Araştırma Makalesi (Research Article)

Effect of Different Nitrogen Levels on Nut Yield and Some Quality Properties of Sweet Chestnut (Castanea sativa Mill.)

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Abstract: Chestnut has been used by humanity both as a fuel and as a food source for many years. Chestnut-related fertilization data is still limited today. This research was performed in order to study the fruit yield and some quality characteristics of the nitrogen doses to be applied in the chestnut plantations of İnegöl (Bursa) during two years. In the study, were used sweet chestnut (Castanea sativa Mill. cv. Sarıaasılama) trees 20 years old. During 2012 and 2013 the doses of 0.5, 1.0, 1.5 and 2.0 kg N / tree were applied to the chestnut experimental orchard, except for the control. However, 0.75 kg P / tree and 1.5 kg K / tree basic fertilization were applied to each tree. In the experiment, each parcel was composed of 3 trees and 3 replications. According to the results of the study, the highest fruit yield was recorded as 31.8 and 32.8 kg / tree in 1.5 kg N / tree in both application years respectively. Total protein and starch in the highest fruit were determined as 12.8 and 36.3 g / 100 g in 1.0 kg N / tree dose in the 2013 year, respectively. The total sugar content in fruit was recorded as 10.8 g / 100 g in 1.5 kg N / tree in 2013. When all of these parameters are examined, the amount of nitrogen to be applied to a mature chestnut tree at 20 years old is determined as 1.5 kg N / tree / year.

Farklı Azot Seviyelerinin Tatlı Kestane (Castanea sativa Mill.) Meyve Verimi ve Bazı Kalite Özelliklerine Etkisi

Öz: Kestane yıllar boyunca insanlık tarafından hem bir yakacak hem de bir besin kaynağı olarak kullanılmıştır. Kestane ile ilgili gübreleme verileri bugün hala sınırlıdır. Bu çalışma, İnegöl\'ün (Bursa) kestane plantasyonlarında iki yıl boyunca uygulanacak azot dozlarının meyve verimini ve bazı kalite özelliklerini incelerek amacıyla yapılmıştır. Araştırmada, 20 yaşındaki tatlı kestane (Castanea sativa Mill. cv. Sarıaasılama) ağaçlar kullanılmıştır. 2012 ve 2013 yıllarında, kestane deneme bahçesine kontrol hariç, 0.5, 1.0, 1.5 ve 2.0 kg N / ağaç dozları uygulanmıştır. Bununla birlikte, her bir ağaça 0.75 kg P / ağaç ve 1.5 kg K / ağaç temel gübreleme uygulanmıştır. Deneyde, her bir parsel 3 ağaç ve 3 tekerlendirme oluşmaktadır. Araştırma sonuçlarına göre, en yüksek meyve verimi her iki uygulama yılında 1.5 kg N / ağaç konusunda, sırasıyla 31.8 ve 32.8 kg / ağaç olarak kaydedilmişdir. En yüksek meyvede toplam protein ve nişasta, 2013 yılında 1.0 kg N / ağaç konusunda sırasıyla 12.8 ve 36.3 g / 100 g olarak tespit edilmiştir. Meyvede toplam şekerkar oranı ise yine 2013 yılında 1.5 kg N / ağaç konusunda 10.8 g / 100 g olarak belirlenmiştir. Tüm bu parametreler incelendiğinde 20 yaşındaki olgun bir kestane ağacına uygulanacak azot miktarı 1.5 kg N / ağaç / yıl olarak tespit edilmiştir.
1. Introduction

Chestnut belongs to the *Fagaceae* family. The 13 known species of chestnut are usually distributed in different parts of the Northern Hemisphere. East Asia (China, Korea, Japan), Turkey, South Europe and North America are the natural diffusion areas. (Özkarakça et al, 1995). Chestnut (*Castanea sativa* Mill.) is one of the very significant tree nuts in the globe (Ertan et al., 2015). Many species of the Chestnut tree are grown in limited regions of the globe for wood or comestible nut production (Portela et al., 2007). However, some herdsman 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, the 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 tons), Bolivia (84 800 tons), Turkey (64 750 tons), and Republic of Korea (56 200 tons) (FAO, 2019).

Chestnuts are rich in starch and sugars, primarily monosaccharides and disaccharides such as sucrose, glucose, fructose, and raffinose (Bernardez et al., 2004; De la Montana Miguelez et al., 2004). Besides, chestnuts differ from other nuts for their low-fat content which makes them ideally suited for high complex carbohydrate and low-fat diets (Bounous, 2009).

The chestnuts can grow efficient crops of nuts without ever being fertilized, but to get the very ultimate yields a program of organized fertilization will be essential. The higher expenses can be readily balanced by the high price of the crop. If inorganic fertilizers are used then regular with soil tests must determine the amounts and methods. No matter what kind of fertilizer is used, it must be applied in the spring or at the beginning of June. Fertilizers applied after June will cause rapid growth of fresh shoots that will be damaged in winter (Wahl, 2002).

Due to the accumulation of potential in nut, nitrogen fertilizers can be expected to affect many quality parameters, primarily protein content in chestnut. It has been reported that low levels of nitrogen in chestnuts while cause poor growth and reduced flowering, low phosphorus levels cause a decrease in the number of developing female flowers. Besides, if the content of boron in the soil is less than 3 ppm and more than 17 ppm, especially in Chinese Chestnuts, it causes the discharge of burrs, and there is a problem of iron deficiency and besides, chestnuts have high zinc needs in calcium-rich soil (Rutter et al., 1990).

On the other hand, there is little or limited information on mineral fertilization of chestnut trees (Portela et al., 2007). In the past, only organic fertilization (farm manure) was applied to chestnut trees not fertilized with chemical fertilizers. Regularly balanced mineral fertilization is a new topic (Arrobas et al., 2017).

Nitrogen is present in the structure of many important organic compounds in the plant. Proteins, amino acids, nucleic acids, enzymes, chlorophyll, ATP, ADP are important organic compounds containing nitrogen (Akteş and Ateş, 1998; Boşgelmez et al., 2001; Güzel et al., 2004; Gardiner and Miller, 2008; McCauley et al., 2009). Nitrogen plays an important role in many physiological and biochemical events taking place in the plant. Nitrogen has a great effect on the synthesis of proteins and chlorophyll. It is the basic constituent of the plant cell wall. Nitrogen has a significant role in the respiration of the roots, timely flowering, formation and ripening of the fruit (seed). Nitrogen well-fed trees are increasing resistance to pests (Kantarci, 2000; Fageria, 2009).

In this study, the purpose was to determine the effectiveness of nitrogenous mineral fertilizer applications on fruit yield and some quality properties in mature Sarıasılama chestnut (Candy type) trees.

2. Materials and Methods

The study was pursued in the chestnut orchards of İnegöl district of Bursa province in the sweet chestnut (*Castanea sativa* Mill. cv. Sarıasılama) trees of 20 years old. The research was planned as a randomized parcels design with three replications. There are three trees in each parcel. In the study, nitrogen fertilizers were applied in 2012 and 2013. Nitrogen fertilizer applications were
adjusted to be N₀: 0, N₁: 0.5, N₂: 1.0, N₃: 1.5, N₄: 2.0 kg N / tree. However, basic fertilizers were applied for nitrogen application treatments as 0.75 kg P / tree and 1.5 kg K / tree.

All fertilizers (treatments and basic) were applied to under the canopy of chestnut tree in April mixed in 0-30 cm soil depth. In the experiment, urea (CH₄N₂O) was used as a nitrogen source, triple superphosphate (Ca(H₂PO₄)₂·H₂O) as a phosphorus source, and potassium chloride (KCl) as a potassium source.

The region is located in the Marmara and the Aegean climate transitional zone. In the 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 of the study area are shown in Figure 1 (During chestnut vegetation periods).

![Figure 1. Climate data at the research area over two years (2012 and 2013). The values shown (bars or symbols) are means ± standard deviation (SD). *: Climate data are taken from Inegol Meteorology Station (17670).](image)

The soil samples of the chestnut orchard were taken in March month. The physical and chemical soil properties of the research area are shown in Table 1. Soil texture analysis was done by the hydrometer method (Bouyoucos 1955), soil pH and EC were measured in water (1:2.5 soil: distilled water), CaCO₃ equivalent by Scheibler calcimeter method. Organic matter and organic carbon contents were determined by modified Walkley-Black method (Walkley and Black, 1934), total nitrogen (N%), according to the modified macro Kjeldahl method (Bremner, 1965), phosphorus by the Olsen sodium bicarbonate method (Olsen and Sommers, 1982), potassium according to 1 N Ammonium acetate (NH₄OAC) method (Nielsen, 1972; Pratt, 1965) and trace elements by DTPA soil test of Lindsay and Norvell (1978).

According to the outcome of the soil tests, the chestnut orchard soil was determined that the soil texture was loamy, the pH was slightly acidic and (5.87). Organic carbon contents (0.78%) and organic matter contents (1.35%) were insufficient. It was found that the total salinity of the soil (0.018%) was low levels. Also, the nitrogen, phosphorus and zinc concentrations were found to be insufficient and potassium, iron and copper were found to be sufficient in the soil. Furthermore, Mn content was excessive. The lime content of the soils was below 1% (non-calcareous soil) (Ülgen and Yurtseven, 1995).
Table 1. Some soil characteristics of research area (0-30 cm)

| Soil characteristics | Soil characteristics |
|----------------------|----------------------|
| Clay (%)             | Total N (%)          |
| 12.3                 | 0.068                |
| Silt (%)             | P (ppm)              |
| 27.9                 | 6.28                 |
| Sand (%)             | K (ppm)              |
| 59.8                 | 139.2                |
| pH                   | Fe (ppm)             |
| 5.87                 | 41.3                 |
| Organic matter (%)   | Zn (ppm)             |
| 1.35                 | 0.39                 |
| Organic carbon (%)   | Mn (ppm)             |
| 0.78                 | 52.5                 |
| Total salinity (%)   | Cu (ppm)             |
| 0.018                | 0.79                 |

Chestnuts were collected during the harvest period when fruits reached a physiological maturity stage where the chestnut burrs began to separate and the nuts had grown. To calculate the total yield of each tree, nuts were collected by swinging trees and harvesting by hand. The samples of about 120-150 g fruit that were casually sampled were crushed with muller after their burrs and seed shells were removed and analysis was realized. The dry matter ingredients of the samples were determined by drying them night long in the air oven at 105 °C (Ertürk et al., 2006). Total protein content quantity was calculated using the Kjeldahl method (AOAC 1990). Dinitrophenol method was used in the analysis of total carbohydrates and total sugar (Ross, 1959) utilizing the Beckman Du 530 model spectrophotometer. The starch content was calculated by multiplying the value obtained by subtracting the total sugars from carbohydrate contents by the coefficient 0.94 (Ertürk et al., 2006).

Statistical analyses were conducted using analysis of variance (ANOVA) with IBM SPSS 22 Statistics Software. Applied nitrogen doses means were matched with Duncan’s multiple range test (P≤0.05, P≤0.01).

3.3. Results
3.1. Fruit yields

Nitrogen fertilization effects on yield in the present study were observed to be more significant than those on other crops (Scholefield & Stone 1995; Liu et al. 2004). Fruit yields in the nitrogen experimental orchard were determined between 23.4- 32.2 kg / tree. In the experimental orchard, the highest fruit yield was obtained for N3+PK dose (first year: 31.8, second year: 32.2 kg / tree) and the lowest fruit yield for control (first year: 23.4, second year: 23.5 kg / tree) (Figure 2). As it is known, usually, when N applied to plants, it causes in increased nutrients in leaf tissues and an increase of yields (Rodrigues et al 2006; Centeno & Campo 2011). A fruit yield increase of 36% was observed in chestnut plants compared to control (N0+PK) in N3+PK (1.5 kg / tree) treatment. Similar studies in which nitrogen doses were applied, for example in garden cress (Yağmur et al. 2019) and chickpeas (Kayan et al. 2018) indicated that nitrogen increased the yield.

Figure 2. The effect of different nitrogen doses on the chestnut yields. The letters represent the results of the Duncan test (*, P ≤ 0.05) for the nut yields. The values are shown (bars or symbols)
are means ± standard deviation (SD). (CV\textsubscript{2012}: 9.1, CV\textsubscript{2013}: 10.5). Year x Nitrogen: (*, \( P \leq 0.05 \)).

3.2. Fruit total protein contents

The protein amount of the fruit is an important criterion for fruit quality. In the chestnut fruit, a 50\% total protein increase was recorded in N\textsubscript{2+PK} treatment compared to the control. The total protein content of chestnut fruit varies between 4.88 and 10.87 g / 100 g (Ertürk et al 2006). The total protein content in the nitrogen application orchard was recorded between 8.0- 12.8 g / 100 g (Figure 3). The highest protein content was determined for N\textsubscript{2+PK} and N\textsubscript{3+PK} doses (first year: 12.2 and 12.0 g / 100 g, second year: 12.8 and 12.7 g / 100 g, respectively) and the lowest was recorded for control (first year: 8.0, second year: 8.6 g / 100 g). In the studies conducted, the amount of nitrogen in the soil and many cultural processes are reported to affect the protein ratio and quality (Bushuk, 1982; Atlı, 1999; Çağlayan and Elgün, 1999).

The increase in the protein content of the fruit continued until the N\textsubscript{3PK} dose and decreased at the next dose. This may be due to the negative interaction of phosphorus and potassium fertilizers given in basic fertilization with the last dose of nitrogen. As is known, high amounts of nitrogenous fertilization causes the plant to not benefit from enough phosphorus and potassium (Kacar and Katkat, 1998).

![Figure 3](image-url)  
Figure 3. The effect of different nitrogen doses on the total protein in the chestnut fruits. The letters represent the results of the Duncan test (**, \( P \leq 0.01 \)) for the fruit total protein. The values are shown (bars or symbols) are means ± standard deviation (SD). (CV\textsubscript{2012}: 2.6, CV\textsubscript{2013}: 3.1). Year x Nitrogen: (ns)

3.3. Fruit starch contents

In the chestnut fruit, a 36\% starch increase was recorded in N\textsubscript{2+PK} treatment compared to the control. The increase of starch in fruit increased with potassium applied in basic fertilization. Potassium activity in starch synthetase enzyme activity is very high up to a certain level (Preusser et al. 1981). The starch is transport to the storage organs especially in the presence of sufficient potassium (Kacar and Katkat, 1998). The highest starch content was obtained for N\textsubscript{2+PK} (1.0 kg / tree) dose (first year: 35.9, second year: 36.3 g / 100 g) and the lowest starch content for control (first year: 27.0, second year: 27.3 g / 100 g). The starch content in the experimental orchard was recorded between 27.0- 36.3 g / 100 g (Figure 4). The starch content of the fruit decreased in the last two nitrogen doses. Similary, Dede et al. 1999; Karadoğan et al., 1997 reported that increasing nitrogen doses had a negative effect on the starch rate in a similar way to these findings. As the amount of nitrogen applied to plants increases, carbohydrate, starch and sugar content decreases (Kacar and Katkat, 1998).
3.4. Fruit total sugar contents

Depending on the nitrogen doses applied, basic fertilizers (P and K) used caused an increase in fruit sugar ratio. In the chestnut fruit, a 35% total sugar increase was recorded in N$_3$+PK treatment compared to the control. Nutrition of plants with phosphorus and potassium positively affects sugar metabolism (Mengel, 1991). The highest total sugar content was obtained in application orchard, in the N$_2$+PK and N$_3$+PK doses (first and second year: 10.1, second year: 10.7 and 10.8 g / 100 g, respectively) and the lowest total sugar content was determined for control (first year: 7.8, second year: 8.0 g / 100 g). The total sugar content in the application orchard was recorded between 7.8 - 10.8 g / 100 g. At the last dose, the sugar content decreased (Figure 5). Similary, many researchers have reported that sugar content decreases at high nitrogen doses (Turhan, 1992; Demirer et al., 1994; Turhan and Özgümüş, 1992). The excess of nitrogen is extending the vegetative development period of the plant, delays blooming and decreases sugar synthesis (Aktas and Ates, 1998; Boşgelmez et al., 2001; Fageria et al., 2011).
4. Discussions and Conclusions

Chestnut trees in the region have been deprived of fertilization and other cultural maintenance for many years and have always been recognized as a forest tree. In this research, significant increases in chestnut fruit yield and quality characteristics have been determined along with balanced fertilization and maintenance processes. The fruit yield in chestnut orchards were increased by approximately 36%. A good balanced fertilization program could increase tree growth rates, fruit yield, and strength to biotic and abiotic stress (Wahl, 2002).

In many studies, it has been reported that phosphorus and potassium used in nitrogen applications and basic fertilization cause significant increases in both yield and fruit quality (Arrobas et al., 2017; Brohi et al., 1994; Bremner, 1965; Fageria, 2009; Fageria et al., 2011; Kantarcı, 2000; Kacar and Katkat, 1998; McCauley et al., 2009). According to the results, the highest fruit yield was determined for N3+PK (1.5 kg N / tree) dose as an average of 32.2 kg / tree, highest total starch and total protein contents were recorded for N2+PK (1.0 kg N / tree) dose as an average of 36.3 and 12.8 g / 100 g respectively and the highest total sugar content was obtained for N3+PK (1.5 kg N / tree) dose as an average of 10.8 g / 100 g.

However, when year x nitrogen interactions were examined, statistical significance was recorded in other parameters (yield, total sugar, and starch) than fruit protein content. When the meteorological data are considered, both the amount of precipitation and temperature averages were decreased in the second year compared to the first year.

In addition, the chestnut plant, which positive reacted to the nitrogen fertilization of the first year, had a positive also result with the second year fertilization. This may be due to the nitrogen residue in the soil. Moreover, in addition to nitrogen fertilization in the first year, fertilization in the second year resulted in improvement in all parameters with dose increases.

Also, there is no continuous soil cultivation in the chestnut orchards. In this way, nitrogen losses are prevented (Brohi et al., 1994). Although, the continuous, unidirectional and high doses of inorganic fertilizers bring more harm than benefits over many years (Müftüoğlu and Demirer, 1998).

In the light of all these data, the amount of nitrogen which should be an application to a sweet chestnut (Castanea sativa Mill. cv. Sarıaasılama) tree was determined for the highest fruit yield and quality characteristics as 1.5 kg / tree. Also, in addition to nitrogen fertilization, 0.75 kg P and 1.5 kg K per tree should be applied for basic fertilization.

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