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THE EFFECT OF TREATED WASTEWATER LEVELS ON THE ROOTING OF BLACKBERRY (*Rubus fruticosus* L.) GREEN CUTTINGS

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Abstract: The world population is growing, leading to a gradual decrease in available water supplies. Reusing wastewater is seen as beneficial for environmental conservation and mitigating water contamination. Recycled wastewater includes essential nutrients for agricultural growth, and its reuse can lower the need for fresh water in dry areas. This study examined the impact of diluted treated wastewater at various ratios on the roots and shoot growth of blackberry green cuttings. For this purpose, Jumbo blackberry green cuttings located at the Bilecik Şeyh Edebali University Agricultural Practice and Research Center were used in 2023. In September, cuttings were taken and treated with a control (0 ppm IBA) and a 4000 ppm dose of indole-3-butyric acid (IBA). The cuttings were then planted in rooting tables with perlite and bottom heat (22±2 °C). From the time of planting, the cuttings were irrigated with treated wastewater from the university's wastewater treatment facility, diluted in five different doses (0, 25, 50, 75, 100 %). In the cuttings removed from the rooting medium after 90 days, the following were determined: survival rate (%), rooting rate (%), callus formation rate (%), number of roots (per cutting), root length (cm), root diameter (mm), fresh and dry root weight (g), chlorophyll a (µg/g DW), chlorophyll b (µg/g DW), and total chlorophyll content (µg/g DW). At the end of the trial, the positive effects of treated wastewater on rooting had been determined. The rooting rate was determined to be best in the Control (0 ppm IBA)+TWW100 application (73%). The research suggests that blackberry cuttings can be rooted using treated wastewater without the need for IBA

Keywords: Chlorophyll, IBA, Jumbo, Rooting rate

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1. Introduction

Water is acknowledged as the most crucial resource for sustainable agricultural development globally (Davies and Simonovic, 2011; Ungureanu et al., 2020). It is a crucial resource for supporting economic growth and social well-being. Factors such as fast worldwide population growth, changes in land use, the expansion of productive activities including agriculture, industry, and tourism, and urban development have contributed to the deterioration of water supplies (Liu et al., 2017; Zhang et al., 2017). Recently, there has been a consistent rise in the demand for water resources. Global water usage has increased by an average of 1% annually from 1980 to the present (Velasco et al., 2018). The growth is projected to persist until 2050, with an anticipated surge of 20-30% above present levels in the next several years (Bouwer, 2002).

The rising need for water resources, coupled with the incapacity to fulfill this need, can be eased by utilizing wastewater for irrigation, thus reducing strain on freshwater resources (Toze, 2006), and can also reduce the need for fertilizers due to their nutrient content (Angelakis, 1999). Both treated and untreated wastewater is utilized for irrigation in certain arid and semi-arid regions for various reasons. Research indicates that regions irrigated with wastewater see heightened soil biological activity and nitrogen cycling (Filip et al., 1999; Speir, 2002; Chen et al., 2008).

Wastewater can decrease heavy metal levels based on its composition (Charerntanyarak, 1999; Brown et al., 2000; Rae and Gibb, 2003; Lesage et al., 2007) and microbial pathogens (Zhang and Farahbakhsh, 2007; Reinoso et al., 2008), as well as increase organic and mineral components (Mujeriego and Asano, 1999; Toze, 2006; Muga and Mihelcic, 2008).

The blackberry plant, scientifically known as *Rubus fruticosus*, belongs to the *Rosaceae* family and is part of the aggregate fruit group (Padmanabhan et al., 2016). Its adaptability in different environments and its sweet yet somewhat tangy flavor have caused a rise in popularity in recent times.

Blackberry fruit is a rich source of carbohydrates, protein, ascorbic acid, sugars, carotenoids, minerals, and vitamins (Zia et al., 2014). Blackberry plants are commercially propagated using cuttings from breeding plants (Lopez-Medina and Moore, 1997; Bray et al., 2003;

Takeda et al., 2011).

Various irrigation methods are used in blackberry cultivation. Blackberries intended for fresh consumption are typically irrigated with drip systems, whereas those for processed consumption are mainly watered with overhead sprinklers, mobile pipes, or huge gun systems (Strik and Finn, 2012).

Global warming has caused more hot weather, resulting in drought as a major environmental stress that greatly hinders plant growth and development in many areas. Therefore, the need for water resources is increasing. Wastewater is now being utilized as a solution to address the challenges of global water scarcity.

Worldwide studies have reported examples of wastewater reuse in the irrigation of table grapes (Petousi et al., 2019), olives (Petousi et al., 2015), and even vegetables (Christou et al., 2017; Farhadkhani et al., 2018; Libutti et al., 2018; Mehmood et al., 2019). However, no studies have investigated the use of treated wastewater for irrigating blackberry cuttings.

This study examined the impact of treated wastewater, diluted at different ratios, on the roots and shoot growth of blackberry green cuttings. The study investigated the feasibility of utilizing treated wastewater in agriculture and its effects on rooting. It also determined how propagation through cuttings responded to these waters.

2. Materials and Methods

This study was carried out in 2023 at the Bilecik Şeyh Edebali University Agricultural Application and Research Center, within a high tunnel rooting system. The material used was the Jumbo blackberry variety obtained from the research center. The specific origin of the Jumbo blackberry variety is uncertain, but it has been grown in America since 1920 and has shown positive results in adaptation tests undertaken in our nation (Akbulut et al., 2003; Cangi and İslam, 2003; Gerçekcioğlu et al., 2003). The treated wastewater used in the study was obtained from the Bilecik Şeyh Edebali University wastewater treatment facility and diluted according to the doses used in the study. The chemical values of the treated wastewater are provided in Table 1.

Table 1. Chemical analysis results of the treated wastewater used in the study

Chemical properties of treated wastewater[1]	Average values	Limit value[2]
Total Suspended Solids (mg l^{-1})	20.35	70
Chemical Oxygen Demand (mg l^{-1})	58.84	180
Biological Oxygen Demand (mg l^{-1})	34.00	50
pH	8.42	6-9

[1] Conducted within the scope of the Ministry of Environment, Urbanization and Climate Change qualification certificate and TÜRKAK.
[2] Limit values, water pollution control regulation.

The green cuttings were taken on September 15th, a time identified by Edizer (2011) as the most suitable for vegetative multiplication in the Jumbo blackberry variety, as stated in his research on assessing the vegetative propagating potential of this variety. 15-20 cm cuttings from one-year-old branches were treated with a 0.3% fungicide (Benlate) and let to dry for about ten minutes before planting.

In the experiment, along with a control treatment, five different levels of treated wastewater (0%, 25%, 50%, 75%, and 100%) were used in conjunction with the 4000 ppm IBA dose, which was determined to be the best rooting rate for green cuttings (Edizer, 2011) (Table 2). The basal sections of the cuttings were immersed in the IBA solution utilizing the quick dip technique (Zenginbal and Eşitken, 2016), subsequently planted in rooting tables that contain perlite with bottom heat provision (22±2 °C). Irrigation was conducted at the designated water levels at the time of planting.

Table 2. The IBA dose applied in the study and the concentrations of treated wastewater

Applications		Definition of treated wastewater (TWW) concentration
Control (0 ppm IBA)+TWW0	4000 ppm IBA+TWW0	100% pure water was used
Control (0 ppm IBA)+TWW25	4000 ppm IBA +TWW25	25% treated wastewater and 75% pure water was used
Control (0 ppm IBA)+TWW50	4000 ppm IBA +TWW50	50% treated wastewater and 50% pure water was used
Control (0 ppm IBA)+TWW75	4000 ppm IBA +TWW75	75% treated wastewater and 25% pure water was used
Control (0 ppm IBA)+TWW100	4000 ppm IBA+TWW100	100% treated wastewater was used

TWW0= 100 % pure water, TWW25= 25% treated wastewater and 75 % pure water, TWW50= 50% treated wastewater and 50 % pure water, TWW75= 75% treated wastewater and 25 % pure water, TWW100= 100% treated wastewater.

Blackberry cuttings were placed on propagation rooting tables with perlite for 90 days. After the study ended, the cuttings were removed to assess the following parameters: survival rate (%), callus formation rate (%), number of roots (per cutting), root length (cm), root thickness (mm), fresh and dry root weight (g), chlorophyll a (g l^{-1}), chlorophyll b (g l^{-1}), and total chlorophyll content (g l^{-1}).

The trial was conducted according to a randomized block trial design with 3 replications, and each replication consisted of 15 cuttings. Following the analysis of variance, the means of the treatments were compared using the Least Significant Difference (LSD) multiple comparison test. The statistical analyses were performed using the MSTAT-C software package (Michigan State University v. 2.10).

3. Results and Discussion

Statistically significant differences were found in all categories except for the ratio of live cuttings and callus formation when analyzing the data acquired from the use of treated wastewater on blackberry cuttings. Survival was determined by counting callused and rooted cuttings. Figure 1 shows that the survival rate of cuttings and the rate of callus formation were comparable. There was no statistical difference between the treatments, except for when 4000 ppm IBA+TWW100 was applied. The control group exhibited a 100% survival rate and callus ratio at all concentrations of treated wastewater (Figure 1). Edizer (2011) conducted a study on blackberry green cuttings in September and discovered that the survival rate of cuttings and callus formation was comparable at a 4000 ppm IBA dose. They were reported to have achieved a 100% success rate in both the control group and the group treated with a 4000 ppm dose. Our investigation showed a 100% success rate in survival cuttings and callus formation while using the control application. However, a decrease in success rate was noted in applications other than the 25% treated wastewater (4000 ppm IBA+TWW25) at the 4000 ppm dose.

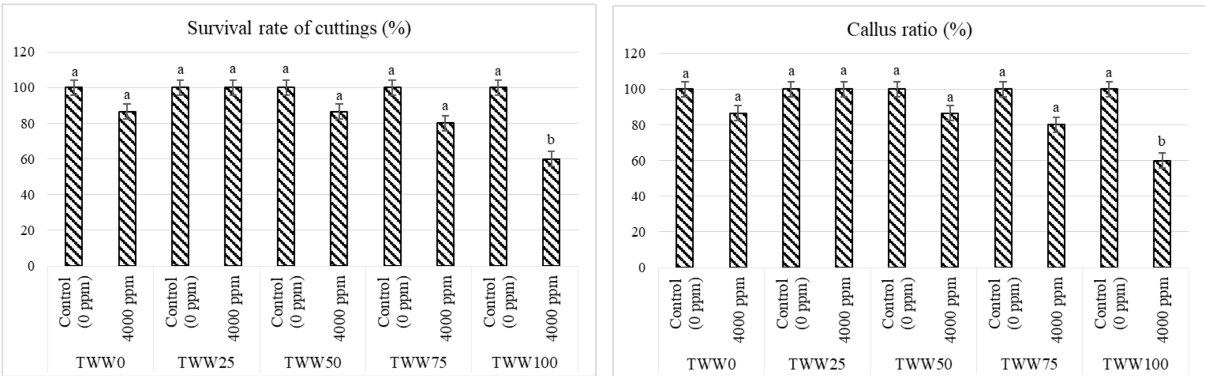

Figure 1. The effect of treated wastewater on the survival rate(%) and callus ratio (%) of Jumbo blackberry cuttings.
TWW0= 100 % pure water, TWW25= 25% treated wastewater and 75 % pure water, TWW50= 50% treated wastewater and 50 % pure water, TWW75= 75% treated wastewater and 25 % pure water, TWW100= 100% treated wastewater.

The effects of treated wastewater applications on the rooting rate, root number, root length, root diameter, root fresh and dry weight of blackberry cuttings are presented in Tables 3 and 4. The highest rooting rate average was found in the Control group (0 ppm) at 67.21%. After analyzing the mean concentrations of treated wastewater, it was discovered that concentrations of 50%, 75%, and 100% are statistically equivalent, with the optimal value identified as 75% (71.00%) (Table 3). The findings from the control group, 4000 ppm IBA + TWW50, and 4000 ppm IBA + TWW75 applications are statistically similar when analyzing the interaction table for rooting rate and treated wastewater. The control group achieved the maximum rooting rate of 73% with the application of 100% wastewater (Control (0 ppm IBA)+TWW100). (Table 4). As mentioned in previous studies, it has been observed that wastewater has an effect on rooting. Yıldız et al. (2009) reported a 24% rooting rate as a result of applying 6000 ppm IBA, Roussos et al. (2020) achieved an 85% rooting rate following the application of 2000 ppm IBA, and Edizer (2011) reported a 100% rooting rate in green cuttings after applying 4000 ppm IBA. The study results showed that the rooting rate of 35% achieved with the treatment of 4000 ppm IBA+TWW0 was low. This is believed to be caused by temperature and environmental factors, as mentioned in the literature (Cigdem et al., 2022).

The analysis of the mean values for the applications for the number of roots revealed that the combination of a 4000 ppm IBA dose and 100% treated wastewater yielded the most favorable outcomes (Table 3). The analysis of the interaction table revealed that the number of roots per cutting ranged from 3.83 to 10.03. The Control (0 ppm IBA)+TWW75 application has the fewest roots (3.83 per cutting), whereas the 4000 ppm IBA+TWW100 application has the most roots (10.03 per cutting). Treated wastewater has been found to have a beneficial impact on root growth at the 4000 ppm IBA concentration (Table 4).

The most favorable results for root length and root diameter averages were found to be 3.21 cm and 0.67 mm, respectively, in the Control (0 ppm IBA) group. Upon examining the average concentrations of treated wastewater, it was found that the best root length was obtained at 75% concentration (TWW75), while the best root diameter was obtained from both 25% (TWW25) and 75% (TWW75) concentrations (Table 3). The best root length was determined in the Control (0 ppm IBA)+TWW75 interaction, while the lowest root length was found in the 4000 ppm IBA+TWW25 interaction (Table 4). The root diameter interaction with treated wastewater showed that the Control (0 ppm IBA)+TWW0 and Control (0 ppm IBA)+TWW25 applications had statistically similar diameters of 0.84–0.82 mm. Reductions were observed in the interaction between treated wastewater and the 4000 ppm IBA dose (Table 4). The beneficial impacts of treated wastewater combined with 4000 ppm IBA on rooting rate and root quantity have resulted in a reduction in root length and diameter. In *Helianthus annuus* (Fozia et al., 2008), *Phaseolus vulgaris* (Bhardwaj et al., 2009), and *Pisum sativum* (Hattab et al., 2009) plants, a decrease in root length was also observed, which is reported to likely result from the accumulation of toxic elements in the root zone. Kocak et al. (2005) reported that the nutrients contained in wastewater become toxic to plants after irrigation with wastewater.

The control (0 ppm IBA) treatment yielded superior results in terms of root fresh and dry weights compared to the 4000 ppm IBA dose. The concentrations of treated wastewater had no statistically significant effect on root fresh weight. For root dry weight, it was determined that the 75% (TWW75) concentration yielded the best result. Upon examining the interaction between root fresh weight and treated wastewater, it was determined that the control (0 ppm IBA)+TWW50 and 4000 ppm IBA +TWW25 applications, respectively, yielded the best results. For root dry weight, it has been observed that the Control (0 ppm IBA)+TWW0 application was statistically the best. Baskaran et al. (2009) reported that diluted sugar factory wastewater maintained optimum nutrient levels, which in turn increased the dry weight of *V. radiata* plants. Plant biomass decreased when exposed to concentrated sugar mill wastewater. Marwari and Khan (2012) found that irrigating plants with 20-30% polluted water led to a reduction in both fresh and dry biomass. Dagianta et al. (2014) reported that the application of wastewater along with fertilization reduced the dry matter content of the biomass in peppers. Anwar et al. (2016) noted that the biomass of mint, coriander, and fenugreek was negatively affected when irrigated with wastewater. Ganjegunte et al. (2017) reported that there was no significant difference in biomass production of grasses irrigated with wastewater and freshwater within a specific year. It has been determined that the data obtained from these studies is consistent with our findings.

Table 3. Average values of the examined features

Applications	RR	RN	RL	RD	RFW	RDW
Control (0 ppm IBA)	67.21^{a}	6.41^{b}	3.21^{a}	0.67^{a}	0.41^{a}	0.08^{a}
4000 ppm IBA	53.05^{b}	8.41^{a}	2.87^{b}	0.55^{b}	0.27^{b}	0.06^{b}
	RR	RN	RL	RD	RFW	RDW
TWW0	49.56^{b}	7.22^{b}	2.72^{c}	0.61^{b}	0.28	0.09^{b}
TWW25	51.00^{b}	7.64^{ab}	2.84^{c}	0.72^{a}	0.35	0.03^{e}
TWW50	60.00^{ab}	7.44^{b}	3.20^{b}	0.52^{c}	0.41	0.05^{d}
TWW75	71.00^{a}	6.17^{c}	3.46^{a}	0.69^{a}	0.28	0.13^{a}
TWW100	69.09^{a}	8.58^{a}	2.98^{bc}	0.50^{c}	0.40	0.07^{c}

$^{a-e}$ Means with different letters in the same column are significantly different at P<0.05. RR= Rooting rate (%), RN= Root number (per cutting), RL= Root length (cm), RD= Root diameter (mm), RFW= Root fresh weight (g), RDW= Root dry weight (g). TWW0= 100 % pure water, TWW25= 25% treated wastewater and 75 % pure water, TWW50= 50% treated wastewater and 50 % pure water, TWW75v 75% treated wastewater and 25 % pure water, TWW100= 100% treated wastewater.

Table 4. Average values of doses and interactions of the examined traits

Applications	RR	RN	RL	RD	RFW	RDW
Control (0 ppm IBA)+TWW0	63.33 ab	9.44 a	2.93 d	0.84 a	0.53 ab	0.16 a
Control (0 ppm IBA)+TWW25	66.00 ab	5.62 d	3.22 a-d	0.82 ab	0.11 de	0.02 ı
Control (0 ppm IBA)+TWW50	60.00 ab	6.02 cd	3.40 ab	0.45 e	0.69 a	0.06 e
Control (0 ppm IBA)+TWW75	66.00 ab	3.83 e	3.55 a	0.66 cd	0.29 cd	0.11 c
Control (0 ppm IBA)+TWW100	73.00 a	7.14 bc	2.95 d	0.61 cd	0.47 abc	0.10 d
4000 ppm IBA+TWW0	35.00 c	5.00 de	2.52 e	0.36 e	0.03 e	0.01 j
4000 ppm IBA +TWW25	46.00 bc	9.65 a	2.45 e	0.65 cd	0.58 a	0.04 f
4000 ppm IBA +TWW50	60.00 ab	8.86 a	3.01 cd	0.57 d	0.13 de	0.03 h
4000 ppm IBA +TWW75	66.00 ab	8.50 ab	3.36 abc	0.72 bc	0.27 cde	0.15 b
4000 ppm IBA+TWW100	66.00 bc	10.03 a	3.02 bcd	0.39 e	0.34 bcd	0.02 g

a-ı Means with different letters in the same column are significantly different at $P<0.05$. RR= rooting rate (%), RN= root number (per cutting), RL= root length (cm), RD= root diameter (mm), RFW= root fresh weight (g), RDW= root dry weight (g). TWW0= 100 % pure water, TWW25= 25% treated wastewater and 75 % pure water, TWW50= 50% treated wastewater and 50 % pure water, TWW75= 75% treated wastewater and 25 % pure water, TWW100= 100% treated wastewater.

The total chlorophyll concentration of plants is a crucial indicator of their physiological state and photosynthetic efficiency. The chlorophyll contents are shown in Figure 2. In the control (0 ppm IBA) dose application, it was observed that the total chlorophyll amount increased as the concentration of treated wastewater increased, while in the 4000 ppm IBA dose application, a decrease is observed up to the TWW100 concentration. The highest total chlorophyll content was achieved with the 4000 ppm IBA+TWW100 combination (Figure 2). Chlorophyll a content showed a consistent rise at all dosages and concentrations, except for the control (0 ppm IBA) +TWW100 application. The highest chlorophyll b level was found in the 4000 ppm IBA+TWW0 treatment. The increase in chlorophyll content in the cuttings' leaves when treated with wastewater is believed to be caused by the nutrients present in the wastewater, as indicated by the results. Nutrients act as building blocks for proteins and enzymes that play a crucial role in the proper formation of pigment biosynthesis. Liu et al. (2002) observed a decrease in chlorophyll levels when seedlings were irrigated with wastewater. Manisha and Angoorbala (2013) obtained a decrease in chlorophyll content with increasing concentrations of wastewater. Faizan et al. (2014) reported higher chlorophyll a, chlorophyll b, and total chlorophyll content in okra irrigated with treated wastewater. Hassena et al. (2018) determined that young olive plants irrigated with treated wastewater showed an improvement in growth, soluble sugars, photosynthetic rate, and in the content of chlorophyll a, b, and total chlorophyll. The use of treated wastewater is thought not only to reduce the need for fertilizers due to its watering purpose but also because of its nutritive properties. Moreover, it offers a solution to reduce environmental pollution (Seleiman et al., 2021).

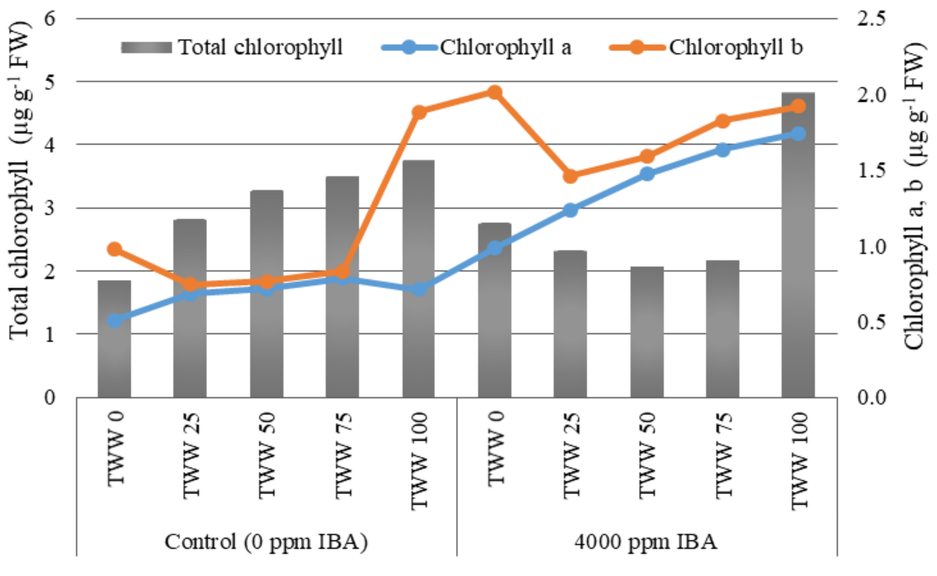

Figure 2. The effect of treated wastewater on chlorophyll a (µg g-1 FW), chlorophyll b (µg g-1 FW), and total chlorophyll (µg g-1 FW) content. TWW0= 100 % pure water, TWW25= 25% treated wastewater and 75 % pure water, TWW50= 50% treated wastewater and 50 % pure water, TWW75= 75% treated wastewater and 25 % pure water, TWW100= 100% treated wastewater.

4. Conclusion

Treated wastewater is used to increase efficiency in terms of irrigating crops and increasing biomass in arid regions. The study shows that treated wastewater can be used for rooting of the green cuttings. The control dose (0 ppm IBA) produces better outcomes in several rooting features compared to IBA applications, which are crucial for rooting.

An advantage in fertilizing has been identified, while also avoiding harm to the environment and plants. It has been discovered that the correct irrigation dose differs depending on the species thus; distinct investigations are required for each variety.

Author Contributions

The percentage of the author(s) contributions is present below. All authors reviewed and approved final version of the manuscript.

	A.C.	M.K.	S.O.E.
C	20	40	40
D		40	60
S			100
DCP	40	40	20
DAI		80	20
L	30	35	35
W	10	50	40
CR		40	60
SR		50	50
PM	30	40	30

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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