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Preliminary Study on Investigation of the Use of Effective Microorganism Applications in Walnut

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Abstract

It is observed that beneficial microorganisms are used effectively in agricultural practices that are important for human and environmental health. These microorganisms, which are known biological agents and microbial fertilizers, are important in terms of plant development, quality and reduction of input, thanks to the increase in the amount of nutrients in plants. In this study, it was goaled to determine the effects of EM.FPE and EM.5 as foliar sprays and EM.A as soil application on shoot length, some fruit parameters and yield and leaf nutrient elements in Chandler walnut variety. In the experiment, the longest shoots were measured with 55 cm in EM.A application. Similarly, the highest values in nut weight (12.85 g) and yield (2.28 kg tree^{-1}) were obtained in the same application. All applications positively affected kernel browning. These were found to increase leaf macro and micro nutrient content, except Cu and Fe.

Keywords: *Juglans regia* L., Microorganisms, Fruit properties, Nutrient, Yield

Introduction

The world population is increasing rapidly in spite of the decrease in agricultural areas and natural resources. Accordingly, increasing food demand can be achieved with increased yield. This situation depends on adequate intake of nutrients, in general (Zaman et al., 2014). Excessive use of chemical inputs that affect the product quantity and quality causes a decrease in soil fertility, economic and environmental damages. Today, the awareness of human and environmental health comes to the forefront, and it is important to use microbial fertilizers as an alternative to chemical fertilizers.

Microorganisms have been used in human, animal and environmental health, food processing, food safety and quality, genetic engineering and biotechnology for many years (Higa and Parr, 1994). The physiological effects of microorganisms promoting plant growth have attracted attention since the beginning of the 20th century (Bona et al., 2016; Parewa et al., 2014; Ruzzi and Aroca, 2015). These microorganisms are produced biologically active substances, which have an effect on growth and development (Çakmakçı, 2005). In perennial plants such as fruit trees, the effects of these applications were determined on vegetative development, fruit characteristics, yield, nutrient content and disease resistance (Demirci and Hancıoğlu, 2005; Ertürk et al., 2012; Güneş et al., 2015; İpek et al., 2014; Karakurt et al., 2011; Karlıdağ et al., 2013; Thakur et al., 2015).

Intake of some nutrients in the soil is limited or difficult, so that foliar applications are even more important for plant nutrition. Applications that benefit to the plant in a shorter period and have a direct effect on the amount of product are widely used. In fruit species, microorganisms are applied to the leaves and flowers as well as soil applications as alone or

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combination (Atılgan et al., 2019; Eşitken et al., 2010).

Microorganisms that are important for sustainable agriculture and environment are expressed as "Effective Microorganisms (EM)" (Higa and Parr 1994). "EM" technology is not a single type of microorganism; it represents various microorganisms. This group includes photosynthetic and lactic acid bacteria, yeasts, fungi and effective enzymes. "EM" is used in agriculture for various purposes (Acarsoy Bilgin, 2019; Anonymous, 2020; İşçi et al., 2019).

Recently, it is pointed out that microorganisms can be useful in different plant species, climate and soil conditions (Çakmakçı, 2005). In this regard, there are a very limited number of researches on walnuts, although many fruit species have been studied. In our country, new plantations have been established with Chandler walnuts, which have high quality fruit characteristics. In this trial planned in the mentioned walnut variety, it was aimed to determine the use of effective microbial fertilizers on shoot length, some fruit parameters, yield and leaf nutrient elements.

Materials and Methods

The current study was conducted in Manisa/Demirci location in 2018 year. Chandler walnut variety (6-years-old) was used as plant material. This variety is foliar and blooms in the late period. Fruits ripening in the middle season are large, oval and smooth. The shell breaks more quickly and easily (Özçağıran et al., 2014).

EM.FPE (2 cc lt^{-1}) and EM.5 (2 cc lt^{-1}) as foliar sprays and EM.A (2 cc lt^{-1}) as soil application were treated to plant material. These microbial fertilizers belong to Agriton Company. The applications were carried out 3 times such as before the blooming male flowers, after the blooming male flowers and hazelnut-sized fruits for each microorganism. The content of the applied microbial fertilizers is given below (Table 1).

Table 1. Microbial fertilizers and their contents.

Fertilizers	Content	Application method
EM.FPE	Lactic acid bacteria (*Lactobacillus casei, Lactobacillus fermentum, Lactobacillus plantarum, Lactobacillus rhamnous*), Yeast (*Saccharomyces cerevisiae*) Phototrophic bacteria (*Rhodopseudomonas palustris*)	Foliar
EM.5	Lactic acid bacteria (*Lactobacillus delbrueckii, Lactobacillus plantarum, Lactobacillus rhamnous, Lactobacillus casei*), Yeast (*Saccharomyces cerevisiae*) Phototrophic bacteria (*Rhodopseudomonas palustris*)	Foliar
EM.A	Lactic acid bacteria (*Lactobacillus fermentum, Lactobacillus plantarum, Lactobacillus rhamnous, Lactobacillus casei, Lactobacillus delbrueckii*), Yeast (*Saccharomyces cerevisiae*) Phototrophic bacteria (*Rhodopseudomonas palustris*) Others	Soil

The shoot length (cm) was measured in terms of morphological features. The harvested fruits were separated from green peels and dried in the shade. Average nut and kernel weight were determined on precision electronic scale (0.01 g) then the kernel ratio (%) was calculated. The widths, height, length of the nut and shell thickness were stated using a digital caliper sensitive to 0.01 mm (Şen, 1980). Fruit color was measured by a CR400 model Minolta Colorimeter in CIE L* a* b*. For yield, the total amount of nut was recorded in each tree at harvest time (kg). In fruit which is accepted as 4 parts the shrinkage ratio of each part was determined as % (Aşkın and Gün, 1995; Beyhan, 1993; Şen, 1980). In addition, kernel browning ratio (%) was found. For nutrient analysis, the dried leaf samples were ground. The Kjeldahl method for N; the colorimetric method for P; the flame fotometric method for Ca and K; atomic absorption spectrophotometer for Mg, Fe, Cu, Zn and Mn analyses were used (Kacar and İnal, 2008).

The experiment was carried out according to the design of the random blocks, with 3 replications and 3 trees per replication. 10 samples were evaluated for each replication. The data were subjected to analysis of variance using SPSS 20 statistical package program. Significant differences between averages were defined by Duncan test at the $P<0.05$ significant level.

Results and Discussion

Some properties of Chandler walnut variety according to applications are given in Table 2. According to this, the longest shoots were measured with 55 cm in EM.A application. This was followed by control (47 cm) and EM.5 (42.72 cm), respectively. The shortest shoots were determined in the EM.FPE (36.93 cm) microbial fertilizer. Considering some fruit properties, while there was a statistical difference between applications in terms of nut weight and kernel browning, other features did not change (Table 2). In terms of nut weight, the heaviest fruits were obtained in application EM.A (12.85 g). After that EM.FPE (12.58 g) and EM.5 applications (12.47 g) took place. In contrast, the control group was observed the last row (11.97 g). It was seen that the applications had a positive effect on the kernel browning. Although it was not statistically important, the value of shell thickness, nut width, length and height was found to be lower than the others. Applications led to increased yield per tree. In this way, EM.5 (2.52 kg) and EM.A (2.28 kg) applications were the first group, while EM.FPE (1.28 kg) and control (0.78 kg) were in the second group.

There are many studies on the effects of plant growth promoting rhizobacteria (PGPR) in fruit species (Aslantaş et al., 2007; Eşitken et al., 2003; Dede, 2013). In this study, in which the effects of microbial fertilizer applications on some properties of Chandler walnut variety were tested, EM.A and EM.5 applications increased the shoot length and this effect was detected in different bacterial applications on sweet cherry (Atılgan et al., 2019) sour cherry (Arıkan and Pırlak, 2016), apple (İpek et al., 2016), strawberry (Parewa et al., 2014), raspberry (İpek et al., 2018) and hazelnut (Ertürk et al., 2011). It was reported that this increase may be caused by the change of hormone level (İpek et al., 2014). In our study, bacterial applications had a significant effect on nut weight. Fruit sizes are not statistical but relatively increased compared to control. It was stated that the effect of *Pseudomonas fluorescens* bacteria varies for nut weight, whereas significant increase for fruit width and length was determined on the same walnut variety. In this respect, alone and combination bacteria applications are important in terms of apple fruit weight and yield (Aslantaş et al., 2007; Eşitken et al., 2006; Karakurt, 2006; Karlıdağ et al., 2007; Pırlak et al., 2007). These treatments led to an increase in yield on quince (Gerçekoğlu et al., 2018) and sour cherry (Arıkan and Pırlak, 2016). Likewise, on strawberry (Ağgün et al., 2018) and raspberry (İpek et al., 2018), the positive effect of root bacteria application was mentioned on these properties. It was emphasized that combined microbial fertilizer application (EM.5 + EM.FPE) differed with regard to fruit weight, width and length and yield compared to control in olives tree. Thus, it was concluded that combined application is more effective (Acarsoy Bilgin, 2019).

Table 2. Shoot length, some fruit properties and yield according to applications

Applications Properties	Control	EM.FPE	EM.5	EM.A
Shoot length (cm)	47.00 ab	36.93 b	42.72 ab	55.00 a
Nut weight (g)	11.97 b	12.58 ab	12.47 ab	12.85 a
Kernel weight (g)	5.98^{ns}	5.99	5.83	5.99
Kernel ratio (%)	49.99^{ns}	47.62	46.73	46.66
Shell thickness (mm)	1.51^{ns}	1.66	1.68	1.70
Nut width (mm)	34.45^{ns}	33.58	33.98	34.48
Nut length (mm)	32.33^{ns}	32.71	32.84	33.11
Nut height (mm)	40.10^{ns}	41.61	40.99	41.24
Kernel L*	53.91^{ns}	55.05	56.07	54.07
Kernel a*	7.32^{ns}	7.43	7.39	7.97
Kernel b*	28.99^{ns}	28.97	28.79	28.51
Shrinkage ratio (%)	11.67^{ns}	8.33	8.33	13.33
Kernel browning (%)	20 a	0 b	0 b	0 b
Yield (kg tree^{-1})	0.78 b	1.28 b	2.52 a	2.28 a

The differences in the means were determined by the Duncan test according to P≤0.05. ns: Not significant

As a result of microbial fertilizer applications, macro and micro nutrient contents determined in Chandler walnut leaves are given in Table 3. It was seen that these applications had an important effect on the nutrient content. The effect of the applications on the N, P, K, Ca and Mg content of the leaf was statistically significant at the level of 5%. EM.FPE application with the highest value in terms of all macro elements took the first place. On the other hand, the nutrient content was the lowest in untreated trees. Leaf N nutrient content ranged from 2.42% (control) to 3.04% (EM.FPE). While all applications formed the same statistical group, control was included in different groups. Compared with limit values (2.47-2.98%), N content was determined to be sufficient except untreated trees (Mills and Jones, 1996). Thus, applications had a positive effect. Leaf P nutrient content varied from 0.09% to 0.14%. EM.FPE application, in which the highest content was determined, ranked the first row and formed a separate statistical group. This was followed by EM.5 and EM.A with the same value (0.11%). Even though applications increase P content, limit values (0.16-0.24%) were not reached (Mills and Jones, 1996). The highest value in terms of leaf K nutrient content belongs to EM.FPE application with 1.88%. This application was in the same group with the others, except untreated trees. Control located at the second group with 1.55%. This element content was found above the limit values (1.32-1.47%) in all applications (Mills and Jones, 1996). Leaf Ca nutrient content was similar to K. Accordingly; the Ca content variation range was determined as 1.43% (control) - 2.60 (EM.FPE). It was noteworthy that the applications exceed the limit values of 1.90-2.01% (Mills and Jones, 1996). Leaf Mg nutrient content was determined with the highest values in EM.FPE (0.74) and EM.A (0.70%) applications, respectively. EM.5 and control were in the same group. It was seen that Mg content was the limit value (0.51-0.63%) and above in all applications including control.

Similar to the macro element content of the leaves, the effect of the applications was statistically important on the Fe, Cu, Zn, Mn and B content of the leaf (Table 3). The highest contents were determined in EM.FPE application, except Zn. In contrast, the lowest value was found in the control group for other elements except Fe and Cu. Leaf Fe element content ranged from 74.97 ppm (EM.5) to 138.20 ppm (EM.FPE). Although EM.FPE application limit values (69-129 ppm) are exceeded, other applications are also included in limit values (Mills and Jones, 1996). For Cu content, EM.FPE was in the first place and in a different group with 8.83 ppm. Other applications were in the same group. EM.5 had the lowest value. All applications are seen below the limit values of 10-12 ppm (Mills and Jones, 1996). Leaf Zn content reached the highest value with EM.A (23.58 ppm). This was followed by EM.FPE with 20.67 ppm. Control and EM.5 were in the same group. This content was determined below the limit values (Mills and Jones, 1996). The leaf Mn nutrient content significantly increased compared to control in EM.FPE (447.20 ppm) and EM.A (393.47 ppm) applications, respectively. The lowest Mn content was found in the untreated trees with 129.27 ppm. EM.5 constituted the second group. Microbial fertilizer applications have increased the Mn content too above the limit values (Mills and Jones, 1996). Leaf B content varied between 26.59 ppm and 41.23 ppm. Accordingly, EM.FPE located the first group and the others were the second group.

In this study conducted by us, applications were determined to have a statistically significant effect in terms of nutrient content. Similarly, it was reported that the increase in the content of leaf nutrients was important as a result of different bacterial applications in Heritage raspberry (İpek, 2019) and Italian grape (Erdoğan et al., 2018). Also, confirming our findings, leaf P and Fe content increased by bacterial inoculations on apple (Pırlak et al., 2007), cherry (Eşitken et al., 2006), hazelnut (Ertürk et al., 2011) and strawberry (İpek et al., 2014). These applications were reported to affect the P and K content on pomegranate, in addition (Fayed, 2010). It was stated by İpek (2019) that leaf nutrient content varies according to bacterial strains, fruit species and varieties. It is seen that the use of microbial-based fertilizers has become widespread in recent years (Küçük, 2019). Through bacteria, nutrients are transformed into a form that plants can use, thereby increasing nutrient uptake (Malua and Vassilev, 2014). Consequently, in our study, EM applications contributed positively to nutrient content and fruit properties.

Table 3. Macro and micro nutrient content

Applications Properties	Control	EM.FPE	EM.5	EM.A
N (%)	2.42b	3.04 a	2.76a	2.79a
P (%)	0.09 c	0.14 a	0.11 b	0.11 b
K (%)	1.55 b	1.88 a	1.77 a	1.85 a
Ca (%)	1.43 b	2.60 a	2.31 a	2.40 a
Mg (%)	0.56 b	0.74 a	0.57 b	0.70 a
Fe (ppm)	122.13 ab	138.20 a	74.97 c	112.47 b
Cu (ppm)	7.03 b	8.83 a	6.40 b	7.27 b
Zn (ppm)	16.44 c	20.67 b	17.96 c	23.58 a
Mn (ppm)	129.27 c	447.20 a	277.77 b	393.47 a
B (ppm)	26.59 b	41.23 a	27.14 b	28.78 b

The differences in the means were determined by the Duncan test according to $P \leq 0.05$.

Conclusion

Due to environmental and human health awareness, studies focused on protecting the environment, reducing the use of chemical fertilizers. Sustainable agricultural techniques are of great importance. In this regard, microorganisms used as bio fertilizers have a positive effect on many properties in horticultural plants. In this study, where effective microorganisms were tested, the best results were obtained from EM.A application in terms of shoot length, nut weight, kernel browning and yield. In addition, all applications positively affected nutrient content compared to control, except Cu and Fe. In this context, it is thought that it would be beneficial to plan the applications according to the methods and doses.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

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