



THE EFFECT OF DIFFERENT DOSES OF NITROGEN (N) APPLICATION ON THE ANTIOXIDANT ACTIVITY AND PHENOLIC MATERIAL INGREDIENT OF SORREL (*RUMEX ACETOSA* L.)

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Abstract

In this study, the effect of different doses of nitrogen (N) application on the antioxidant activity and phenolic material ingredient of sorrel (*Rumex acetosa* L.) are investigated. In the test, four various doses of nitrogen NH₄NO₃ fertilizer (N₀: 0 kg/da, N₁: 4 kg/da-16ppm, N₂: 6 kg/da-24ppm, N₃: 8 kg/da-32ppm) have been applied to the plants. As a result, it has been determined that the antioxidant activity and phenolic compounds of Sorrel (*Rumex acetosa* L.) have parallels with the increase in the application of nitrogen. Increasing the amount of phenolic compounds and antioxidant group compounds, in which they are included, leads to positive impact on above-mentioned effects of Rumex. In this context, it can be said that increasing phenolic compounds and antioxidant activity, which are obtained as a result of this study, with different dose N applications can increase the positive effects of this plant to human health.

Keywords: Nitrogen (NH₄NO₃), Sorrel (*Rumex acetosa* L.), antioxidant activity, phenolic material ingredient, functional food

Özet

Bu çalışmada kuzukulağına (*Rumex acetosa* L.) uygulanan farklı dozlardaki azot gübresinin antioksidan aktivitesine ve kuzukulağında muhtevi uçucu madde içeriğine etkisi araştırılmıştır. Araştırmada bitkilere (N₀: 0 kg/da, N₁: 4 kg/da-16ppm, N₂: 6 kg/da-24ppm, N₃: 8 kg/da-32ppm) dört farklı dozda gübreleme uygulanmıştır. Sonuç olarak, azot gübresi miktarındaki artışa paralel olarak antioksidan aktivitesinde ve kuzukulağında muhtevi uçucu madde içeriği miktarında artış gözlemlenmiştir. Yukarıda da bahsedildiği gibi, uçucu bileşiklerin ve antioksidan grubundaki bileşiklerin artması, Rumex'in yukarıda bahsedilen etkilerini arttırmaktadır. Bu bağlamda; uçucu bileşiklerin ve antioksidan seviyelerinin farklı azot gübrelemesi uygulamalarına bağlı olarak artması, bu bitkinin insan sağlığı için elverişliliğini de arttırmaktadır.

Anahtar kelimeler: Azot (NH₄NO₃), Kuzukulağı (*Rumex acetosa* L.), antioksidan aktivitesi, uçucu madde içeriği, işlevsel besin.

INTRODUCTION

Sorrel (*Rumex acetosa* L.) belongs to *Polygonaceae* family and is known as *Rumex acetosa* L. Although it is

not cultivated, it is commonly and spontaneously produced in most places of our country. It has an importance among the herbs that are sold in spring, summer, and fall seasons (Baytop 1984, Tabata et al.

1994, Vural et al. 2000, Karataş 2013). It has been known that when consumed as raw, sorrel leaf decreases blood sugar, and its extract shows anti-tumor, anti-microbial, antiviral, antibacterial, anti-inflammatory, anti-dermatitis, and diuretic effects (Demirezer 1993, Litvinenko et al. 2003, Mothana et al. 2010, Karataş 2013). Like sorrel (*Rumex acetosa* L.), other raw vegetables should be consumed not only to form a diet and meal, but also as purifier, remedial, and protector against diseases as the key for a healthy life. For these reasons, vegetables are examined in terms of their substances, and the term “functional nourishment” has been introduced to us. Functional nourishment is identified as nutrients and nutrient components that provide benefits for human physiology and metabolic functions and thus, constitute a protection against diseases and a healthier life besides fulfilling the need for the essential nutrients. These nutrients are expected to provide benefits for one or more body functions by reducing the risk of becoming sick (Boyacıoğlu 2013, Akan et al. 2013, Sönmez and Ellialtıoğlu 2014). In order to reach a high level of yield in vegetable farming (Stewart et al. 2005), the maximum growth (Badr and Fekry 1998, Arisha and Bardisi 1999, Arisha and Gad 2003, Dauda et al. 2008) and the amount of inorganic fertilizers as the primary source of herb nutrients are important and emphasized increasingly in the recent years (Adediran et al. 2004, Naeem et al. 2006). Plants take nitrogen as inorganic ion (NH_4^+ and NO_3^-) or organic nitrogen (N) (Barker 1975, Gagnon and Berrouard 1994, Montagu and Goh 1990, Sandoval-Villa et al. 1999). Nitrogen has an important role in the yield formation and quality of the plant (Bellitürk and Sağlam 2005, Lemaire and Gastal 2009). It is a well-known fact that the application of increasing amounts of nitrogen fertilizer affects the plant yield in a positive way. However, increasing nitrogen fertilization may affect some agronomic characteristics of the product, macro or micro nutrient content, vitamin and protein account and quality positively or negatively. Nitrogen is an important part of many genetic and metabolic compounds such as chlorophyll and amino acids in plant cells. In excessive nitrogen applications, the unused nitrogen in soil causes environmental pollution (Zand-Parsa et al. 2006, Gollany et al. 2004, Beman et al. 2005) and deposition of detrimental compounds in vegetables (Ruiz and Romero 1999). For this reason, the management and programming of nitrogen fertilization is essential in vegetable farming. The color, the content of nutrient, and the taste of tomato are related to the content of antioxidant of the plant (Dumas et al. 2003). When the usable content of

nitrogen is limited in the plant, there is more change in phenolic compounds (Haukioja 1998). The phenolic compounds in herbal materials are divided into two parts as “phenolic acids” and “flavonoids” (Cemeroğlu et al 2007). The phenolic compounds have antioxidant effect and this effect is caused by such elements as the purification of free radicals (Rice-Evans et al., 1995, Pekkarinen et al. 1999), composing compounds with metal ions and, preventing or diminishing singlet oxygen composition (Rice-Evans et al. 1995). The phenolic compounds are generally found in leaf, flower, and arboreal part of the plant (Kähkönen et al. 1999). In in vitro studies which deal with the effects of phenolic compounds on health, it has been proved that these compounds have the characteristics of free radical sweeper, enzymatic activity regulator, cell proliferation inhibitor, antibiotic, anti-allergic, anti-diarrheal, anti-ulcerative, anti-neurodegenerative (Alzheimer and Parkinson protector) (Dzoyem and Eloff 2015), and anti-inflammatory (Bravo 1998, Barut Uyar et al. 2013). It has been reported that many medical plants (Kumar et al. 2008, Koleva et al. 2002, Güvenç et al. 2005), vegetables (Çoruh et al. 2007, Brighente et al. 2007, Baravalia et al. 2009) and spices (Hinneburg et al. 2006) are the significant sources of phenolic compounds and have the antioxidant activity.

The conducted studies have proved that the nutrients which are rich for phenolic compounds prevent the free radical formation because of their antioxidant effect (Barut Uyar and Ören 2013). Herbal antioxidant compounds improve the immune system by preventing the free radical formation thus; help the repression of oxidative stress based diseases (Fusco et al. 2007, Adiloğlu et al. 2013, Afacan et al. 2014a). In this respect, for thousands years plants have been the source of medical agents, and many researches have been made on antioxidant compounds of various plants, the antioxidant activity of these compounds and their effects on human health (Humeera et al. 2013, Milella et al. 2014, Afacan et al. 2014b, Jameel et al. 2015).

The target of this study is to reveal the effect of different doses of nitrogen (N) as NH_4NO_3 application on the antioxidant activity and phenolic material ingredient of sorrel (*Rumex acetosa* L.)

MATERIAL AND METHOD

The research was planned in May 2015 to be conducted at the Laboratory of Soil Science and Plant Nutrition Department of Namık Kemal University

(Tekirdağ), according to randomized blocks experimental design with two replications.

Imported turf was used as production environment in plantation. (Klasmann TS1-Deilmann, Potground H, Germany). Some features of the used turf were the following: 160-260 mg/L N, 180-280 MG/L N, 180-280 mg/L P₂O₅, 200-150 mg/L K₂O, 80-150 mg/L Mg, Ph: 6, %0.8 N, %70 organic substance, %35 C.

In the experiment, four doses of NH₄NO₃ fertilizer (N₀: 0 kg/da, N₁: 4 kg/da-16ppm, N₂: 6 kg/da-24ppm, N₃: 8 kg/da-32ppm) were used in solution state and a single dose of fertilizer was applied to Rumex, 40 days after the seeding process. The experiment was done in rectangular parcel lands (45x30x15 cm³). Shortly after the harvest, plants were washed with distilled water two times, they were dried in 65 °C drying-oven till their weight get stabilized, they were ground and prepared for the analysis. Dried samples were analyzed in the Laboratory of Faculty of Agriculture in Namık Kemal University.

Extraction of Sorrel (*Rumex acetosa* L.)

Samples

Dried Rumex samples were treated methanol extraction for five hours by total phenolic compounds assay and DPPH method in order to be used in antioxidant activity determination. After removing methanol from the obtained extracts, they were diluted by definite proportions of methanol and used in the analysis (Humeera et al. 2013).

Determination of Total Phenolic Compounds

By using 760 nm wave length gallic acid standard, the total amount of phenolic compounds is determined as mg/g with Folin- Ciocalteu reagent (Frank et al. 2004, Cemeroglu et al 2007).

Determination of Antioxidant Activity by DPPH Method

DPPH (1,1-diphenyl-2-picrylhydrazyl) method is based on the measurement of reduction ability of antioxidant compounds, which exist in the sample against DPPH radical, a purple colored compound (Frank et al. 2004, Cemeroglu et al 2007). Alterations in the absorbance values of radical solution were determined as 517 nm wave length. The results of the analysis were evaluated by "EC₅₀" value (Cemeroglu 2010).

RESULTS AND DISCUSSION

Total phenolic material amounts (TPMA) of the Rumex extracts, to which four different doses of

NH₄NO₃ had been applied in solution state, were evaluated by using gallic acid standard graphic (Figure 1). The phenolic compound amount, the equivalent of absorbance values, which were obtained for samples, in terms of gallic acid, was found by means of standard curve and total phenolic material amount was calculated as gallic acid equivalent. Total phenolic material amounts (TPMA) of the samples were given in Table 1.

When Table 1 is examined, it is found that according to check sample (N₀) the highest TPMA can be seen in the second nitrogen application (N₂) with the value of 72.70 ± 1.8 mg TPMA/g. When TPMA increased values of the other doses were examined according to control, the increase in the values of the phenolic material could be seen in the first (N₁, 54.95 ± 2.1 mg/g) and in the third doses (N₃ 52.20 ± 1.1 mg/g) when they were compared to check samples. Total phenolic material concentration of Rumex show differences according to kind, solvent and standard solution that are used in the analysis (Uyar and Ören 2013).

As a result of the analysis done with different kinds of Rumex, total phenolic material content of it was determined as a value between 12.27 mg and 26.78 mg tannic acid equivalent per 100g by Jimoh et al. (2008), 160.53 mg /100gr by Uyar and Ören. (2013), 121.80 ± 0.03 mg gallic acid (GA) by Khan et al. (2014). In his study about the examination of antioxidant activity of some plants, whose leaves are consumed as salad-spice, İşbilir (2008) stated that total phenolic material amounts of the extracts were in the range between 49,63±2,5- 127,55±14,48 mg GA/g, they were changed between 24,08±1,67-76,03± as a catechol equivalent and the highest amounts were taken from the extracts of Sorrel and poppy.

Although the obtained values were lower than the values, which were found by Uyar and Ören 2013, and Khan et al. (2014), they were between the values stated by İşbilir (2008) and they showed parallelism with that study.

Table 2 shows the mg amounts which provide the inhibition of the %50 Rumex samples' DPPH (1,1-diphenyl-2-picrylhydrazyl) radicals.

When Table. 2 is examined, it is seen that with the increase of nitrogen amounts, which were applied in different levels, antioxidant activities of the samples were increased. The highest increase in the antioxidant activity was found with the value of 0.17± 0.02 mg (32ppm per 8 kg) dried plant weight in the numbered N₃ sample which had the most intense nitrogen application. According to check sample (N₀), as a result

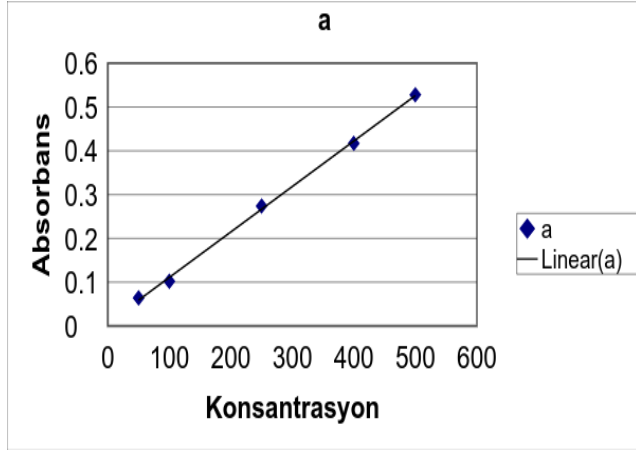


Figure1. Gallic Acid Standard Curve (Absorbance/ Gallic Acid (GA) Concentration mg/L)

Table1.Total phenolic material amounts of Sorrel (*Rumex acetosa* L.) samples (TFMM mg GA/g)

Sorrel Samples	TFMM (mg/g)
N ₀	34.45 ± 0.1
N ₁	54.95 ± 2.1
N ₂	72.70 ± 1.8
N ₃	52.20 ± 1.1

Table 2.EC₅₀ values of *Rumex* (*Rumex acetosa* L.) samples (mg dry weight)

Sorrel Samples	EC ₅₀ (mg)	Antioxidant Activity %
N ₀	0.33 ± 0.17	-
N ₁	0.24 ± 0.16	27
N ₂	0.19 ± 0.15	42
N ₃	0.17 ± 0.02	48

of nitrogen applications, percentage increments in antioxidant activity were determined as %27, %42 and %48 for N₁, N₂ and N₃ respectively. Similarly to the phenolic material amounts in the study, the increase in the antioxidant activities of *Rumex* samples shows parallelism with the increasing N doses.

It has been concluded that the application of different nitrogen doses has no effect on *Rumex*'s total phenolic material content and antioxidant activity. By contrast with that, in their study, which they examined the effect of nitrogen state, in the bloom period, on the anthocyanin and phenolic development during grape maturation Keller and Hrazdina stated (1998) before pre-bloom stage of grapes, medium level of nitrogen application affects positively the polyphenol synthesis. In the research done by Delgado et al. (2003), they stated that with the increase of nitrogen application, anthocyanin amount and in parallel with that, increase in the color intensity could be observed.

There are different types of *Rumex* which are used for some diseases like inflammation and constipation. It is indicated that the effect of *Rumex acetosa* to bodyweight, serum, amino acid and mineral levels (Ladeji et al. 1995), psychopharmacologic and purgative effect of *Rumex nepalensis* (Ghosh et al. 2002; Ghosh et al. 2003), antioxidative effect of *Rumex patienta* (Demirezer et al. 2001), antifertility effect of *Rumex steudeliin*, antimicrobial and anti-inflammatory effect of *Rumex nervosus* antidiarrheal effect of *Rumex maritimus* antiviral effect of *Rumex bequaerti* are derived from the phenolic compounds they include.

Increasing the amount of phenolic compounds and antioxidant group compounds, in which they are included, leads to positive impact on above-mentioned effects of *Rumex*. In this context, it can be said that increasing phenolic compounds and antioxidant activity, which are obtained as a result of this study, with different dose N applications can increase the positive effects of this plant to human health. For this reason it's thought that concrete results can be obtained through the studies related to stated matters by supporting the biological activity researches of human.

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Submitted: 22.10.2015

Accepted: 11.12.2015