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Effect of Bacterial Inoculation on Morphological and Pomological Characteristics of Three Strawberry (*Fragaria x ananassa* Duch.) Cultivars Under Cadmium Toxicity*

Murat ŞAHİN[1], Lütfi PIRLAK[2]**

[1]*Siirt University, Faculty of Agriculture, Department of Horticulture, Siirt, TÜRKİYE*
[2]*Selçuk University, Faculty of Agriculture, Department of Horticulture, Konya, TÜRKİYE*

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ORCID ID (By author order)
orcid.org/0000-0002-5667-5483 orcid.org/0000-0003-3630-3591
**Corresponding Author: muratsahin@siirt.edu.tr

Abstract: Cadmium (Cd) is one of the main elements that cause heavy metal pollution, which is one of the important types of environmental pollution. There is a constant quest to reduce or eliminate the effects of Cd pollution. Plant growth-promoting rhizobacteria (PGPR) is one of these possible solutions. PGPRs not only increase plant growth but also protect plants against organic and inorganic stresses. In this study, the effects of three different *Pseudomonas* strains (MS-7, MS-12, and MS-13) on morphological and pomological characteristics of three different strawberry cultivars (Rubygem, Kabarla, and YFL) exposed to three different Cd doses (0, 100 and 300 mg kg^{-1}) were investigated to determine the effectiveness of PGPR against Cd toxicity in strawberry. To this end, root collar diameter (RCD), root length (RL), root fresh weight (RFW), root dry weight (RDW), shot fresh weight (SFW), shot dry weight (SDW), leaf area (LA), mean fruit weight (MFW), mean fruit length (MFL), and mean fruit diameter (MFD) were examined. It was observed that the effects of different *Pseudomonas* strains were cultivar-specific and affected some parameters more. Rubygem MS-7 bacterial strain preserved SDW (3.21 g) and MS-12 bacterial strain preserved RFW (13.01 g) at 300 mg kg^{-1} Cd dose significantly better against Cd toxicity than other bacterial strains. In Kabarla MS-7 bacterial strain preserved RDW (3.72 g) at 300 mg kg^{-1} Cd dose and MS-12 bacterial strain preserved SFW (15.27 g) at 100 mg kg^{-1} Cd dose significantly better against Cd toxicity than other bacterial strains. Likewise, in YFL, MS-13 bacterial strains preserved MFW (7.509 g) and RL (30.00 cm) at 300 mg kg^{-1} Cd dose, and MS-7 bacterial strain preserved LA (57.87 cm^2) at 100 mg kg^{-1} Cd dose significantly better against Cd toxicity than other bacterial strains. The results of the study showed that formulations containing *Pseudomonas sp.* can be used as an agricultural improver in areas with heavy metal pollution. As a result of the study, it was observed that PGPR applications were effective in preserving the morphological and pomological characteristics that decreased with the increase in Cd dose.

Keywords: Strawberry, cadmium toxicity, morphological characteristics, pomological characteristics, *Pseudomonas*

1. Introduction

Strawberry (*Fragaria x ananassa* Duch.) is one of the most produced and consumed fruits worldwide with an annual production of more than 8.8 million tons as of 2020 (Anonymous, 2022). Due to ever-increasing consumption, there is a trend for extending production areas, which causes additional fertilizer use and chemical pollution. It is possible that the lands used for strawberry production could be contaminated with heavy metals.

Chemicals of natural or anthropogenic origin that are mixed and accumulate in the soil by human hands and change the natural soil balance are considered pollutants (Yaron et al., 1996; Macfarlane and Burchett, 2001). All soils naturally contain more or less various polluting compounds. These compounds consist of metal, inorganic ions, salts, and organic compounds (Doble and Kumar, 2005; Rajkumar et al., 2009). A significant part of these compounds are formed through microbial activity and decomposition of organic matter that enters the soil. In addition, these compounds can reach the soil by precipitation, wind, and surface and groundwater runoff (Yaron et al., 1996; Chipasa, 2003). When soil pollutants exceed a certain level, the soil becomes polluted. Heavy

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metal pollution is very critical among these pollutants and become widespread with industrialization (Hasan et al., 2009; Angelone and Bini, 2017).

Metals with a density greater than 5 g cm^{-3} are defined as heavy metals. This makes 53 of the 90 naturally occurring elements be considered heavy metals (Abdelatey et al., 2011). Some of these elements are not toxic to plants at low concentrations and in some cases, they are among the essential plant nutrients, like iron (Fe), zinc (Zn), molybdenum (Mo), nickel (Ni), manganese (Mn), copper (Cu), and cobalt (Co) (Nies, 1999). On the other hand, some metals (Fe, Mo, and Mn) are considered important micronutrients, while some others (Zn, Ni, Cu, and Co) are considered trace elements (Arnon and Stout, 1939; Bakkaus et al., 2005).

Of these, cadmium (Cd) is one of the heavy metals that are not necessary for plants and cause pollution by mixing with soil and water in various ways (Garrido et al., 1998; Benavides et al., 2005). As a result, Cd taken from the soil by plants causes various negative effects on plant growth and development. Some of the damages caused by Cd in plants are chlorosis (Griffiths et al., 1995; Larsen et al., 1998), reduction of mineral intake (Das et al., 1997; Moreno et al., 1999), reduction of photosynthesis (Chugh and Sawhney, 1999; Vassilev et al., 2005), reduction of plant weight (Ekmekçi et al., 2008; Vijendra et al., 2016; Wu et al., 2021), reduction of root growth (Jinbiao et al., 2001) and harms on fruit characteristics (Lin et al., 2013; Zhang et al., 2020).

Plant growth promoting rhizobacteria (PGPR) are bacteria found naturally in the soil, colonizing plant roots (Kloepper, 1978; Kloepper et al., 1980), stimulating growth (Kuffner et al., 2008; Ryan et al., 2009), and showing biocontrol activity against disease and pests (Raj et al., 2003; Guo et al., 2004). Many studies are showing that PGPRs increase heavy metal uptake from soil (Rajkumar and Freitas, 2008; Kamran et al., 2015; Pan et al., 2017; Rojjanateeranaj et al., 2017). However, there are not many studies investigating the effects of PGPRs applied to plants under heavy metal stress on plant growth and development (García-Fraile et al., 2012; Guo and Chi, 2014). It is reported that some *Pseudomonas* strains can survive under high Cd concentrations (Rajkumar and Feritas, 2008; Rojjanateeranaj et al., 2017). In this study, the effects of bacterial strains (*Pseudomonas* ssp.) on morphological and pomological characteristics of three different strawberry (*Fragaria x ananassa* Duch.) cultivars grown under Cd toxicity were investigated.

2. Materials and Methods

This study was carried out in the plant growth room of Siirt University Faculty of Agriculture, Department of Agricultural Biotechnology in 2020.

2.1. Materials

2.1.1. Plant materials

In this study, three different strawberry (*Fragaria x ananassa* Duch.) cultivars were used as plant material. Rubygem was selected as the short-day, while Kabarla and YFL were selected as neutral-day strawberry cultivars. All three cultivars are commonly grown throughout Türkiye.

2.1.2. Bacterial strains

The bacterial strains used in the study were obtained from Çukurova University Faculty of Sciences and Arts, Department of Biology, and belong to the genus *Pseudomonas*. The supplied bacterial strains were selected according to their resistance to Cadmium and were named *Pseudomonas* MS-7, MS-12, and MS-13.

2.1.3. Cadmium preparation and application to plants

Cadmium sulfate ($3CdSO_4.8H_2O$; 98%) was used as the source of toxicity as 100 ml per pot, corresponding to 100 and 300 mg Cd per 1 kg of plant growth medium.

2.2. Methods

2.2.1. Growth medium and cultivation of plants

The growth medium used in this study was prepared from a mixture of peat, clay, and river sand with a ratio of 2:1:1. The prepared mixture was filled in 2-liter plastic pots with 1250 g of growth medium in each pot (After drying at room temperature "about 30 C" for a long time). The growth medium filled in the pots was irrigated and the strawberry seedlings, with roots shortened to 5 cm and kept in a solution containing fungicide against root diseases, were planted in the pots. After planting, the pots were irrigated again (100 ml per pot). The plants were irrigated as needed with half-strength Hoagland solution [modified from Epstein (1972)] and grown until the 10 true leaf stage.

2.2.2. Preparation of bacterial solutions and application to plants

Bacterial strains were supplied as a liquid culture and rejuvenated in a fresh liquid culture. The culture medium was kept in the liquid medium and grown until 10^8 cfu ml^{-1} concentration. 100 ml of the prepared solution was applied to the applications containing bacteria, while control plants were supplied with regular tap water.

2.2.3. Experimental design

The experiments were set up with three strawberry cultivars (Rubygem, Kabarla and YFL) according to randomized complete blocks design with split-split plots and 3 replications (3 plants per replication; 9 plants per treatment). In the study, strawberry cultivars were placed in the main plots (due to the difference in light duration), bacterial applications in the split plots and Cd doses in the split-split plots. The applications included in the study consisted of the absence of bacteria (B-), MS-7 bacterial strain, MS-12 bacterial strain, and MS-13 bacterial strain applications and 0, 100, and 300 mg kg^{-1} Cd doses and their combinations.

2.2.4. Traits examined in the study

In this study, to determine the effects of bacterial applications against Cd toxicity; morphological traits, root collar diameter (RCD), root length (RL), root fresh weight (RFW), root dry weight (RDW), shoot fresh weight (SFW), shoot dry weight (SDW), and leaf area (LA), and pomological traits, mean fruit weight (MFW), mean fruit length (MFL) and mean fruit diameter (MFD) were investigated.

2.2.5. Statistical analysis

The experimental data were collected and subjected to analysis of variance for a split-split plot design using the SPSS software version 23 (IBM Corp.). The means were separated by Duncan's multiple comparison test at a $p \leq 0.05$ significance level.

3. Results and Discussion

3.1. Root traits

Since cadmium pollution originates from the soil, the first plant organ that naturally encounters this heavy metal is the roots (Andresen and Küpper, 2013; Hassan and Mansoor, 2014) and the first negative effect of cadmium is seen on the roots (Lux et al., 2011; Shah et al., 2011; Souguir et al., 2011; Tripathi et al., 2012). Cadmium causes various effects on root collar diameter (Shah et al., 2011; Şahin et al., 2017; Balcı, 2018; Mahdavi and Kermandar, 2018), root length (Jiang et al., 2001; Shah et al., 2011; Şahin et al., 2017), root fresh weight (Malan and Farrant, 1998; Shah et al., 2011), root dry weight (Cieśliński et al., 1996; Malan and Farrant, 1998) and root morphology (Lux et al., 2011; Souguir et al., 2011; Triphati et al., 2012).

In this study, cultivar, bacterial applications, Cd doses and the interactions between cultivar x bacterial application, cultivar x Cd doses, bacterial application x Cd doses, and between cultivar x bacterial application x Cd doses were found to be statistically significant on RCD ($p<0.001$). Among the strawberry cultivars, the highest and lowest RCD values were detected in Rubygem and YFL cultivars, respectively. Bacterial application increased RCD compared to the B- application while increasing the dose of Cd decreased it. Considering the cultivar x Cd interaction, RCD decreased significantly with the increase of Cd dose, especially in YFL, while the decrease in Rubygem remained lower. While bacterial applications in all cultivars had a positive effect on RCD, this effect was more pronounced in YFL. In Cd doses x bacteria interaction, RCD increased in all bacterial strains and all Cd doses compared to B- application and RCD decreased with the increase in Cd dose. Depending on the dose, the reactions in each cultivar and bacterial applications were quite different. In Kabarla and YFL, B- and MS-13 applications decreased the RCD value due to the increase in dose, while in Rubygem it decreased at first and then increased in B- application, and decreased with the increase in dose in MS-13 application. On the other hand, in the MS-7 bacteria application, RCD increased at first and then decreased due to the increase in Cd dose in Kabarla and YFL. In the Rubygem, RCD was increased with increasing doses. In the MS-12 bacterial strain, it increased at first and then decreased in the Rubygem, it decreased at first and then increased in the Kabarla and decreased with increasing dose in YFL (Table 1). In this study, RCD was decreased with the increase in Cd dose. Similar results were found by Balcı (2018), but the results were found to be statistically insignificant (despite a 5 to 10% decrease). In addition, the RCD values of *Prunus cerasus* (Şahin et al., 2017), *Washingtonia filifera* (Mahdavi and Kermandar, 2018), and *Eucalyptus camaldulensis* (Shah et al., 2011) were decreased significantly with increasing Cd doses. The reduction of stem and root collar diameters under cadmium toxicity has been explained by researchers in 3 ways. Plant stem diameter and root collar diameter may decrease due to; 1) cadmium reduces the production of cell wall components and impairs polysaccharide metabolism (Punz and Sieghardt, 1993), 2) cadmium reduces water and nutrient uptake (Sheoran et al., 1990), and 3) small cells and narrow intercellular space formation as a result of the decrease of cell turgor potential and cell wall elasticity under cadmium stress (Barcelo et al., 1988). Bacterial strains used against cadmium stress slightly increased RCD in this study. Similarly, studies on tomato (Moustaine et al., 2017), hazelnut (Rostamikia et al., 2016), and Lodgepole pine seedlings (Chanway et al., 1991) have shown that

PGPRs have a positive effect on the RCD. The negative effect of cadmium on the RCD was somewhat reduced with the applied bacteria, especially in the MS-7 bacterial strain. On the other hand, the RCD of the plants without bacteria was significantly reduced. Balcı (2018) reported that 24-Epibrassinosteroid protects the RCD against cadmium stress in strawberry.

Table 1. The effect of cadmium stress and bacterial applications on RCD (mm)[1]

Cultivars (C)	Cd doses	Bacterial applications (BA)					
		B-	MS-7	MS-12	MS-13	Mean (CxCd)	Mean (C)
Rubygem	0	9.01^{g-l}	9.14^{e-i}	9.26^{c-f}	9.81^{a}	9.30^{A}	
	100	8.96^{h-m}	9.34^{cde}	9.36^{cd}	9.16^{d-h}	9.20^{A-C}	
	300	9.18^{d-g}	9.41^{bc}	8.99^{g-l}	9.13^{e-i}	9.18^{BC}	
Mean (CxBA)		9.05^{C}	9.30^{AB}	9.20^{B}	9.37^{A}		9.23A
Kabarla	0	9.30^{c-f}	9.30^{c-f}	9.14^{e-h}	9.17^{d-h}	9.23^{AB}	
	100	8.96^{h-m}	9.58^{b}	8.93^{i-n}	8.96^{h-m}	9.11^{C}	
	300	8.71^{no}	9.13^{e-i}	9.11^{f-j}	8.88^{k-n}	8.96^{D}	
Mean (CxBA)		8.99^{C}	9.34^{A}	9.06^{C}	9.00^{C}		9.08B
YFL	0	8.32^{qr}	8.86^{l-n}	8.75^{mno}	8.42^{pq}	8.59^{E}	
	100	7.89^{t}	9.09^{f-k}	8.55^{op}	8.05^{st}	8.39^{F}	
	300	7.34^{u}	8.90^{j-n}	8.21^{rs}	7.94^{t}	8.10^{G}	
Mean (CxBA)		7.85^{F}	8.95^{C}	8.50^{E}	8.14^{D}		8.36C
		Mean (CdxBA)				Mean (Cd)	
	0	8.88^{DE}	9.10^{B}	9.24^{BC}	9.13^{B}	9.04A	
	100	8.60^{G}	9.34^{A}	8.95^{CD}	8.72^{FG}	8.90B	
	300	8.41^{H}	9.14^{B}	8.77^{EF}	8.65^{FG}	8.74C	
Mean (BA)		8.63D	9.19A	8.92B	8.84C		
LSD value		C= 0.063***, Cd= 0.063***, BA= 0.072***, CxCd= 0.108***, CxBA= 0.125***, CdxBA= 0.125***, CxCdxBA= 0.215***					

[1]: Differences between means denoted by the same letters in the same group, in the same row, and in the same column are insignificant, ***: Significant at $p<0.001$

The effect of cultivar, bacterial applications and interactions between cultivar x bacterial application, cultivar x Cd doses, bacterial application x Cd doses, and cultivar x bacterial application x Cd doses were found to be significant ($p<0.001$) on RL. Among the cultivars, the longest roots were detected in the Rubygem and the shortest in the YFL. Among bacterial applications, only the MS-13 bacterial strain increased RL slightly. Besides, the effect of Cd doses on RL was found to be statistically insignificant. RL slightly decreased with the increase in Cd dose in Rubygem, it increased in Kabarla, first increased and then decreased in YFL. In cultivar x bacterial interaction, bacterial strains increased root length in YFL, decreased it in Rubygem, and only the MS-13 bacterial strain increased in Kabarla. On the other hand, in the Cd dose x bacteria interaction, the root length first increased and then decreased with the increase in the Cd dose in B- and MS-13 bacteria applications. RL decreased with the increase in Cd dose in Rubygem, while it decreased at first and then increased in Kabarla and increased at first and then decreased in YFL. In the Rubygem, although bacterial applications reduced RL at all doses, it preserved and slightly increased RL in the Kabarla, especially in the MS-13 bacterial strain. In the YFL, bacterial strains increased RL compared to B- application in almost all applications (Table 2).

Root length, which decreased with the increase in Cd dose in the Rubygem, first decreased and then increased in the Kabarla. In the YFL, it first increased with the increase in Cd dose and decreased at 300 mg kg^{-1} Cd dose. When previous studies were examined, the RL decreased with the increase in Cd dose (Kamran et al., 2015; Zhang et al., 2015; Rojjanateeranaj et al., 2017; Mahdavi and Kermandar, 2018) but, in some studies, it was increased at low Cd doses and decreased again with increasing dose (Jiang et al., 2001; Liu et al., 2003; Wang et al., 2007; Balcı, 2018). There were contrasting reports in the literature about the role of Cd on RL. Some studies reported increased RL with Cd application (Moustaine et al., 2017; Nawaz and Bano, 2020; Wu et al., 2021), while others reported a decrease (Kaymak et al., 2008; Zhang et al., 2015; Hashem et al., 2016) and some studies reported that Cd does not affect root length (Sharafzadeh, 2012) in plants. In the present study, the effect of bacterial applications on root length under Cd stress was different on each cultivar. Similar to the current study, there were various reports of bacteria application causing an increase (Prapagdee et al., 2012, 2013; Kamran et al., 2015; Rojjanateeranaj et al., 2017; Wu et al., 2021), initial increase then decreases with dose increase (Jiang et al., 2001; Wang et al., 2007) or decrease (Kamran et al., 2015; Zhang et al., 2015; Hashem et al., 2016;

Table 2. The effect of cadmium stress and bacterial applications on RL (cm)[1]

Cultivars (C)	Cd doses	Bacterial applications (BA)					
		B-	MS-7	MS-12	MS-13	Mean (CxCd)	Mean (C)
Rubygem	0	33.22^{a}	32.50^{ab}	29.83^{fg}	30.11^{ef}	31.42^{A}	
	100	31.83^{bc}	29.39$^{f\text{-}h}$	28.39$^{i\text{-}k}$	30.89^{de}	30.13^{B}	
	300	30.00^{ef}	29.78^{fg}	31.28^{cd}	30.06^{ef}	30.28^{B}	
Mean (CxBA)		31.69^{A}	30.56^{B}	29.83^{C}	30.35^{B}		30.61A
Kabarla	0	27.17^{lmn}	27.11^{lmn}	25.95^{p}	26.11^{op}	26.59^{E}	
	100	26.11^{op}	26.97$^{m\text{-}o}$	27.06^{mn}	31.04^{cd}	27.80^{C}	
	300	27.82$^{j\text{-}m}$	27.11^{lmn}	27.72$^{j\text{-}m}$	29.44^{fgh}	28.03^{C}	
Mean (CxBA)		27.03^{F}	27.07^{F}	26.91^{FG}	28.87^{D}		27.74B
YFL	0	25.83^{pq}	26.61$^{n\text{-}p}$	27.61^{klm}	28.94$^{g\text{-}i}$	27.25^{D}	
	100	28.56$^{h\text{-}j}$	28.00$^{j\text{-}l}$	27.33^{lmn}	28.94$^{g\text{-}i}$	28.21^{C}	
	300	24.98^{q}	28.28$^{i\text{-}k}$	26.11^{op}	30.00^{ef}	27.34^{D}	
Mean (CxBA)		26.46^{G}	27.63^{E}	27.02^{F}	29.30^{D}		27.60B
		Mean (CdxBA)				Mean (Cd)	
	0	28.74^{B}	28.74^{B}	27.80^{DE}	28.39^{BC}	28.42	
	100	28.83^{B}	28.12^{CD}	27.59^{E}	30.29^{A}	28.71	
	300	27.60^{E}	28.39^{BC}	28.37^{BC}	29.83^{A}	28.55	
Mean (BA)		28.39B	28.42B	27.92C	29.51A		
LSD value		C= 0.258***, Cd= ns, BA= 0.298***, CxCd= 0.447***, CxBA= 0.516***, CdxBA= 0.516***, CxCdxBA= 0.893***					

[1]: Differences between means denoted by the same letters in the same group, in the same row, and in the same column are insignificant, ***: Significant at p<0.001, ns: Not significant

Rojjanateeranaj et al., 2017) the RL under Cd stress. However, in most of the studies, bacteria (Prapagdee et al., 2012, 2013) mycorrhiza (Zhang et al., 2015; Hashem et al., 2016), and other substances (brassinosteroid, citric acid, silicon, melatonin, etc.) (Balcı, 2018; Wu et al., 2021) applied against Cd stress increased the RL under stress. However, bacterial strains were ineffective against Cd stress in *Glycine max* L., as reported by Rojjanateeranaj et al. (2017). From this point, the results from the cv. YFL overlaps with the literature, while the results were only from one bacterial strain in the other two cultivars.

The effects of cultivar, bacterial application, Cd doses and three interactions (cultivar x bacterial application, bacterial application x Cd doses, and cultivar x bacterial application x Cd doses) were significantly (p<0.001) different in RFW. Among the cultivars, the highest RFW value was observed in Kabarla and the lowest value was observed in the Rubygem. Among bacterial treatments, MS-12 and MS-13 bacterial strains increased RFW, while MS-7 did not. Increasing the Cd dose (especially the high dose) decreased the RFW drastically. Cultivar x Cd dose interaction was found to be insignificant. From the cultivar x bacteria interactions, bacterial strains caused different effects on cultivars. MS-12 and MS-13 bacterial strains in Rubygem, MS-7 and MS-12 bacterial strains in Kabarla, and MS-13 bacterial strains in YFL increased RFW. In Cd doses x bacteria interaction, MS-12 and MS-13 bacterial strains increased RFW at all Cd doses compared to the B- application. Depending on the increase in the cadmium dose, RFW decreased in all applications, and MS-12 and MS-13 bacterial strains increased RFW compared to B- application. In the Rubygem, all bacterial strains increased RFW at 0 and 300 mg kg^{-1} Cd doses, while it decreased it slightly at 100 mg kg^{-1} Cd dose. In all bacterial applications except B- application, RFW decreased with the increase in Cd dose, and in B- application, it increased at first and then decreased with increasing dose of Cd. However, especially high doses of bacteria applications preserved RFW. In the Kabarla, all bacterial strains increased RFW at all Cd doses (except for MS-13 bacterial strain at 100 and 300 mg kg^{-1} Cd doses) compared to B- application. However, only the MS-13 bacterial strain was effective in the YFL and significantly increased RFW at all Cd doses. Other bacterial strains were not effective compared to the B- treatment (Table 3). In this study, RFW was decreased with increasing cadmium dose, and similar results were reported in *Vigna ambacensis* (Al-Yemeni, 2001), garlic (Jiang et al., 2001), tomato (Hashem et al., 2016), dog grape (Wang et al., 2020), and mustard (Ahmad et al., 2011). But, the RFW of strawberry seedlings increased at 1.5 ppm Cd dose compared to control and decreased with increasing Cd dose to 3 ppm. However, the value obtained from the 3 ppm Cd dose was higher than the control (Balcı, 2018). In a study conducted on garlic, it was reported that RFW on the lowest Cd dose (1 ppm) was similar to the control (Jiang et al., 2001). Moreover, in a study Cd applications increase root growth (Chen et al., 2014). In this study, the increase in the 100 mg kg^{-1} Cd dose

Table 3. The effect of cadmium stress and bacterial applications on RFW (g)[1]

Cultivars (C)	Cd doses	Bacterial applications (BA)					
		B-	MS-7	MS-12	MS-13	Mean (CxCd)	Mean (C)
Rubygem	0	12.70^{klm}	14.43^{g}	14.44^{g}	15.49^{f}	14.27	
	100	14.06$^{g\text{-}i}$	12.02^{m}	13.25$^{i\text{-}k}$	13.83$^{g\text{-}j}$	13.29	
	300	10.19^{n}	10.63^{n}	13.01$^{j\text{-}l}$	12.40^{lm}	11.55	
Mean (CxBA)		12.31^{H}	12.36^{H}	13.57^{G}	13.91^{FG}		13.04C
Kabarla	0	18.20^{b}	19.43^{a}	19.72^{a}	19.69^{a}	19.26	
	100	17.76^{bcd}	18.01^{bc}	20.18^{a}	17.34^{cde}	18.32	
	300	17.05^{de}	17.58$^{b\text{-}e}$	17.21^{cde}	16.05^{f}	16.97	
Mean (CxBA)		17.67^{C}	18.34^{B}	19.04^{A}	17.70^{C}		18.19A
YFL	0	15.85^{f}	15.63^{f}	17.73$^{b\text{-}e}$	18.23^{b}	16.82	
	100	15.48^{f}	13.65$^{g\text{-}j}$	15.60^{f}	16.91^{e}	15.41	
	300	14.43^{gh}	13.48$^{h\text{-}k}$	13.24$^{i\text{-}k}$	16.05^{f}	14.23	
Mean (CxBA)		15.16^{E}	14.25^{F}	15.52^{E}	17.06^{D}		15.50B
		Mean (CdxBA)				Mean (Cd)	
	0	15.59^{D}	16.50^{C}	17.30^{B}	17.81^{A}	16.80A	
	100	15.77^{D}	14.56^{E}	16.34^{C}	16.03^{CD}	15.68B	
	300	13.80^{F}	13.90^{F}	14.49^{E}	14.83^{E}	14.25C	
Mean (BA)		15.05B	14.99B	16.04A	16.22A		
LSD value		C= 0.241***, Cd= 0.241***, BA= 0.279***, CxCd= ns, CxBA= 0.483***, CdxBA= 0.483***, CxCdxBA= 0.836***					

[1]: Differences between means denoted by the same letters in the same group, in the same row, and in the same column are insignificant, ***: Significant at p<0.001, ns: Not significant

compared to the control in Rubygem and the low Cd dose application and the control being in the same group in YFL can be explained by the above explanation. In this study, MS-12 and MS-13 bacterial strains, which were applied against Cd stress, increased the RFW and in previous studies, RFW increased by AMF (Hashem et al., 2016; Wang et al., 2020) and RFW increased by bacteria (Sheng and Xia, 2006; Prapagdee et al., 2013) under Cd stress. The bacterial strains used in the present study were found to be effective at low Cd doses in some cultivars and high Cd doses in others. These results were similar to mycorrhiza in tomato (Hashem et al., 2016), salicylic acid in mustard (Ahmad et al., 2011), mycorrhiza and earthworms in dog grape (Wang et al., 2020) which were reported to increase RFW at various rates under Cd stress. Also in the current study, different bacteria strains in different cultivars were more effective on RFW.

The effects of cultivar, bacterial application, Cd doses and three interactions [cultivar x Cd doses ($p<0.05$), cultivar x bacterial application, bacterial application x Cd doses, and cultivar x bacterial application x Cd doses] were significantly ($p<0.001$) different in RDW. The highest RDW was found in Kabarla and the lowest in Rubygem, and bacterial strains, especially MS-12 and MS-13, increased RDW. Due to the increase in the cadmium dose, RDW decreased compared to the average of all applications. In cultivar x Cd dose interactions, RDW decreased in all cultivars due to the increase in Cd dose, while the lowest proportional decrease was observed in Kabarla and the highest decrease was observed in YFL. When the cultivar x bacteria interaction was evaluated, all bacterial treatments (except MS-7 in YFL) reduced RDW compared to the B- treatment. Although it varies between cultivars, the most effective bacterial strain was MS-12. In Cd x bacteria interaction, all bacterial strains increased RDW compared to B- application at a dose of 0 mg kg^{-1} Cd, while the effect changes with the dose increase. MS-13 emerged as the most effective bacterial strain at a dose of 300 mg kg^{-1} Cd. Among the strawberry cultivars, the highest RDW was observed in Kabarla and the lowest in the Rubygem. Although there were differences in bacteria applied to strawberry cultivars, in general, RDW decreased with the increase in dose. While the applied bacterial strains were effective at 0 and 300 mg kg^{-1} Cd doses in the Rubygem, they were effective at 0 and 100 mg kg^{-1} Cd doses in the Kabarla. In the YFL, bacterial strains applied especially at high Cd dose decreased RDW compared to B- application. At 0 and 100 mg kg^{-1} Cd doses, MS-12 and MS-13 bacterial strains showed better or similar results compared to B- application. In the present study, RDW also decreased with the increase in cd dose, similar to the RFW (Table 4). In previous studies, similar results were obtained on garlic (Jiang et al., 2001), strawberry (Cieśliński et al., 1996), soybean (Malan and Farrant, 1998), corn (Ekmekçi et al., 2008), and many other plants (Rajkumar and Freitas, 2008; Gao et al., 2010; Zhang et al., 2015; Hashem et al., 2016; Gunathilakae et al., 2018). However, although there were studies indicating that the

Table 4. The effect of cadmium stress and bacterial applications on RDW (g)[1]

Cultivars (C)	Cd doses	Bacterial applications (BA) B-	MS-7	MS-12	MS-13	Mean (CxCd)	Mean (C)
Rubygem	0	2.38^{h-j}	2.62^{g-i}	2.63^{gh}	3.10^{ef}	2.68^{D}	
	100	2.65^{g}	2.45^{g-j}	2.36^{i-j}	2.47^{g-j}	2.48^{E}	
	300	1.85^{l}	2.10^{kl}	2.48^{g-j}	2.26^{jk}	2.17^{F}	
Mean (CxBA)		2.29^{H}	2.39^{GH}	2.49^{FG}	2.61^{EF}		2.45C
Kabarla	0	3.18^{ef}	3.93^{ab}	3.88^{ab}	3.67^{bc}	3.66^{A}	
	100	3.29^{de}	3.61^{c}	3.98^{a}	3.27^{def}	3.54^{A}	
	300	3.16^{ef}	3.72^{bc}	3.12^{ef}	3.13^{ef}	3.28^{B}	
Mean (CxBA)		3.21^{C}	3.75^{A}	3.65^{A}	3.36^{B}		3.49A
YFL	0	3.20^{ef}	3.17^{ef}	3.56^{c}	3.53^{cd}	3.36^{D}	
	100	3.14^{ef}	2.46^{g-j}	3.16^{ef}	3.06^{ef}	2.96^{C}	
	300	3.02^{f}	2.50^{g-j}	2.45^{g-j}	3.05^{ef}	2.75^{B}	
Mean (CxBA)		3.12^{CD}	2.71^{E}	3.06^{D}	3.22^{BC}		3.02B
		Mean (CdxBA)				Mean (Cd)	
	0	2.92^{EFG}	3.24^{BC}	3.36^{AB}	3.43^{A}	3.24A	
	100	3.03^{DE}	2.84^{FG}	3.17^{CD}	2.94^{EF}	2.99B	
	300	2.67^{H}	2.77^{GH}	2.68^{H}	2.82^{FGH}	2.74C	
Mean (BA)		2.87B	2.95B	3.07A	3.07A		
LSD value		C= 0.076^{***}, Cd= 0.076^{***}, BA= 0.087^{***}, CxCd= 0.131^{*}, CxBA= 0.151^{***}, CdxBA= 0.151^{***}, CxCdxBA= 0.261^{***}					

[1]: Differences between means denoted by the same letters in the same group, in the same row, and in the same column are insignificant, *: Significant at $p<0.05$, ***: Significant at $p<0.001$

severity of RDW increases at low Cd doses (Jiang et al., 2016; Balcı, 2018; Gunathilakae et al., 2018), the general opinion is that RDW decreases under Cd stress. In this study, RDW increased at 100 mg kg^{-1} Cd dose in Rubygem and Kabarla, and it was in the same statistical group at 0 and 300 mg kg^{-1} Cd doses in Kabarla. But, in the YFL, a decrease was observed. Similarly, in a study conducted by Cieśliński et al. (1996) with 3 different strawberry cultivars, RDW increased while pH was 5.1 at 15 and 30 mg kg^{-1} Cd doses in the Selva, however, RDW increased pH was 6.8 at 15 mg kg^{-1} Cd dose in Totem and all Cd doses in Rainier. Also, in a study conducted by Balcı (2018), RDW increased at 1.5 and 3 ppm Cd doses. Especially in brassinosteroid applications, RDW increased with the increase in Cd dose. Looking at the current and previous studies, it can be said that some strawberry cultivars increase the root biomass, especially at low Cd doses. The positive effect of Cd at low doses on plant development is a phenomenon worth further in-depth research. Many different methods have been studied to protect plants against Cd stress like brassinosteroid (Balcı, 2018), citric acid (Gao et al., 2010), silicon (Treder and Cieśliński, 2005), selenium (Khan et al., 2015), hydrogen sulfide (Kaya and Aslan, 2020), mycorrhiza (Hashem et al., 2016; Jiang et al., 2016), and bacteria (Prapagdee et al., 2013; Guo and Chi, 2014). Many of these methods increased RDW under Cd stress. Although the bacterial strains used in the current study increased RDW in Rubygem (0 and 300 mg kg^{-1}) and Kabarla (0 and 100 mg kg^{-1} except for the MS-7 bacterial strain), they did not increase it in YFL. In previous studies, it has been stated that mycorrhiza applications increase RDW in the presence and absence of Cd and depending on the increase in Cd dose (except at low doses), although RDW decreases in mycorrhiza applications, higher RDW occurs compared to plants without mycorrhizae (Gao et al., 2010; Zhang et al., 2015; Hashem et al., 2016; Jiang et al., 2016; Gunathilakae et al., 2018). Similarly, it has been reported in previous studies that bacterial applications also increase RDW under Cd stress and different bacterial strains respond differently (Rajkumar and Freitas, 2008; Prapagdee et al., 2013; Guo and Chi, 2014). In terms of the effect of Cd and bacteria applications on RDW, the present study overlaps with the previous ones.

3.2. Shoot growth parameters

Although the first negative effects of cadmium are seen in the roots, vegetative organs of some plants are more affected by Cd toxicity (Jiang et al., 2001; Gao et al., 2010; Zhang et al., 2015). However, there are also studies reporting that shoots are more affected by Cd toxicity (Cieśliński et al., 1996; Hashem et al., 2016; Balcı, 2018; Wang et al., 2020). Moreover, root and shoot were similarly affected by Cd toxicity in a study on *Eichhornia crassipes* (Mart.) Solms conducted by Gunathilakae et al. (2018). Cd prevents the development of shoots by preventing nutrient uptake (Yoshihara et al., 2006; Mendoza-Cozatl et al., 2008; Küpper and Kochian, 2010), water uptake (Perfus-Barbeoch et al., 2002), by inhibiting the growth of roots that feed shoots and leaves (Zhang et al., 2015; Rojjanateeranaj et al., 2017), and creating a toxic effect by reaching the shoots in some plants (Küpper et al., 1996; Baryla et al., 2001; Gao et al., 2010; Tripathi et al., 2012; Hashem et al., 2016).

The effects of cultivar, bacterial application ($p<0.05$), Cd doses and interactions between cultivar x Cd doses, cultivar x bacterial application, bacterial application x Cd doses, and cultivar x bacterial application x Cd doses were significantly ($p<0.001$) different in SFW. The highest SFW value was obtained from the Rubygem, and the lowest value was obtained from the YFL. According to the average of all applications, MS-12 and MS-13 bacteria applications increased the SFW value relatively compared to other bacteria applications. In addition, with the increased Cd dose, the SFW increased at first slightly and then decreased sharply, compared to the average of all applications. In the interaction of cultivar x Cd dose, SFW increases slightly (100 mg kg^{-1} Cd) with the increase of Cd dose in all cultivars and then decreases sharply (300 mg kg^{-1} Cd). In the cultivar x bacteria interaction, in Kabarla all bacterial treatments increased SFW, while Rubygem did not show any effect (except for the reduction seen in the MS-7 bacterial strain). However, the MS-7 bacterial strain was the most effective application in YFL as well. In the interaction of Cd dose x bacteria, MS-7 and MS-13 bacteria increased at 100 mg kg^{-1} Cd dose compared to SFW 0 mg kg^{-1} Cd dose. Despite the decrease in SFW at high doses, the values obtained from all bacterial applications were higher than the B- application. In the Rubygem the SFW value obtained from all bacterial strains at 0 mg kg^{-1} Cd dose was below the B- application, while the values obtained from all bacterial strains in the application of MS-13 at 100 mg kg^{-1} Cd dose and all bacterial strains at 300 mg kg^{-1} Cd dose was higher than the B- application. In the Kabarla, the values obtained from bacterial applications at all Cd doses (except for the MS-7 bacterial strain at a dose of 0 mg kg^{-1} Cd) were higher than the B- application. In particular, MS-12 and MS-13 bacterial strains were found to be highly effective at 0 and 100 mg kg^{-1} Cd doses. In the YFL, bacterial strains at 0 mg kg^{-1} Cd dose reduced SFW, while bacterial strains MS-12 and MS-13 preserved SFW at 100 and 300 mg kg^{-1} Cd doses (Table 5).

In this study, SFW first increased with the increase in Cd dose and decreased to a lower level than the control at high doses. When previous studies were examined, Cd applications decreased SFW (Jiang et al., 2001; Tripathi et al., 2012; Kamran et al., 2015; Hashem et al., 2016; Balcı, 2018). To reduce the negative effects of cadmium, many methods such as various chemicals, mycorrhiza and bacteria have been tried. Some of these studies were found to be effective (Pan et al., 2017; Wang et al., 2020), or less effective (Kamran et al., 2015; Hashem et al., 2016) while others were found to be ineffective (Balcı, 2018). It was observed that the bacterial strains used in this study protected SFW in all cultivars, especially at low doses and were highly effective in Kabarla and YFL at high doses. It has been seen in the studies that bacterial applications increase SFW and also stated that PGPRs protect the plant against Cd toxicity (Rajkumar and Freitas, 2008; Prapagdee et al., 2012; Kamran et al., 2015; Pan et al., 2017).

When we analyzed the effects of all factors [Cultivar, bacterial application ($p<0.01$), Cd dose and interactions ((cultivar x Cd doses, $p<0.05$), cultivar x bacterial applications, Cd doses x bacterial applications and cultivar x bacterial application x Cd doses) were significant] on SDW, we observed significant differences ($p<0.001$). Among the cultivars, the highest value was obtained from Kabarla and the lowest value was obtained from the YFL. In addition, bacterial applications have slightly increased the SDW. According to the average of all applications, Cd doses increased the SDW value at first and decreased it a little at the higher Cd dose. In the cultivar x Cd dose interaction, SDW first increased with the increase in Cd dose in all cultivars and decreased slightly with the continuation of the dose increase (except for YFL). Values obtained from high doses in Rubygem and Kabarla were higher than the values obtained in the absence of Cd. Bacterial strains decreased SDW in Rubygem and YFL compared to B- treatment, while it increased it significantly in the Kabarla. While high-dose SDW decreased slightly in B- application from Cd bacteria interaction, a significant increase was observed in 3 bacterial strains at 100 mg kg^{-1} Cd dose compared to the absence of Cd. At a high Cd dose, SDW was found to be lower only in the MS-12 bacterial strain than in the absence of Cd (the opposite was true for the other two bacterial strains). Bacterial applications reduced SDW in Rubygem at 0 mg kg^{-1} Cd, while at 100 and 300 mg kg^{-1} Cd doses, especially with MS-12 and MS-13 bacterial strains, SDW was increased compared to both B- and 0 mg kg^{-1} Cd doses. In addition, bacterial applications increased at first and then decreased the SDW due to the increase in Cd dose in the Rubygem. In Kabarla, B-, MS-7, and MS-12 (except 300 mg kg^{-1} Cd dose) bacterial applications, slightly increased SDW due to the increase in Cd dose but decreased in MS-13 bacteria application. In addition, bacterial applications at all cadmium doses (except for MS-12 and MS-13 bacterial strains at 300 mg kg^{-1} Cd) significantly increased

Table 5. The effect of cadmium stress and bacterial applications on SFW (g)[1]

Cultivars (C)	Cd doses	Bacterial applications (BA)					
		B-	MS-7	MS-12	MS-13	Mean (CxCd)	Mean (C)
Rubygem	0	15.68^{a}	13.60^{f-i}	14.62^{bcd}	13.97^{c-h}	14.47^{AB}	
	100	14.54^{bcd}	14.11^{c-g}	14.42^{b-f}	15.23^{ab}	14.58^{A}	
	300	13.43^{g-i}	13.54^{g-i}	14.42^{b-f}	14.21^{c-g}	13.90^{CDE}	
Mean (CxBA)		14.55^{AB}	13.75^{C}	14.49^{AB}	14.47^{AB}		14.32A
Kabarla	0	12.85^{i-l}	12.80^{i-l}	15.74^{a}	14.81^{bc}	14.05^{BCD}	
	100	13.57^{f-i}	13.82^{d-h}	15.27^{ab}	14.47^{b-e}	14.28^{ABC}	
	300	13.39^{g-j}	13.90^{d-h}	13.65^{e-i}	13.63^{e-i}	13.64^{DEF}	
Mean (CxBA)		13.27^{CD}	13.51^{C}	14.89^{A}	14.30^{B}		13.99B
YFL	0	14.11^{c-g}	13.46^{g-i}	13.13^{h-k}	12.56^{jkl}	13.32^{F}	
	100	13.86^{i-l}	14.52^{bcd}	12.44^{klm}	14.18^{c-g}	13.50^{EF}	
	300	11.69^{mn}	12.16^{lm}	11.22^{n}	12.08^{lm}	11.79^{G}	
Mean (CxBA)		12.89^{D}	13.38^{CD}	12.27^{E}	12.94^{D}		12.87C
		Mean (CdxBA)				Mean (Cd)	
	0	14.21^{ABC}	13.29^{FG}	14.50^{AB}	13.78^{CDE}	13.94A	
	100	13.66^{DEF}	14.15^{A-D}	14.05^{BCD}	14.63^{A}	14.12A	
	300	12.84^{G}	13.20^{FG}	13.10^{G}	13.31^{EFG}	13.11B	
Mean (BA)		13.57B	13.55B	13.88A	13.91A		
LSD value		C= 0.246***, Cd= 0.246***, BA= 0.284*, CxCd= 0.427***, CxBA= 0.493***, CdxBA= 0.493***, CxCdxBA= 0.853***					

[1]: Differences between means denoted by the same letters in the same group, in the same row, and in the same column are insignificant, *: Significant at $p<0.05$, ***: Significant at $p<0.001$

SDW compared to B- application. In the YFL, on the other hand, although the bacterial applications generally decreased the SDW compared to the B- application, it increased with MS-12 and MS-13 applications at 100 mg kg^{-1} and MS-13 at 300 mg kg^{-1} Cd doses. In addition, in the B- and MS-12 bacterial applications, SDW decreased with the increase of the Cd dose, while it increased at first and then decreased in the MS-7 and MS-12 bacterial strains (Table 6). When previous studies are examined, although there were studies indicating that SDW is increased at low Cd doses (Jiang et al., 2001; Gunathilakae et al., 2018), the general opinion is that Cd toxicity reduces SDW (Cieśliński et al., 1996; Gao et al., 2010; Khan et al., 2015; Zhang et al., 2015; Hashem et al., 2016; Balcı, 2018; Kaya and Aslan, 2020; Zhang et al., 2020). Although SDW increased with the increase of Cd dose in the Kabarla, it decreased in the other two cultivars. Many studies are showing that PGPR applications have a positive effect on the shoot and leaf dry weight of plants (Rajkumar and Freaitas, 2008; Prapagdee et al., 2012, 2013). However, in the current study, bacterial applications at 0 mg kg^{-1} Cd dose in Rubygem and YFL reduced SDW somewhat. However, bacterial applications preserved SDW, which decreased with increasing Cd dose. At 0 mg kg^{-1} Cd dose, SDW was higher than bacterial applications in untreated plants, while bacterial applications with an increase in Cd dose led to an increase, especially in the Rubygem. At a dose of 300 mg kg^{-1} Cd, the other two bacterial strains showed similar results with the absence of bacteria, while the MS-7 bacterial strain increased SDW. In general, the best results were obtained from MS-7 and MS-13 bacterial strains. It has been stated in many studies that various chemicals (Khan et al., 2015; Kaya and Aslan, 2020; Zhang et al., 2020; Wu et al., 2021), mycorrhiza (Zhang et al., 2015; Hashem et al., 2016; Jiang et al., 2016; Gunathilakae et al., 2018), and bacteria (Rajkumar and Freitas, 2008; Prapagdee et al., 2013; Kamran et al., 2015; Pan et al., 2017; Rojjanatearanaj et al., 2017) used against Cd toxicity preserve the SDW. However, it was also stated that some chemicals, strains of bacteria, and mycorrhizae failed to achieve success in some species and cultivars, such as *Solanum nigrum L.* – citric acid (Gao et al., 2010), Indian mustard (*Brassica juncea* (L.) Czernj. Cosson)- J62 strain, (Jiang et al., 2008), *G. max* (L.) Merr. - YL-6 strain (Guo and Chi, 2014), *Sedum plumbizincicola* – NSX1, NCR4 and CCM2 strains (Liu et al., 2015), *Populus x generosa* - *Glomus intraradices* and *Salix viminalis* L. - *Glomus intraradices* species (Bissonnette et al., 2010). In this respect, the data obtained from the present study are compatible with the literature.

The results obtained for the LA were significant ($p<0.001$) for the cultivar, bacterial application, Cd doses, cultivar x bacterial applications, Cd dose x bacterial application, and cultivar x Cd doses-bacteria application interactions. The highest value among the cultivars was obtained from Rubygem and the lowest value was obtained from Kabarla. However, among bacteria applications, the MS-7 strain increased LA and the MS-13 strain decreased it. According to the average of all treatments, LA

Table 6. The effect of cadmium stress and bacterial applications on SDW (g)[1]

Cultivars (C)	Cd doses	Bacterial applications (BA)					
		B-	MS-7	MS-12	MS-13	Mean (CxCd)	Mean (C)
Rubygem	0	3.23^{def}	2.84^{klm}	$2.97^{i\text{-}k}$	2.84^{klm}	2.97^{E}	
	100	$3.19^{e\text{-}h}$	3.26^{de}	2.90^{jkl}	3.32^{ce}	3.17^{C}	
	300	$3.02^{i\text{-}j}$	$3.21^{d\text{-}g}$	$3.09^{f\text{-}i}$	$3.22^{d\text{-}g}$	3.14^{C}	
Mean (CxBA)		3.15^{C}	3.11^{C}	2.99^{DC}	3.13^{C}		3.09B
Kabarla	0	2.70^{mn}	3.06^{hi}	3.52^{b}	3.41^{bc}	3.17^{C}	
	100	3.05^{hi}	3.29^{ce}	3.90^{a}	3.34^{cd}	3.39^{A}	
	300	3.25^{de}	3.49^{b}	$3.08^{g\text{-}i}$	$3.21^{d\text{-}g}$	3.26^{B}	
Mean (CxBA)		3.00^{D}	3.28^{B}	3.50^{A}	3.32^{B}		3.27A
YFL	0	3.29^{cde}	2.78^{lm}	2.84^{klm}	2.79^{lm}	2.93^{E}	
	100	3.01^{ij}	$3.22^{d\text{-}g}$	2.79^{lm}	3.24^{de}	3.06^{D}	
	300	2.76^{lm}	2.74^{mn}	2.60^{n}	$2.90^{j\text{-}l}$	2.75^{F}	
Mean (CxBA)		3.02^{D}	2.91^{E}	2.75^{F}	2.98^{DE}		2.91C
		Mean (CdxBA)				Mean (Cd)	
	0	3.08^{DE}	2.89^{GF}	3.11^{D}	3.01^{E}	3.02B	
	100	3.08^{DE}	3.26^{AB}	3.20^{BC}	3.31^{A}	3.21A	
	300	3.01^{E}	3.15^{CD}	2.92^{F}	3.11^{D}	3.05B	
Mean (BA)		3.06B	3.10AB	3.08B	3.14A		
LSD value		C= 0.041^{***}, Cd= 0.041^{***}, BA= 0.047^{**}, CxCd= 0.071^{*}, CxBA= 0.081^{***}, CdxBA= 0.081^{***}, CxCdxBA= 0.141^{***}					

[1]: Differences between means denoted by the same letters in the same group, in the same row, and in the same column are insignificant, *: Significant at $p<0.05$, **: Significant at $p<0.01$, ***: Significant at $p<0.001$

decreased due to the increase in cadmium dose. Depending on the dose increase in the cultivar x Cd dose interaction, LA decreased in Rubygem and Kabarla, while it slightly decreased at the low Cd dose in YFL (compared to the absence of Cd). However, the sharpest proportional decrease in Cd dose increase was also seen in YFL. When the interaction of cultivar x bacteria applications was examined, no positive effect of bacterial applications on LA was observed in cultivars other than YFL and in some cases it led to a decrease. In YFL, especially the MS-7 bacterial strain was quite effective. From the interaction of Cd dose x bacteria applications, LA decreased with the increase of Cd dose in all bacterial applications (except the MS-7 bacterial strain). However, bacterial applications at high Cd doses preserved LA better than the absence of bacteria. Bacterial applications decreased LA in Rubygem at all Cd doses (except for MS-12 bacterial application at 0 mg kg^{-1} Cd dose) compared to B- application. In addition, LA decreased in all bacterial applications (except MS-7 bacterial strain) due to the increase in Cd dose. In the Kabarla, bacterial applications reduced LA compared to B- application in all bacterial strains (except for the application of MS-7 bacteria at a dose of 100 mg kg^{-1} Cd). In B- and MS-12 bacterial strains, LA increased at first and then decreased due to the increase in Cd dose, while it decreased due to the increase in Cd dose in MS-7 and MS-13 bacterial applications. In contrast to other strawberry cultivars, bacterial strain significantly increased LA in all Cd doses (except for MS-13 bacterial strain at 0 mg kg^{-1} Cd dose) compared to B- treatment in the YFL. However, with bacterial applications, LA was significantly increased at 100 mg kg^{-1} Cd dose, compared to the values obtained from the 0 mg kg^{-1} Cd dose and decreased significantly when the dose increased to 300 mg kg^{-1} Cd. In the present study, Cd applications significantly reduced leaf area. Although leaf area did not decrease much at 100 mg kg^{-1} Cd dose, it decreased by 10% at 300 mg kg^{-1} Cd dose compared to 0 mg kg^{-1} Cd dose (Table 7). Similarly, many studies reported that Cd toxicity reduces LA (Ghorbanli et al., 1999; El-Beltagi et al., 2010; Rehman et al., 2011; Loi et al., 2018). However, it has been stated in many studies that PGPR applications increase LA under stress or ideal conditions (Karakurt and Aslantaş, 2010; Ipek et al., 2014; Sen and Chandrasekhar, 2014; Khan et al., 2018). In the current study, bacterial applications except for MS-12 in Rubygem and MS-7 bacterial strain in YFL did not increase leaf area, especially MS-13 bacterial strain decreased LA in all strawberry cultivars. Similarly, leaf area decreased at 100 mg kg^{-1} Cd dose in Rubygem and 300 mg kg^{-1} Cd dose in Rubygem and Kabarla in all bacterial strains. However, in the YFL, all bacterial strains protected the leaf area against Cd stress, especially at 100 mg kg^{-1} Cd dose. It has been stated in previous studies that gibberellic acid (Ghorbanli et al., 1999), selenium and sulfur (Khan et al., 2015) mycorrhiza (de Andrade et al., 2008; Aloui et al., 2011), and bacteria (Pal et al., 2018; Awan et al., 2020; Tanwir et al., 2021) applications protect the LA against Cd toxicity, but there were differences between bacterial strains (Pal et al., 2018).

Table 7. The effect of cadmium stress and bacterial applications on LA (cm^2)[1]

Cultivars (C)	Cd doses	Bacterial applications (BA)					
		B-	MS-7	MS-12	MS-13	Mean (CxCd)	Mean (C)
Rubygem	0	63.07^{b}	59.01^{d}	65.23^{a}	61.95^{bc}	62.44^{A}	
	100	61.38^{c}	59.15^{de}	58.62^{de}	58.12^{de}	59.32^{B}	
	300	58.50^{de}	55.25^{g}	56.29^{fg}	55.95^{g}	56.50^{C}	
Mean (CxBA)		60.98^{A}	57.97^{B}	60.05^{A}	58.67^{B}		59.42A
Kabarla	0	48.75^{ij}	46.11$^{m\text{-}p}$	47.52$^{j\text{-}m}$	44.61$^{p\text{-}r}$	46.75^{E}	
	100	46.62$^{l\text{-}o}$	47.10$^{k\text{-}n}$	46.49$^{l\text{-}o}$	46.27^{mno}	46.62^{EF}	
	300	45.14$^{o\text{-}q}$	44.26^{qrs}	42.92^{s}	43.46^{rs}	43.95^{G}	
Mean (CxBA)		46.83^{E}	45.82^{F}	45.65^{FG}	44.78^{G}		45.77C
YFL	0	51.00^{h}	56.27^{fg}	50.94^{h}	47.90$^{i\text{-}l}$	51.53^{D}	
	100	48.35$^{i\text{-}k}$	57.87^{ef}	52.33^{h}	49.27^{i}	51.95^{D}	
	300	41.02^{t}	52.48^{h}	44.31^{qrs}	45.60$^{n\text{-}q}$	45.85^{F}	
Mean (CxBA)		46.79^{E}	55.54^{C}	49.19^{E}	47.59^{D}		49.78B
		Mean (CdxBA)				Mean (Cd)	
	0	54.27^{A}	53.96^{A}	54.57^{A}	51.49^{CD}	53.57A	
	100	52.12^{BC}	54.71^{A}	52.48^{B}	51.22^{CD}	52.63B	
	300	48.22^{E}	50.66^{D}	47.84^{E}	48.34^{E}	48.76C	
Mean (BA)		51.53B	53.11A	51.63B	50.35C		
LSD value		C= 0.470***, Cd= 0.470***, BA= 0.542***, CxCd= 0.813***, CxBA= 0.939***, CdxBA= 0.939***, CxCdxBA= 1.625***					

[1]: Differences between means denoted by the same letters in the same group, in the same row, and in the same column are insignificant, ***: Significant at p<0.001

3.3. Fruit characteristics

The interactions between two factors (cultivar and Cd doses) and interactions (cultivar x Cd doses, cultivar x bacterial applications, Cd doses x bacterial applications, and cultivar x Cd doses x bacterial applications) were found to be statistically significant ($p<0.001$) on MFW. The heaviest fruits were obtained from the Kabarla and the smallest fruits were obtained from the YFL. According to the averages of all applications, bacterial applications had a slight positive effect on MFW and were found to be statistically insignificant, while MFW decreased with the increase in Cd dose. In the interaction of cultivar x Cd dose, MFW decreased at similar rates with the increase of Cd dose in all cultivars. In the interaction of cultivar x bacteria applications, a decrease was observed in Rubygem with the effect of bacterial strains, while a significant increase was observed in all bacterial strains in Kabarla (except MS-7) and especially in YFL cultivar. In the interaction of Cd x bacteria applications, while all bacterial applications increased MFW in the absence of Cd compared to B- application, a slight decrease was observed with the increase of Cd dose (especially at high Cd dose). Bacterial applications decreased MFW in Rubygem at all Cd doses (except 0 mg kg^{-1} Cd MS-7 bacterial strain) compared to B- application (especially at 300 mg kg^{-1} Cd dose). In addition, MFW decreased significantly due to the increase in Cd dose in all bacterial applications (especially bacterial strains). In the Kabarla, unlike the Rubygem, the application of bacterial strains at 0 and 100 mg kg^{-1} Cd doses significantly increased MFW compared to the B- treatment. At a dose of 300 mg kg^{-1} Cd, only the MS-12 bacterial strain was effective compared to the B- application. The values obtained from other applications were found to be quite low compared to the B- application. In addition, MFW decreased significantly with the increase in Cd dose in all bacterial applications, similar to the Rubygem. In the YFL, MFW was found to be higher in all bacterial strain applications (except for MS-12 bacterial strain at a dose of 100 mg kg^{-1} Cd) compared to B- application. While all bacterial strains at 0 mg kg^{-1} Cd dose had a very high increase, the MS-13 bacterial strain at 300 mg kg^{-1} Cd dose produced the same effect (Table 8).

It has been stated in previous studies that cadmium applications have a negative effect on MFW (Moral et al., 1996; Malan and Farrant, 1998). In the current study, the MFW decreased significantly due to the increase in Cd dose, and a similar situation was observed in all three strawberry cultivars. In previous studies, it was stated that PGPR applications increased the MFW in many plant species and cultivars (Pırlak and Köse, 2009; Karakurt et al., 2011). Bacterial applications cause this effect by increasing the uptake of water and nutrients dissolved in water (O'connell, 1992; Aslantaş et al., 2007), protecting plants against various biotic and abiotic stresses (Dobbelaere et al., 2002; Dey et al., 2004), and increasing plant growth and development with the metabolites they produce (Jeon et al., 2003; Zahir et al., 2004; Egamberdiyeva, 2005; Aslantaş et al., 2007). In the current study, bacterial strains that did not show any effect against Cd stress in Rubygem,

Table 8. The effect of cadmium stress and bacterial applications on MFW (g)*[1]*

Cultivars (C)	Cd doses	Bacterial applications (BA)					
		B-	MS-7	MS-12	MS-13	Mean (CxCd)	Mean (C)
Rubygem	0	9.63^{e-h}	9.84^{d-g}	9.12^{h-j}	9.55^{e-h}	9.53^{C}	
	100	8.97^{i-k}	8.50^{klm}	8.49^{klm}	8.45^{k-n}	8.60^{E}	
	300	8.44^{k-n}	7.91^{n-q}	7.46^{qr}	7.62^{pq}	7.86^{F}	
Mean (CxBA)		9.01^{D}	8.75^{DE}	8.36^{FG}	8.54^{EF}		8.66B
Kabarla	0	10.88^{c}	11.53^{b}	12.75^{a}	11.39^{bc}	11.64^{A}	
	100	10.08^{de}	9.93^{def}	10.26^{d}	10.94^{c}	10.30^{B}	
	300	9.16^{h-j}	8.25^{l-o}	9.49^{f-i}	8.76^{jkl}	8.92^{D}	
Mean (CxBA)		10.04^{C}	9.90^{C}	10.83^{A}	10.37^{B}		10.29A
YFL	0	9.20^{h-j}	9.56^{e-h}	9.48^{f-i}	9.31^{g-i}	9.39^{C}	
	100	7.88^{o-q}	8.09^{m-p}	7.72^{o-q}	8.08^{m-p}	7.94^{F}	
	300	6.64^{s}	6.96^{rs}	6.97^{rs}	7.50^{qr}	7.02^{G}	
Mean (CxBA)		7.91^{H}	8.20^{GH}	8.06^{GH}	8.30^{FG}		8.12C
		Mean (CdxBA)				Mean (Cd)	
	0	9.90^{C}	10.31^{AB}	10.45^{A}	10.09^{BC}	10.19A	
	100	8.98^{DE}	8.84^{E}	8.82^{E}	9.16^{D}	8.95B	
	300	8.08^{F}	7.71^{G}	7.98^{FG}	7.96^{FG}	7.93C	
Mean (BA)		8.99	8.95	9.07	9.08		
LSD value		C= 0.122^{***}, Cd= 0.122^{***}, BA= ns, CxCd= 0.212^{***}, CxBA= 0.244^{***}, CdxBA= 0.244^{***}, CxCdxBA= 0.423^{***}					

[1]: Differences between means denoted by the same letters in the same group, in the same row, and in the same column are insignificant, ***: Significant at $p<0.001$, ns: Not significant

showed some positive effects at 0 mg kg^{-1} Cd dose and 100 mg kg^{-1} Cd dose (MS-12 and MS-13) in the Kabarla. In YFL strawberry cultivar, bacterial strains increased MFW at all Cd doses. Especially MS-13 bacterial strain significantly preserved MFW at 300 mg kg^{-1} Cd dose. In previous studies, it was stated that bacterial applications differ according to plant species/variety, and bacterial strain (Pishchik et al., 2002; Karakurt et al., 2011). However, bacterial applications against Cd stress generally increased fruit weight (Moral et al., 1994; Shen et al., 2012; Kanchana et al., 2014). In addition, researchers reported that mycorrhiza (Tahiri et al., 2022), brassinosteroid (Hayat et al., 2012), and sodium selenite (Zhang et al., 2020) protect plants against Cd stress.

The MFL was also significantly affected by cultivar, bacterial applications ($p<0.05$), Cd doses and interactions (cultivar x Cd doses, cultivar x bacterial applications, Cd doses x bacterial applications, and cultivar x bacterial applications x Cd doses) ($p<0.001$). The longest fruits were obtained from Kabarla and the shortest fruits were obtained from the Rubygem. Although bacterial applications were not very effective on MFL, the MS-12 bacterial strain produced the longest fruits. MFL decreased significantly due to the increase in Cd dose compared to the average of all applications. In the cultivar x Cd dose interaction, MFL decreased significantly in all cultivars with the increase in Cd dose. The highest proportional decrease was observed in the Kabarla and 100 mg kg^{-1} Cd dose. Bacterial applications were found to be somewhat effective in the interaction of cultivar x bacteria applications in Kabarla, while it was ineffective in the other two cultivars. In the interaction of Cd dose x bacteria applications, MFL decreased with the increase of Cd dose in all bacterial applications. Bacterial strains, which provided close results with B- application in the absence of Cd, caused a slight decrease in MFL with the increase in Cd dose. Bacterial strain applications were ineffective in the Rubygem, especially at 300 mg kg^{-1} Cd dose. However, the MS-12 bacterial strain increased the MFL at all Cd doses compared to B- application. Moreover, in all bacterial applications, MFL was significantly reduced due to the increase in Cd dose. In the Kabarla, the MS-12 bacterial strain significantly increased MFL, especially at 0 and 100 mg kg^{-1} Cd doses compared to other bacterial strains and B- application, and decreased it slightly compared to B- application at 300 mg kg^{-1} Cd dose. Other bacterial strains, on the other hand, increased MFL compared to B- application only at 0 mg kg^{-1} Cd dose. In addition, MFL was significantly shortened with the increase of Cd dose, similar to the Rubygem. In the YFL, unlike other strawberry cultivars, the MS-12 bacterial strain shortened MFL at all Cd doses compared to B- application, while MS-7 and MS-13 bacterial strains at all Cd doses (0 mg kg^{-1} Cd dose MS-13 bacterial strain) increased MFL compared to B- application. As in other strawberry cultivars, MFL decreased significantly due to the increase in Cd dose in all bacterial applications (Table 9).

Table 9. The effect of cadmium stress and bacterial applications on MFL (mm)[1]

Cultivars (C)	Cd doses	Bacterial applications (BA)					
		B-	MS-7	MS-12	MS-13	Mean (CxCd)	Mean (C)
Rubygem	0	31.55^{f-i}	31.22^{g-k}	31.80^{fg}	31.33^{g-j}	31.47^{C}	
	100	29.90^{l-n}	29.42^{m-o}	30.35^{j-m}	29.58^{mn}	29.82^{D}	
	300	28.32^{pq}	27.38^{q-s}	28.59^{op}	27.02^{s}	27.83^{E}	
Mean (CxBA)		29.92^{EF}	29.34^{G}	30.25^{DE}	29.31^{G}		29.71C
Kabarla	0	33.87^{cd}	36.32^{b}	38.23^{a}	34.49^{c}	35.73^{A}	
	100	32.02^{fg}	31.66^{f-h}	32.46^{ef}	31.70^{f-h}	31.96^{BC}	
	300	30.70^{i-l}	29.95^{l-n}	30.24^{k-m}	29.00^{n-p}	29.97^{D}	
Mean (CxBA)		32.19^{BC}	32.64^{BC}	33.64^{A}	31.73^{C}		32.55A
YFL	0	32.51^{ef}	33.13^{de}	32.33^{ef}	31.60^{f-i}	32.39^{B}	
	100	29.86^{l-n}	30.70^{h-l}	29.06^{n-p}	30.18^{l-m}	29.95^{D}	
	300	28.08^{p-r}	28.55^{op}	27.17^{rs}	29.43^{m-o}	28.31^{E}	
Mean (CxBA)		30.15^{E}	30.80^{G}	29.52^{FG}	30.41^{DE}		30.22B
		Mean (CdxBA)				Mean (Cd)	
	0	32.65^{B}	33.56^{A}	34.12^{A}	32.47^{B}	33.20A	
	100	30.59^{C}	30.60^{C}	30.62^{C}	30.49^{C}	30.58B	
	300	29.03^{D}	28.63^{D}	28.67^{D}	28.49^{D}	28.70C	
Mean (BA)		30.76BC	30.93AB	31.14A	30.48C		
LSD value		C= 0.286***, Cd= 0.286***, BA= 0.331*, CxCd= 0.496***, CxBA= 0.573***, CdxBA= 0.573***, CxCdxBA= 0.992***					

[1]: Differences between means denoted by the same letters in the same group, in the same row, and in the same column are insignificant, *: Significant at $p<0.05$, ***: Significant at $p<0.001$

In the present study, the effect of Cd applications on MFL was negative and MFL was significantly decreased with increasing Cd doses in all strawberry cultivars. In previous studies, it was stated that Cd stress decreases MFL and negatively affects fruit characteristics (Cieśliński et al., 1996; Moral et al., 1996; Hayat et al., 2012). Researchers attributed this effect to poor nutrition of fruits due to reduced root and shoot growth (Moral et al., 1994; Hayat et al., 2012). In the current study, also, fruit characteristics may have been damaged, especially due to damage to the roots, similar to previous studies. However, it has been reported that bacterial applications improve fruit characteristics of plants (Pırlak and Köse, 2009) and increase fruit size in strawberries (Kurokura et al., 2017; Seema et al., 2018) and some other species (Karakurt et al., 2011; Shen et al., 2012). In the current study, especially in Rubygem, none of the bacterial strains had a positive effect on MFL at a dose of 0 mg kg^{-1} Cd, whereas only the MS-7 bacterial strain showed a positive effect on YFL. However, in the Kabarla, all bacterial strains significantly increased MFL. As stated in previous studies, the effect of bacterial applications on fruit characteristics is significantly affected by plant species, plant cultivars, bacterial species, and bacterial strains (Karakurt et al., 2011; Shen et al., 2012; Kanchana et al., 2014). In the literature, it has been stated that bacteria (Pishchik et al., 2002; Kanchana et al., 2014), mycorrhiza (Tahiri et al., 2022), and some other applications (Hayat et al., 2012; Zhang et al., 2020) improve the fruit characteristics of plants under Cd stress and in the current study, this effect was seen only at 100 mg kg^{-1} Cd dose and in bacterial applications. In general, bacterial applications did not cause positive or negative effects, especially at a dose of 300 mg kg^{-1} Cd.

The effect of cultivar, bacterial applications, Cd doses, and interactions (cultivar x Cd doses, cultivar x bacterial applications, Cd doses x bacterial applications, and cultivar x bacterial applications x Cd doses) were found to be statistically significant ($p<0.001$) on MFD. Among the cultivars, the highest value was obtained from Kabarla, while the lowest value was obtained from the YFL. Although the highest value was obtained from the MS-12 bacterial strain, the other two bacterial strains revealed lower MFD values than B- treatment. According to the average of all applications, MFD decreased due to the increase in the Cd dose. In the cultivar x Cd dose interaction, MFD was significantly reduced in all cultivars at all Cd doses. There is no significant difference between the varieties in terms of reduction rate. Considering the interaction of cultivar x bacteria applications, effective results could not be obtained except for the YFL. The increase in MFD was also limited in YFL, except for the MS-13 bacterial strain. In the interaction of Cd dose x bacteria applications, the effect of bacterial strains that slightly increased MFD in the absence of Cd decreased with the increase in Cd dose and turned negative at high doses. There was no significant difference between bacterial applications. Bacterial applications reduced MFD at all Cd doses (except for MS-12 at 0 mg kg^{-1} Cd and MS-7 at 300 mg kg^{-1} Cd) in the

Rubygem. However, MFD decreased due to the increase in Cd dose in all bacterial applications except the MS-7 strain. On the other hand, in the Kabarla (unlike the Rubygem), bacterial strains increased MFD significantly at 0 and 100 mg kg^{-1} Cd doses compared to B- application. However, bacterial strains were ineffective due to the increase in MFD in B- application at 300 mg kg^{-1} Cd dose. Accordingly, while MFD decreased with the increase in Cd dose in bacterial strains, it increased at 300 mg kg^{-1} Cd dose in B- application, which had a similar effect in 0 and 100 mg kg^{-1} Cd doses. In the YFL, bacterial strains were found to be effective at all Cd doses compared to B- application and protected the MFD value against the increase in Cd dose. In all bacterial applications, MFD decreased due to the increase in Cd dose (especially in B- application) (Table 10).

In this study, the effect of Cd applications on MFD was negative, MFD decreased with the increase of Cd dose in Rubygem and YFL strawberry cultivars, but in Kabarla it decreased at first and increased with the Cd dose increase. In previous studies, it was stated that Cd stress had a negative effect on fruit characteristics (Moral et al., 1996; Malan and Farrant, 1998; Hayat et al., 2012; Zhang et al., 2020). However, it has been stated in many studies that bacterial applications increase the fruit characteristics (Pırlak and Köse, 2009; Karakurt et al., 2011; Shen et al., 2012). Bacterial strains applied in studies on strawberry cultivars increased fruit yield, fruit weight, and MFD (Pırlak and Köse, 2009; Kurokura et al., 2017; Seema et al., 2018). In the current study, although no effect of bacteria was detected in Rubygem, a slight increase was observed in Kabarla and YFL. It has been stated that bacterial strains applied to plants under heavy metal stress improve fruit characteristics and increase fruit size (Pishchik et al., 2002; Kanchana et al., 2014). In this study, especially MS-12 and MS-13 bacterial strains reduced fruit width in Rubygem, while the MS-7 bacterial strain did not. However, all bacterial strains preserved MFD at the dose of 100 mg kg^{-1} Cd in Kabarla, and 100-300 mg kg^{-1} Cd in YFL. Researchers have stated that the interaction varies depending on the bacterial strain and the plant species and variety, and as a result, the benefit of the bacteria is revealed (Karakurt et al., 2011; Shen et al., 2012; Kanchana et al., 2014). In this respect, the present study overlaps with the literature in terms of MFD.

4. Conclusions

In the present study, it was observed that Cd toxicity had negative effects on the morphological and pomological characteristics of strawberry cultivars. It was determined that the strawberry was resistant to low Cd dose, but the severity of damage increased with the increase in dose. It was also determined that bacterial strains applied against Cd toxicity reacted according to strawberry cultivars and Cd doses. In addition, it has been determined that bacterial applications protect many characteristics under Cd toxicity. New studies are needed to clarify the subject thoroughly and to determine how effective bacterial applications are against Cd toxicity.

Table 10. The effect of cadmium stress and bacterial applications on MFD (mm)[1]

Cultivars (C)	Cd doses	Bacterial applications (BA) B-	MS-7	MS-12	MS-13	Mean (CxCd)	Mean (C)
Rubygem	0	27.49^{d-f}	26.57^{hi}	27.85^{c-e}	26.26^{i-k}	27.04^{B}	
	100	27.28^{e-g}	25.38^{m}	24.67^{hi}	23.93^{n-p}	25.77^{D}	
	300	25.26^{m}	25.95^{n-p}	23.51^{pq}	23.56^{pq}	24.07^{F}	
Mean (CxBA)		26.67^{B}	25.30^{DE}	25.95^{C}	24.59^{F}		25.63B
Kabarla	0	27.99^{b-d}	28.55^{b}	29.51^{a}	28.42^{bc}	28.62^{A}	
	100	24.70^{d-f}	27.71^{g-i}	26.32^{i}	27.08^{f-h}	26.89^{B}	
	300	26.12^{i-l}	25.23^{m}	25.60^{klm}	25.57^{lm}	25.63^{D}	
Mean (CxBA)		27.17^{A}	26.83^{AB}	27.17^{A}	27.02^{AB}		27.05A
YFL	0	25.22^{m}	26.31^{ij}	26.28^{ij}	26.71^{g-i}	26.13^{C}	
	100	24.26^{no}	24.51^{n}	25.50^{lm}	25.66^{j-m}	24.98^{E}	
	300	22.61^{r}	23.79^{op}	23.08^{qr}	24.47^{n}	23.49^{G}	
Mean (CxBA)		24.03^{G}	24.87^{F}	24.96^{EF}	25.61^{CD}		24.87C
		Mean (CdxBA)				Mean (Cd)	
	0	26.90^{B}	27.14^{B}	27.88^{A}	27.13^{B}	27.26A	
	100	26.32^{C}	25.53^{DE}	26.12^{C}	25.56^{D}	25.88B	
	300	24.66^{E}	24.32^{EF}	24.06^{F}	24.53^{E}	24.87C	
Mean (BA)		25.96AB	25.67C	26.02A	25.74C		
LSD value		C= 0.193***, Cd= 0.193***, BA= 0.223***, CxCd= 0.335***, CxBA= 0.387***, CdxBA= 0.387***, CxCdxBA= 0.670***					

[1]: Differences between means denoted by the same letters in the same group, in the same row, and in the same column are insignificant, ***: Significant at $p<0.001$

Declaration of Author Contributions

The authors declare that they have contributed equally to the article. All authors declare that they have seen/read and approved the final version of the article ready for publication.

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Declaration of Conflicts of Interest

All authors declare that there is no conflict of interest related to this article.

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