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Original article

Effect of melatonin and gibberellic acid foliar application on the yield and quality of Jumbo blackberry species

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ABSTRACT

Not many chemical hormone studies does exist in our country especially in berry fruits. Foliar application of melatonin and gibberellic acid hormones was performed in 2016 and 2017 in order to analyze the effects of different doses of hormone applications on fruit quality and yield. Pheonologic, pomological and some bioactive content analyses of these hormones were studied. According to the research results, while M + GA10 ppm (240.50; 3.9) and M + GA 5 ppm (226.50; 3.6) applications have the highest fruit number and weight (g), the highest fruit size was obtained from GA 5 ppm (21.21 mm fruit length, 16.56 mm fruit width) and M 10 ppm (21.10 mm fruit length, 16.20 mm fruit width) chemical applications in Jumbo blackberry species. The highest values in Ph, soluble solid content (SSC) and titratable acidity averages were obtained from GA 5 ppm (3.69; 10.80; 2.42) and GA 10 ppm (3.68; 10.70; 2.40) applications; the highest total antioxidant activity in bioactive characteristics averages was found in G 10 ppm 143.21 mg/g, the highest total phenolics was identified in G 5 ppm 72.68 ppm/GAE and the highest total flavonoids was determined in G 10 ppm 4925.75 ppm/QE.

1. Introduction

Berry fruits are the species which are demanded by consumers and utilized in various ways. Turkey is within the natural range of these species and we can see different types of one or more species in almost all regions. Economic analyses indicate that berry fruits are among the agricultural products with the highest yield from unit area. They are also more advantageous than many fruit species in terms of short-term fructification and quick delivery of maximum yield (El, 2008). Due to the diversification of fresh or frozen use of berry fruits in the food industry and especially the increase in its importance for dairy products industry, ice cream producers, confectionery and pastry producers; these fruits are processed regularly in the food sector as fresh, mashed, jam, molasses, syrup or fruit juice (Yetgin, 2009; Okatan, 2018). Blackberry from Rosaceae family of Rosales group, Rosaideae sub-family, Rubus genus and Eubautus sub-genus is a fruit type with rich vitamin and mineral content, again with rich antioxidant capacity, which has gained a special importance in recent years (Karakoç, 2011) (see Fig. 1).

Recent reseaches have shown that blackberries contain the chemicals with antioxidant and anticancer properties, which is of great importance in terms of human health, at a higher rate than the quantities mentioned in the literature. Although blackberries contain less amount of A, B, C vitamins, it has been found that they have protective traits against colon cancer and heart diseases due to their high fiber content. It has been observed that berry fruits consist high rates of ellagic acid and anti-cancer features which inhibit tumour development in animals under laboratory conditions (Pehluvan and Güleryüz, 2004). In adition, both fruits and leaves of blackberries, which have antiviral characteristics, prevent Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria (Yiğit and Yiğit, 2014). Depending on the concentration, the blackberry extract destroys the virus population by up to 99%, resulting in its high use in the treatment of intra oral wounds since the 16th century (Danaher et al, 2011).

These fruit species have a large market share in the world as they are widely consumed, particularly in countries with high income levels. However, in Turkey, only wild types of them are consumed and their culture types are not well-known until recent years (Onur, 2006). While cultivation and breeding of this species began a long time ago in other countries, the importance of these species has just recently been realized.

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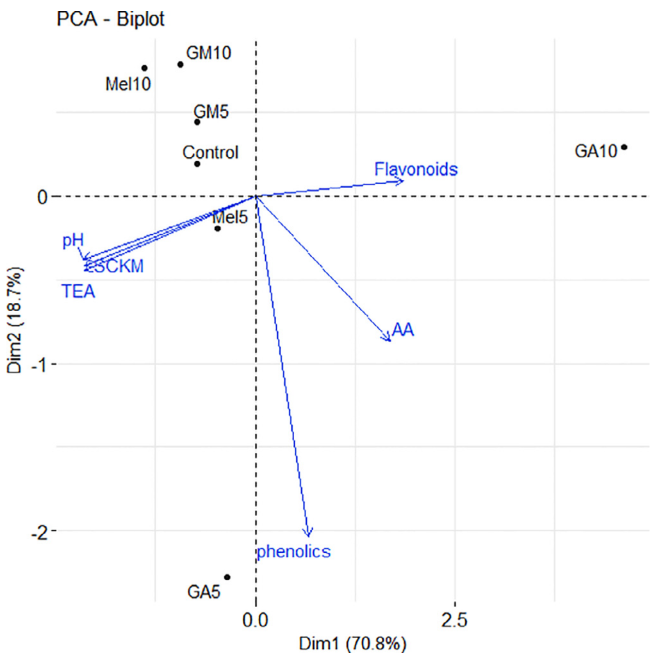

Fig. 1. Basic component analysis of chemical analysis.

Gibberellic acid is responsible for plant growth and leaf enlargement in plants (Matthew et al., 2009). Until recently, the melatonin hormone was thought to exist only in animals. It has later been found that this hormone also occurs in the plants and that it protects antioxidant properties. With the discovery of melatonin in plants, the studies on melatonin has rapidly started to increase (Poeggeler and Hardeland, 1994; Antolínet al., 1997).

As well as making berry fruit cultivation common, developing suitable species, performing adaptation works in different species, the works for raising awareness about cultivation techniques that will guide in berry fruit cultivation and provide the most accurate production also gain importance. The goal of this paper is to analyze the effects of different doses of melatonin and gibberellic acid chemicals on the yield and quality of blackberry plant grown in the ecology of Uşak province in Turkey and to present the first basic study source for blackberry and other berry fruits especially in melotonin application.

2. Materials and methods

2.1. Plant material

Jumbo blackberries were used as plant material in our study carried out in 2016 and 2017. The plant material was conducted in species of Jumbo blackberry found in the 5-year-old Blackberry garden with 5 decare of field in Usak province. Our experiment was established as 3 replications, each of which would have 10 plants. Plants are at the distance of 1.5 m between rows and 0.6 m above rows. Melatonin (M), gibberellic acid (GA) and melatonin + gibber ellic acid (M + GA) are applied through foils as foliar applications. For each application at 2 different doses (5 ppm and 10 ppm) 2.5 ppm M and 2.5 ppm GA for M + GA 5 mixtures and 5 ppm M and 5 ppm GA mixtures for m + GA 10 ppm were applied twice in flowering period and fructification period.

2.2. Chemical material

The melatonin chemical was provided by Sigma company (M5250 SIGMA Melatonin powder, ≥98% (TLC), Germany). Gibberellic acid chemical and all other used materials was from Merck company (Cas-No: 77-06-5, Merck KGaA, Germany.

2.3. Determination of Bioctive contents

2.3.1. Determination of pomological and chemical fruit characteristics

The weight (g), length (mm) and width (mm) of the fruit were measured by applying 3 replications on randomly-selected total 30 blackberries after they were harvested. Soluble solid content (SSC), pH (%) and titratable acidity contents of the fruits were also determined (AOAC, 1995).

2.3.2. Identification of Total Phenolic Content (TPC)

By employing the Folin-Ciocalteu method, The TPC of blackberry juice extract was indicated. 4500 μL deionized water and 500 μL unsubtilised Folin-Ciocalteureagent were laced with 1000 μL extract. Following 60 s, 4000 μL of 7.5% (w/v) aquatic Na_2CO_3 was mixed. And then, the solution was taken to 30 min of maturing period at 30 °C, which was then followed by measuring the absorbance at 765 nm through employing an UV–Vis spectrophotometer. And the result was aligned with a gallic acid calibration curve. The all phenols were identified as gallic acid equivalents (mg gallicacid/g extract), the valves of which have been suggested as medium of triple assessment (Kähkönen et al., 1999).

2.3.3. Identification of Total Flavonoid Content (TFC)

The TFC of the plant extraction was identified by employing aluminum chloride colorimetric assay (Chang et al., 2002). To begin with, 0.5 ml aliquots of the extract and 0.01–1.0 mg/ml of quercetin were mixed with 2 ml of distilled water and then with 0.15 ml of sodium nitrite (5% $NaNO_2$ w/v). Upon waiting for 6 min, 0.15 ml of it (10% $AlCl_3$, w/v) was accompanied. The solutions were made to rest for 6 min more. The last volume was adjusted to 5 ml level by adding instantly the water under distillation, then it was mixed utterly and left to rest up to quarter of an hour. The absorbtion of each composition was identified at the level of 510 nm together with an empty tube as a controller. TFC was determined as mg quercetin equivalent to per gram of sample with the help of calibration curve of quercetin. Every test that indicate the level of extract was conducted for three times (n = 3).

2.3.4. Idenetification of Antioxidant Activity (DPPH)

The DPPH (2,2-diphenyl-1-picrylhydrazyl) was conducted by employing Thaipong et al. technique (Thaipong et al., 2006). The existing solution was made up through solving 24 mg of DPPH into 100 ml of methanol which was then stocked at −20 °C till necessity accurs (Brand-Williams et al., 1995). The working solution was derived by way of stirring 10 ml of existing solution with 45 ml of methanol so as to make the absorbtion of 1.1 ± 0.02 units at 515 nm emplaying the spectrophotometer Shimadzu UV Mini 1240. 150 μL plant extract were put under reaction with 2,850 μL of the DPPH sol for an hour under the darkness. Afterwards, the absorbtion level was applied at 515 nm. The antioxidant level showed a demise in absorbtion value under the equation. The outcomes of the absorbtion were transformed into the table content via a standardized calibration curve. These outcomes was then noted in ascorbic acid equivalents (AAE). The extract which supplies 50% of radical scavenging activity (IC_{50}, the concentration of the sample to scavenge 50% of the DPPH radicals) was counted up by the the graphic of scavenging percentage against extract concentration. In order to achieve this goal, subtilization series (five different concentrations) were made up for every plant sample extract. The resulting valves were counted up and given in μg/ml.

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2.4. Statistical analysis

The normal distribution appropriateness of the variables in the study was analyzed by the Shapiro Wilk test. The applications were compared through Kruskal Wallis method for the variables at which the normal distribution was not ensured. One-way variance analysis was applied for the variables at which the normal distribution was ensured. Homogeneity of the variances was analyzed by Levene test for the variables to which one-way variance analysis was applied. While Tukey test was used for multiple comparison and lettering in the variables that the variance analysis was applied to, the variables that Kruskal Wallis applied were also compared with Whitney *U* test and the lettering was performed according to this comparison. Analyses were performed on SAS 9.4 and R 3.3.4 statistics softwares. Basic components analysis was also applied (PCA).

3. Results and discussion

3.1. Pomologic and chemical properties

Phytochemical chemical analysis averages obtained as a result of chemical application on Jumbo blackberry species are presented in Table 1. According to the results, a significant ($P < 0.05$) difference was found between applications. While the highest fruit amount and weight (g) (240.50; 3.9) was obtained from M + GA10 ppm application, M + GA 5 ppm (226.20; 3.6) application followed after in terms of amount and weight. In the experiment, GA 5 ppm application gives the highest fruit size as 21.21 mm fruit length, 16.56 mm fruit width and GA 10 ppm application shows the second highest fruit size as 21.10 mm fruit length, 16.20 mm fruit width. In one study, it was found that GA3 treatment not only delayed harvest time but also increased fruit size and firmness and decreased resistance to indentation as well as increasing soluble solid content (SSC) (Basak et al., 1998). In some grape species, during or just after full flowering period, 2.5–20 ppm GA application is recommended in order to increase the bunch size and in some species 100 ppm GA application is advised in order to accelerate ripening and increase the fruit size (Akgül, 2008). The study carried out on 14 blackberry species (Arapaho, Novaho, Woldo, Black satin, Chester, Dickson Thornless, Boysenberry, Bartın, Bursa 1, Bursa 2, Bursa 3, Nessy, Jumbo Cherokee) in Giresun by Kurt et al. (2003) indicates that Jumbo species stood out with 79.39 g of weight with the best results in terms of fruit size and yield. Arapaho, Novaho and Cherokee species were the other species adapting to the region. In order to identify the most suitable blackberry species for the ecological conditions of Samsun Çarşamba plain, 14 blackberry species (Chester, Ness, Navaho, Bursa I, Bursa II, Bursa III, Waldo, Jumbo, Bartın, Arapaho, Cherokee, Dickson Thornless, Black Satin, Boysenberry) were put under experiment. In the pomological analysis for 2000–2002, it was found that the heaviest fruits turned out to be Bursa I with 6.4 g and Jumbo with 6.1 g; however, the lightest fruits were titled as Navaho with 3.5 g and Chester with 3.8 g. Given the yield values per decare, Ness, Chester, Bursa I and Jumbo were in the first rank with 587, 533, 333, 332 kg/da respectively, while Cherokee, Waldo and Boysennberry species were in the last rank with 64, 58, 27 kg/da respectively (Akbulut et al., 2003).

Table 2
Chemical characteristics of blacberry (2016–2017).

Application	pH (%)	SSC (%)	Titratable acidity (%)
Control	3.34 cd	10.15 ab	2.34 ab
M 5 ppm	3.64b	9.50c	2.27b
M 10 ppm	3.61b	9.70b	2.36 a
GA 5 ppm	3.69 a	10.80 a	2.42 a
GA 10 ppm	3.68 a	10.70 a	2.40 a
M + GA5ppm	3.48c	9.60b	2.23b
M + GA10ppm	3.47c	10.55 a	2.05c

$p < 0.05$

According to Phytochemical chemical analysis averages obtained as a result of the chemical applications to Jumbo blackberry species in Table 2, a significant ($P < 0.05$) difference was found between applications. According to these results, the highest value in pH, Soluble solid content and titratable acidity averages was obtained in GA 5 ppm (3.69; 10.80; 2.42) and GA 10 ppm (3.68; 10.70; 2.40) applications. Avcı (2013), in his acidity for 2012 search, suggested that 1.26% of acidity value was obtained from Jumbo species and 1.01% of acidity value from Chester species, while Bursa I species had the highest acidity value with 1.56% of acidity. In a study carried out in Isparta, Bursa I species stood out with 1.39% in terms of titratable acidity and the acidity of Jumbo species was 1.26% and the acidity of Chester species was 1.19% (Göktaş et al., 2006). In an adaptation study carried out in Trabzon, Bursa I species was in the first rank with 1.97% of acidity rate among all species. However, the acidity values of Jumbo and Chester species were 1.38% and 1.27% respectively (İslam et al., 2009). When it comes to the fruit weight of blackberry species for 2006, it was found out that Chester had the highest fruit weight with 4.84 g and Dirksen Thornless with 4.36 g and Jumbo with 4.18 g come after it. When total acid amounts of blackbery species was analyzed, it was observed that Dirksen Thornless again had the highest total acidity with 33.50 g/l and Jumbo had 30.00 g/l of acidity. The research for 2006 indicate that Navaho possesed the minimum total acidity as 22.40 g/l and the maximum soluble solid content as 20.70% and Bursa 1 with 18.90%, Bursa 2 with 18.30% and Jumbo with 13.60% followed it respectively (Ağaoğlu et al., 2007). Avcı (2013) found the pH value in Jumbo blackberry species as 3.02 and the acidity value as 1.25. When the species are analyzed in terms of these properties, it can be seen that Chester species is in the first rank with 3.14 followed by Jumbo with 3.02 and Bursa 1 with 2.86. While İslam et al. (2009) in their study in Trabzon reported that there was 3.34 of pH in Jumbo species, 3.20 of pH in Bursa I species and 3.16 of pH in Chester species, Gerçekçioğlu et al. (2003) in their study in Tokat reported that the species with the highest pH was Cherokee 3.47 and pH values of Bursa I and

Table 1
Pomological fruit characteristics of Blacberry (2016–2017).

Application	Fruit Number (piece)	Fruit weight (g)	Fruit width (mm)	Fruit length (mm)
Control	131.88a	2.3ab	15.87ab	19.64bc
M 5 ppm	93.60bc	1.8ab	14.87c	19.45bcd
M 10 ppm	182.38a	2.8a	15.58bc	20.22ab
GA 5 ppm	122.67ab	2.3ab	16.56a	21.21a
GA 10 ppm	19.80c	2.5b	16.20a	21.10a
M + GA5ppm	226.20a	3.6a	15.15bc	18.39 cd
M + GA10ppm	240.50a	3.9a	15.30bc	18.12d

$p < 0.05$.

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Table 3
Average bioctive characteristics of blackberry (2016–2017).

Application	Total antioxidant activity mg/g	Total phenolics ppm/GAE	Total flavonoids ppm/QE
Control	40.20 e	59.66 bc	4038.10b
M 5 ppm	96.99 bc	57.79 bc	3473.57 cd
M 10 ppm	78.35c	51.01 d	2559.09 e
G 5 ppm	110.30b	72.68 a	3343.10 d
G 10 ppm	143.21 a	61.77b	4925.75 a
G + M5ppm	72.99c	54.89c	3458.94 cd
G + M10ppm	50.49 d	54.00c	3551.60c

$p < 0.05$

Jumbo species were 3.28 and 3.15, respectively. While Chester species stood out with 12.6% of soluble solid content in 2012, Avcı (2013) obtained 10.9% and 10.7% of soluble solid content respectively from Jumbo and Bursa I species. While in a study carried out in ecology of Giresun, the highest soluble solid content content was in Navaho with 13.33% and in Chester with 13.13%, soluble solid content of Bursa I and Jumbo species were 9.20% and 9.98%, respectively (Kurt et al., 2003). While the species with the highest soluble solid content in a study carried out in Samsun was Cherokee with 16.0%, Soluble solid content (SSC) of Bursa I, Chester and Jumbo species were 10.1%, 9.9% and 10.3%, respectively (Akbulut et al., 2003). Our results are parallelled with the results of other researchers. It is thought that the differences are due to cultural events and climate variability (see Table 3).

3.2. Bioctive contents

According to the results obtained from bioactive property averages of blackberry in 2016–2017, the highest total antioxidant activity was G 10 ppm 143.21 mg/g, the highest total phenolics was G 5 ppm 72.68 ppm/GAE and total flavonoids was G 10 ppm 4925.75 ppm/QE.

Though a lot of investigations are present on phenolic content and antioxidant activity of blackberry, (Wang et al., 1997; Kalt et al, 1999; Kalt et al, 2001; Moyer et al, 2002; Wada and Ou, 2002; Zheng and Wang, 2003; Siriwoharn et al. 2004; Cho et al., 2005), there dosen't exist enough research in Turkey (Çelik et al., 2008). Koca and Karadeniz (2009) found the total anthocyanin values between 0.95–1.97 and 1.73–3.79 mg gl and total pheonolic values for blackberries between 35.05 and 70.41 mmol g1, 7.41–5 7.92 mmol g1 in a study they carried out on 10 blackberry species (Arapaho, Bartın, Siyah Saten, Bursa 1, Bursa 2, Cherokee, Chester, Jumbo, Navaho and Ness). Hassimoto et al. (2005), found the levels of antioxidant activity as elevated (>70% inhibition), medium (40–70% inhibition), and nominal (<40% inhibition). These findings show that antioxidant content is present adequately and provide large amounts of antioxidant items while other fruits such as strawberry, cherry and blueberry possess medium antioxidant property.

The whole phenolic content varied from 305.38 (blueberry) to 850.52 mg GAE/100 g (blackberry). Given the polyphenol categorization by Vasco et al. (2008) taking nominal as (<100 mg GAE/100 g), and medium as (100–500 mg GAE/100 g) and elevated as (>500 mg GAE/100 g) denominations, blackberry with the phenolic content of (850.52 mg GAE/100 g) and the strawberry with (621.92 mg GAE/100 g) can be regarded as an elevated phenol concentration, which reveals the perfectness of these fruits in terms of phenol source.

Red raspberry having 9.61 of flavonoids, and blackberry 87.03 mg CE/100 g and cherry 59.92 mg CE/100 g and blueberry 47.53 mg CE/100 g and strawberry 38.17 mg CE/100 g can be seen the examples of medium values. Samec and Zegarac (2011).

In basic component analysis, 89.5% of the variances in the data was expressed in terms of applications and the analyzed variables. Titratable Acidity, Soluble solid content (SSC) and TEA variables were found positively high correlated with each other; however, they were found negatively correlated with flavonoids. The variable affecting the change in the data most was phenolics. While the richest application in terms of phenolics was GA5 ppm, the richest application in terms of flavonoid was observed in GA10 ppm. M 10 ppm, MG10 ppm, MG5 ppm and Control Groups were at the same level in terms of the relevant variables and they were accumulated together in PCA.

4. Conclusion

Blackberries are berry fruit species which gain importance gradually in our country and in the world. The studies to introduce the blackberry plant in cultivated plants, whose only wild species have been utilized for years, is an issue which has begun to be handled recently. Numerous adaptation experiments and studies on reclamation of species have been launched throughout the country and the increasing the production of blackberry plants has been the main goal of these studies. However, these efforts are not sufficient for quality products and high yield alone. The effects of various cultural activities such as pruning, irrigation, fertilization, fighting against diseases, cultivation systems applied in growing plant on quality and yield are known and the attempts on those segments have been going on for many years in many plant species. The recent introduction of blackberries into cultivated plants and the necessity of adaptation and breeding studies primarily have not yet allowed adequate research of other cultural practices. In this study on the jumbo blackberry species, the effects of the gibberellic acid and melatonin chemicals applied at different doses on fruit yield and quality were researched. This study is of great importance due to its being the first to investigate the effect of melatonin application through foils on the yield and quality of Jumbo blackberry species. These on-going studies will be very contributive to improve the conditions needed for the production and popularity of blackberry in the future.

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