g kg^{-1} or/ 1.1%) for the chestnut foliar K concentration indicated by Arrobas et al. (2017). Although the foliar K concentration in European chestnut and other nut trees is 2.1-2.2 (Toprak and Seferoğlu, 2013), it has low K concentrations (0.5-0.6%) due to the lack of application of fertilization in trees grown in Missouri (Warmund, 2018). While the P applications significantly reduced the foliar K concentrations in the first year, the K concentrations of the second year remained stable in the leaves. An antagonistic relationship was determined between P levels and foliar K contents.

However, the N doses applied in both application years affected the K concentrations of the leaf in a positive via, and a synergistic relationship was recorded between N and K (Table 2). Especially in plants with high K requirements, N and K were found to be positive in different culture plants (Mengel and Kirkby, 1987; Hakerlerler, 2000). Although the foliar Ca concentrations were not affected by the N levels applied, it was statistically affected negatively by the contents of P and K. In other words, the antagonistic relationship was found between foliar Ca concentrations with increasing phosphorus and potassium levels. The excess of phosphorus can also cause Ca, B, Cu, and Mn deficiencies (Aktaş and Ateş, 1998).

The increasing K levels did not affect the foliar Mg concentrations in the first year but decreased in the second year. The increasing K applications reduce the foliar Ca and Mg concentrations (Yağmur, 2009). Although increasing K levels did not affect the leaf Mg concentrations in the first year, a decrease in Mg content was determined in the second year.

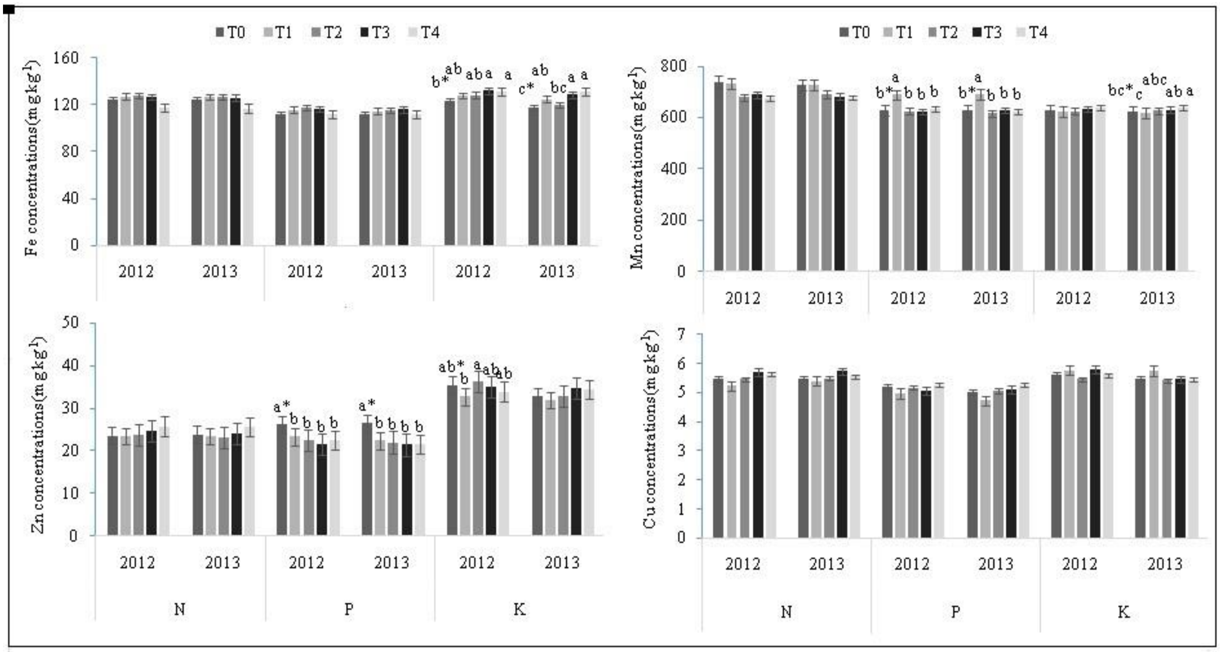

Figure 3. The effect of nitrogen, phosphorus, and potassium application on the foliar micronutrients in the chestnut. The values are shown (bars or symbols) are means ± standard deviation (SD). N, P, and K letters are fertilizer application zone in the chestnut research area (*: $P \leq 0.05$). There was no statistical difference between the treatments not shown with letters. T0: $N_0+P_3K_3$, T1: $N_1+P_3K_3$, T2: $N_2+P_3K_3$, T3: $N_3+P_3K_3$, T4: $N_4+P_3K_3$ = for N application zone, T0: $P_0+N_3K_3$, T1: $P_1+N_3K_3$, T2: $P_2+N_3K_3$, T3: $P_3+N_3K_3$, T4: $P_4+N_3K_3$ = for P application zone, T0: $K_0+N_3P_3$, T1: $K_1+N_3P_3$, T2: $K_2+N_3P_3$, T3: $K_3+N_3P_3$, T4: $K_4+N_3P_3$ = for K application zone.

High K concentration causes Mg and Ca deficiencies in leaves (Aktaş and Ateş, 1998). Increasing K concentrations in leaves cause a decrease in Ca and Mg content due to the antagonistic effect between these elements (Kacar and Katkat, 2009). The increasing P levels decreased the foliar Mg concentrations compared to the first year in the second year. Moreover, significant statistical differences were determined between the increasing N doses and the Mg content of the leaves. Ceylan et al. (2001) reported increasing N applications significantly influenced foliar P, K, Ca, Mg, Fe, Zn, Mn, and Na concentrations. The highest Mg concentration was recorded for T_3 / or N_3 treatment (330 lb N ha^{-1}) as 0.58% in the first year and 0.59% in the second year (Figure 2). In European chestnuts, macroplant nutrient contents such as N, P, K, Ca, and Mg were determined as 2.81%, 0.18%, 2.18%, 0.30%, and 0.48%, respectively (Toprak and Seferoğlu, 2013).

The effects of nitrogen, phosphorus, and potassium applications on macronutrient elements are shown in Figure 3. The N treatments applied to the soil were not statistically significant on the foliar Fe concentrations and were stable in both years (Average: 124 mg kg^{-1}). In a study conducted in pear, researchers determined that increasing nitrogen levels do not show a linear increase or decrease in foliar Fe concentrations (Uysal and Akçay, 2015). However, applied P doses were statistically no significant in the foliar Fe concentrations in both years (Figure 3). The Fe content of the leaves remained generally stable (Average: 114.9 mg kg^{-1}). On the other hand, K applications increased the Fe content of the leaves with statistical significance. As the K levels increased, the foliar Fe concentrations increased, and a synergistic relationship was determined between them. Yağmur (2009) reported that increasing K levels increased foliar Fe content in anise. The foliar Zn concentrations were decreased consistently with increasing P levels, and a statistically antagonistic relationship was determined between both of them.

Using an excessive amount of P fertilizers gives rise to microelement deficiencies such as Zn and Fe (Aktaş and Ateş, 1998). Although increasing N levels increased the foliar Zn content compared to the control, no significant difference was found between the treatments. In contrast, Raese and Drake (1997) stated that the increasing amounts of N applied to the apple reduced the foliar Zn content. Yagmur (2009), although K applications did not have a significant effect on leaf Zn content in anise plant, potassium levels were siğnificantly effective in foliar Zn concentrations. The foliar Zn concentrations were determined as 33.65-33.34 mg kg^{-1} (first year and second year, respectively) in the K experimental zone. The foliar Mn concentrations were recorded in the first and second year of N, P, and K experimental zones, an average of 703 and 701, 639 and 636, 630, and 626 mg kg-1, respectively. These foliar Mn concentrations are much higher than the amount (average 150 mg kg^{-1}) determined by researchers (Arrobas et al., 2017). Increasing P levels reduced the foliar Mn concentrations in an attitude similar to the N application zone. A significant antagonistic relationship was determined between the Mn and P concentrations in the leaf (Figure 3 and Table 2). This decrease is thought to be due to antagonistic relations between P and some microelements (e.g. Fe, Cu, and Mn) (Kacar and Katkat, 2009).

However, in 2012, the K applications did not significantly affect the foliar Zn contents, but this data changed in 2013. The N, P, and K fertilizers applied in all research zones not caused a statistically significant difference in the foliar Cu concentrations (Figure 3). But, the synergetic impact was determined between Cu concentration and N, P in leaves (Table 2). On the contrary, an antagonistic interaction was found between Cu and K in chestnut leaves (Table 2). The highest Cu content in the leaves was recorded in the first and second year of the K experimental zone as 5.65 and 5.52 mg kg-1, respectively. These foliar Cu concentrations are much less than the amount (18- 24 mg kg^{-1}) determined by some researchers (Toprak and Seferoğlu, 2013). In this research, some antagonistic and synergistic effects on nutrient concentrations of chestnut leaves of nitrogen, phosphorus, and potassium fertilizers were determined, and the differences in some nutrient concentrations were statistically significant among the rates of application.

3.1. Correlations Effect Test Between Nutrients And Applications

As expected, in N: P: K fertilization, a strong positive effect was found in foliar N, P, and K concentrations according to Pearson's Correlation model (Table 2). The strongest positive correlations were found as Zn and Cu in nitrogen applications, N and Cu in phosphorus applications, N, Fe, and Mn in potassium applications. The strongest negative correlations were recorded as Fe and Mn in nitrogen applications, K and Ca in phosphorus applications, and Zn in potassium applications.

4. Conclusions

According to the results of the study, the amount of N, P, and K to be applied in the chestnut (Sarıaşılama variety and 20 years old) orchard was determined as 330, 165 and 330 lb ha^{-1}, ($N_3P_3N_3$ or / 2:1:2 ratio) respectively. Fertilizers should be applied every year in the middle of April and under the tree crown diameter and mixed in the soil.

Table 2. Correlations effect test between plant nutrients and N, P, and K applications in chestnut leaves

Plant Nutrients	NF	Significant	PF	Significant	KF	Significant
N	+0.813	**	+0.531	*	+0.522	*
P	-0.059	ns	+0.867	**	-0.253	ns
K	-0.277	ns	-0.856	**	+0.853	**
Ca	+0.280	ns	-0.871	**	-0.331	ns
Mg	+0.150	ns	-0.477	*	+0.083	ns
Fe	-0.528	*	+0.040	ns	+0.799	**
Zn	+0.796	**	+0.296	ns	-0.811	**
Mn	-0.903	**	+0.238	ns	+0.839	**
Cu	+0.647	**	+0.530	*	-0.204	ns

**: 0.05; **:0.01; ns: not significant, NF: Nitrogen fertilization, PF: Phosphorus fertilization, KF: Potassium fertilization.*

Besides, according to applied nitrogen, phosphorus and potassium fertilization, the optimum amounts of required plant nutrients in the leaves of a 20 years old sweet chestnut tree for its healthy nutrition; N: 2.5-2.8%, P: 0.35-0.45%, K: 1.2-1.5%, Ca: 0.50-0.80%, Mg: 0.40-0.60%, Fe: 110-140 mg kg^{-1}, Zn: 25-35 mg kg^{-1}, Mn: 600- 800 mg kg^{-1}, Cu: 5-7 mg kg^{-1} can be considered as limit values.

According to the results of the Pearson correlation analysis of the study, the application of nitrogen fertilizers increased the N, Zn and Cu concentrations in the leaf (strong synergism), decreased the Mn and Fe content (strong antagonism), and did not cause a significant change in the P, K, Ca and Mg concentrations (zero interaction).

Phosphorus fertilization significantly increased the N, P, and Cu concentrations (strong synergism) of the leaves, decreased the K, Ca, and Mg content (strong antagonism), and remained in zero interaction with the Fe and Mn contents. Potassium fertilization increased foliar N, K, Fe and Mn concentrations (strong synergism), however, reduced leaf Zn contents (strong antagonism), and low and nonsignificant interaction determined between P, Ca, Mg and Cu concentrations and K concentration (zero interaction).

This research, which is among the very limited number of studies related to the nutrition of chestnut, maybe a guide for future studies. Finally, further studies on the plant nutrition of chestnut are needed in the future.

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