Journal of Agricultural Sciences
(Tarim Bilimleri Dergisi)

J Agr Sci-Tarim Bili
e-ISSN: 2148-9297
jas.ankara.edu.tr

JAS

Effects of PGPB Inoculations on Plant Growth and Quality of Spray Carnation Cultivation in Greenhouse

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ARTICLE INFO

Research Article

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Received: 08 August 2023 / Revised: 23 February 2024 / Accepted: 24 April 2024 / Online: 22 October 2024

Cite this article

Yıldız Taşkesen E, Çığ A, Türkoğlu N (2024). Effects of PGPB Inoculations on Plant Growth and Quality of Spray Carnation Cultivation in Greenhouse. *Journal of Agricultural Sciences (Tarim Bilimleri Dergisi),* 30(4):668-684. DOI: 10.15832/ankutbd.1339822

ABSTRACT

In order to achieve the desired quality characteristics and good growth in ornamental plants, various applications are carried out. Recently, the importance of beneficial bacteria, which play an extremely important role in sustainable ecology and are environmentally friendly, has been increasingly recognized. However, the effect of beneficial bacteria, which are not sufficiently applied in ornamental plants as well as in other plant groups, on the growth and quality characteristics of spray carnation variety, which is the most preferred among cut flowers, was investigated in this study.

In the greenhouse, the carnation seedlings were treated with *Enterobacter ludwigii* (KF29A), *Pseudomonas fluorescens* (KF31B), *Paenarthrobacter nitroguaiacolicus* (KF3B), *Pseudomonas* sp. strain VG242B (KF5A), *Paenibacillus xylanilyticus* (KF63C), *Pseudoalteromonas tetraodonis* (TV126C) bacteria which have been selected according to nitrogen fixation, phosphate solubilizing, ACC deaminase and siderophore production properties were applied. In the study, in which phenological and morphological observations were made, the effects of bacterial inoculations were tried to be determined.

The effects of beneficial bacteria treatments on the number of petals, the number of nodes, the length between the nodes and the weight of the branches in the carnation plant were statistically insignificant; effects on the parameters of bud first bloom time, full bloom time, time from planting to first harvest, number of flower buds and stem length ($P<0.01$), flower (diameter) width and stem thickness ($P<0.05$) was found to be statistically significant. First bud bloom, full bloom and time from planting to first harvest are 103.38 days, 103.74 days and 106.28 days (KF63C) respectively, maximum number of flower buds is 4.77 (TV126C), flower diameter is 46.73 mm at the widest (KF63C), the highest stem thickness was 3.39 cm (KF3B) and the highest stem length was 56.33 cm (TV126C).

The first flowering time of the buds appeared with a delay of approximately 10-30 days compared to the control with bacterial applications. It is seen that bacterial applications cause an increase on flower stem thickness, flower stem length, flower bud and petal number.

Keywords: Bacteria, Flowering, *Dianthus caryophyllus*, Quality criteria, PGPB

1. Introduction

Cut flower production is one of the sub-group of ornamental plant production activities. It is important in terms of production volume and economic value. Cut flower cultivation began to gain importance worldwide in the 20th century. The most traded cut flower species in the world are roses, chrysanthemums and carnations, respectively. Carnation (*Dianthus caryophyllus* L.), one of the most popular commercial cut flowers in the World and is preferred by many exporting countries due to its wide range of forms and colors, excellent preservation quality and resistance to long-distance transportation (Kumar 2021). Carnation, which is the subject of research, has a very important place in cut flower cultivation and its commercial production is intense. Carnation (*Dianthus caryophyllus* L.) is a species in the Caryophyllaceae (Carnations) family and in the genus *Dianthus* is native to the Mediterranean Region (Besemer 1980; Whealy & Larson 1992). According to Alkaç et al. (2023), the worldwide export of carnation, which is among the most produced cut flowers, was worth 227 million Euros in 2018 (AIPH 2019), and as of 2019, its production is about 635 million in a greenhouse area of 5 thousand decares (TUIK, 2020). Türkiye also made significant contributions to the export of this plant species and ranked 3rd in world carnation exports after the Netherlands and Colombia.
Cut flower production and trade constitute the most important group in the ornamental plants sector in Türkiye, as well as in the World. According to 2022 data in Türkiye, the provinces with the highest production of cut flowers are Antalya (6030.2 da), İzmir (3349.9 da), Isparta (1815.0 da) and Yalova (835.1 da). Carnation production is mostly done in Antalya and İzmir (TUİK 2022).

Table 1- Production amount (units) of the most important cut flower species traded in Türkiye by years

Species/Product Groups	***Production quantity (units)***				***% Share (2022)***	***(2017-21) (%) Rate of Change***	***(2021-22) (%)Rate of Change***
	2017	***2019***	***2021***	***2022***			
Carnation	593 097 350	635 157 850	606 841 140	986 298 552	70.2	9.47	62.5
Gerbera	127 206 050	134 481 050	120 603 008	70 893 208	5.0	-5.2	-11.2
Cut Rose	107 942 520	98 130 020	101 204 410	99 417 885	7.1	-5.1	-1.8
Chrysanthemum	44 476.525	47 677 050	78 649 425	84 133 160	6.0	76.8	7.0
Freesia	17 815 150	17 463 650	11 339 400	14 434 160	1.0	-36.4	27.3
Tulip	44 504 500	40 290 500	27 830 000	4 930 000	0.3	-37.5	-82.3
Solidago	18 968 500	17 386 400	24 595 600	14 186 000	1.0	29.6	-42.3
Lisiantus	13 003 000	12 808 100	20 346 800	14 978 100	1.1	56.0	-26.4
Gypsophila	18 355 290	18 105 690	19 550 940	41 908 240	3.0	6.5	114.4
Narcissus	13 810 250	14 832 000	10 515 000	26 510 000	1.3	-23.9	152.1
Lily	9 552 285	9 282 685	7 916 525	8 806 025	0.6	-17.12	2.1
Matthiola	6 412 940	6 777 238	7 412 290	8 645 290	0.5	15.6	16.6
Gladiolus	7 269 800	6 709 900	4 824 900	5 427 900	0.3	-23.86	12.5
Orchid	1 624 940	1 885 930	1 903 800	2 870 300	0.2	17.16	50.8
Limonium	141 000	133 000	714 350	714 350	0.1	406	0.0
Others	29 107 560	32 212 880	20 735 366	21 037 175	1.6	-28.9	1.01
Total	**1 050 584 960**	**1 093 333 943**	**1 064 982 954**	**1 404 473 345**	**100 0**	**5.63**	**31.4**

(TUİK 2019; 2021; 2022)

When the amount of cut flower production in Türkiye between 2017 and 2021 is examined, it is seen that carnation (986 298 552 pieces) ranks first (Table 1) (TUİK 2021; 2022). Among these, spray carnation emerges as the main product for many cut flower exports.

According to TUİK 2023 data, Türkiye produces carnations in 6 020.87 da of the 58 146.01 da cut flower production area and exports almost all of the carnations produced (TUİK 2023). The prominent province in carnation production and export in Türkiye is Antalya, and its climatic conditions allow production and export for nine months, from the beginning of September to the end of May.

In Antalya, rainfall in the form of torrential downpours can sometimes negatively affect production. The months when production intensifies and the period of maximum rainfall are largely parallel. Rainwater, which is difficult to drain from greenhouses in some enterprises, causes the disease called black spot on flowers. This leads to a significant decrease in the quality of these export-oriented products (Zaman et al. 2011).

Fusarium sp., *Macrophomina* sp., *Rhizoctonia* sp., *Pythium* sp., *Verticillium* sp., *Alternaria* spp., *Sordaria* spp., *Aspergillus* spp., *Penicillium* spp. and *Trichoderma* spp. fungus species were identified in samples taken from plants showing signs of disease in carnation greenhouses in Antalya province (Atakan & Özgönen Özkaya 2018). These soil-borne fungi also cause quality loss in plants. *Fusarium* spp., *Rhizoctonia solani, Pythium* spp. can occur at any time during the production season and cause significant plant losses in greenhouses by causing seedling drying, wilt, root and stem rot in the early post-planting period (McCain 2003; Şevik & Saruhan 2010). Red spider, thrips and greenworm are the main pests of carnations and the most important pest in Antalya province is *Frankliniella occidentalis* (Pergande) (Thysanopiera, Thripidae) (Tunç & Göçmen 1995). This pest sucks plant sap and forms typical white spots on the petals of carnation flowers (Keçeçioğlu 2001). Keçecioğlu & Madanlar (2002) examined the effects of insecticides used with different applications on *F. occidentalis* in a greenhouse in the region. In the study carried out to determine the fertility status of greenhouse soils where carnation are grown in Kepez district of Antalya province, it was determined that the textures of the greenhouse soils ranged from sandy to clayey, pH was generally alkaline and slightly alkaline reaction, and electrical conductivity values varied from non-saline to very high saline. The majority of the soils contain excessive lime and are humus poor in terms of organic matter content. Total N and exchangeable K contents vary from very low to very good, while the amount of available P is adequate and exchangeable Mg and Ca are good (Asri Öktüren et al. 2016). Asri Öktüren et al. (2016) pointed out that nutritional problems that may arise from the antagonistic effect between plant nutrients will be reduced if the producers adjust the pH of the nutrient solutions they use between 6.5-7.0, considering that the clove plant prefers slightly acid and neutral conditions, and recommended that priority should be given to practices aimed at increasing the level of organic matter in the soil in order to eliminate this negative effect that may arise in greenhouse soils, and organic origin fertilizers should be used as much as possible in the fertilization programs to be applied. The researchers stated that although nitrogen fertilizer in chemical form is generally used in greenhouses, 68% of the clove greenhouses examined had low nitrogen content, and this may be due to the high uptake and washing in the form of NO_3^-. In conclusion, in order to increase the quality and yield in carnation cultivation, which has an important place in exports, fertilization programs suitable for soil characteristics should be developed and unbalanced and unconscious fertilization practices should be avoided.

Chemical fertilizers play an important role in increasing production. However, excessive use of chemical fertilizers causes soil fertility to be lost and deteriorated. Theoretically, a significant increase in yield cannot be achieved with high-use artificial fertilizers. It is known that chemical fertilizers harm the environment. Research, advancement and adaptation of biological substitutes have brought the importance and acceptance of organic fertilizers to the agenda. Today, worldwide researches are carried out to create organic fertilizer formulations for clean living spaces and healthy production (Çakmakçı 2005). There is an increasing trend towards organic fertilizers to eliminate the negative effects of chemical fertilizer use all over the world. Among organic fertilizers, plant growth-promoting bacteria (PGPB) are used as raw materials for microbial fertilizers (Meena & Rai 2017). Bacteria constitute the largest part of microorganisms living in the soil. Research has shown that some bacteria support plant development in many ways with different mechanisms of action. These beneficial bacteria were named PGPR (Plant Growth Promoting Rhizobacteria) (Kloepper et al. 1980). They are also known as "Probiotic Rhizobacteria" due to the many benefits they provide to the plant (Ram et al. 2013). PGPRs promote plant development with their action mechanisms such as nitrogen fixation, solubilizing phosphorus and heavy metals, producing hormones, increasing water and mineral uptake, supporting root development, and increasing enzyme activity in the plant. Many researchers are conducting research on the wide range of uses of rhizobacteria. Research has shown that rhizobacteria are responsible for detoxification of heavy metals (Wani & Khan 2010), degradation of pesticides (Ahemad & Khan 2012), salinity tolerance (Mayak et al. 2004), biological control of plant diseases and pests (Hynes et al. 2008; Tozlu et al. 2012), increasing the use of nutrients and minerals by the plant (Çakmakcı 2009), supporting plant development by producing phytohormones and enzymes (Dejordjevic et al. 1987; Ferreira et al. 1987).

Root bacteria (Plant Growth Promoting Rhizobacteria- PGPR), which increase plant growth, have an important place because they increase plant growth and yield (Gül et al. 2008; Çelik et al. 2020). In recent years, it is seen that bacteria that promote plant growth are widely used in fruit and vegetable species that are difficult to root and produce, and in some field crops, while their use in ornamental plants is quite limited.

Sezen & Külekçi (2020) used ornamental plants that are difficult to produce as materials and bacteria that affect the growth parameters of ornamental plants and promote growth. As a result of the research, ornamental plants, and applied bacteria were explained, and the importance of disseminating the use of naturally sourced bacteria that do not harm the environment and support the development of ornamental plants was emphasized.

The colour, height, flower size, yield and growth rate of the varieties vary according to their resistance to diseases, and stress conditions such as cold and heat, salinity and heavy metals. That's why growing and care conditions are very important. Ultimately, the market value of flowers is determined by these factors. The desired level of flowers and quality increases the market value of carnations. Similar to other researchers, it would be logical to investigate the effects of beneficial bacteria on carnation, the most preferred cut flower variety in Türkiye, and to determine the benefits it will provide in its production.

In this context, this study is important in terms of revealing how bacterial applications as biological fertilization affect carnation flowers and accordingly changes in plant and flower quality.

2. Material and Methods

2.1. Materials

2.1.1. Plant material

The carnation seedlings used as material were produced from cuttings taken from fast growing and white colored spray carnation plants grown in the greenhouse (Figure 1).

Figure 1- White-colored spray carnation

2.1.2. Bacteria strains

The bacteria used in the study were obtained from the Department of Field Crops, Faculty of Agriculture, Siirt University. Bacterial isolates are isolates from the rhizospheres of Lake Van Basin (TV group) (Erman et al. 2010) and Siirt province (KF group) within the scope of TOVAG 108O147 TUBITAK project in Türkiye and previously diagnosed with MIS system and Plant Growth Promoting Bacteria (PGPB) activity has been demonstrated in field and greenhouse conditions. These bacterial strains were selected according to their strong or weak effects on nitrogen fixation, phosphate solubilizing, ACC deaminase, and siderophore production (Table 2).

Table 2- Bacterial strains inoculated into the soil and their characteristics

Bacteria Code	***Nitrogen Fixation***	***Phosphate Solubilizing***	***ACC Deaminase***	***Siderophore Production***
Control (no bacteria applied)				
KF3B *Paenarthrobacter nitroguajacolicus*	+	-	+++	+
KF5A *Pseudomonas* sp. strain VG242B	+	++	-	+
KF29A *Enterobacter ludwigii*	+	++	-	+
KF31B *Pseudomonas fluorescens*	+	++	-	+
KF63C *Paenibacillus xylanilyticus*	+	++	++	+
TV126C *Pseudoalteromonas tetraodonis*	Strong	Weak	+	-

2.1.3. Properties of the experimental area

This study was carried out in the 8-decare greenhouse of a private enterprise called Flora City in Aksu district of Antalya, covered with polyethylene and made of iron construction. The area where the study was carried out is at an altitude of 581 meters, at 36° 58' 24" N and 30° 48' 49" E coordinates (Figure 2).

Figure 2- The greenhouse where the experiment was conducted (Google Earth 2023)

3. Methods

3.1. Cultivation of carnation seedlings

The first step before planting clove cuttings in the greenhouse is rooting them with indole butyric acid (IBA) (Figure 3). Cuttings treated with IBA were transplanted into a rooting medium consisting of a mixture of half peat and perlite (Figure 4). When the cuttings started to develop, nitrogen fertilization was applied twice a week to increase the height of the plant. In addition, spraying was done every two days to combat red spider and root rot (active substance: abamectin) and (active substance: tolclofosmethyl). The rooted cuttings (Figure 5) were placed in mulch bags of at least 25, pre-cooled, and then placed in cold storage with 80-85% humidity and 2-4 °C temperature (Figure 6). Then, the carnation seedlings were planted 25 cm apart under a clean and sterilized greenhouse (Figure 7). A trellis system was built in the planting area and meshes were woven row spcae and on the rows. After planting, tip removal was done twice and harvesting was done considering gradation.

Figure 3- IBA treatment of carnation seedlings

Figure 4- Planting of carnation seedlings on tables

Figure 5- Rooted carnation cuttings

Figure 6- Carnation seedlings placed in cold storage

Figure 7- Planting of seedlings

Figure 8- Bacterial contamination of carnation seedlings

3.2. Preparation of stocks of ınoculated bacterial strains

Nutrient agar (Merck-VM71680604) was used as a solid medium for the propagation and production of bacteria. The pH was adjusted to 7.0 by adding 20 g of nutrient agar to one liter of distilled water and the mixture was sterilized by autoclave at 121 °C for 15 minutes. After sterilization, the media were cooled to 50 °C, transferred to Petri dishes, and left to solidify. Stock cultures of bacteria were inoculated on a nutrient agar medium with a loop and incubated for 24 hours at 26 ± 2 °C.

Nutrient broth (Merck-VM775843711) was used as broth. The pH was adjusted to 7.0 by adding 8 g of nutrient broth medium to one liter of distilled water. The mixture was sterilized by autoclave at 121 °C for 15 minutes and left to cool. A single colony was taken from bacteria grown on Nutrient agar medium and transferred to nutrient broth medium under aseptic conditions. Bacteria transferred to the broth were incubated at 26 ± 2 °C for 24 hours and at 120 rpm in a horizontal shaker. After incubation, bacterial concentrations were turbidimetrically adjusted to ~108 cfu/mL.

After shaking overnight and ensuring homogeneity, 10 mL was inoculated into the soil from the root collar in the area where the carnations were planted (Figure 8).

After this first inoculation, the second inoculation was made just before the time of flower bud emergence and in the same amount.

To compare with the effect of bacterial strains, a control group was also formed, and in carnations that were not contaminated with bacteria and in carnations inoculated with bacteria, flower stalk length, number of nodes, the length between internodes, flower stalk thickness, branch weight, flower diameter, flower bud (bud) number, petal measurements, and observations were made on the number of petals, bud first bloom time, full bloom time and the time from planting to first harvest.

3.3. Experimental plan and statistical analysis

The study was conducted according to a randomized plots experimental design and consisted of a control group with no treatment and treatments consisting of inoculation of 6 bacterial strains. Each treatment group consisted of 3 replicates and 15 carnation seedlings were used in each replicate. The data were analysed in the SAS For Academics on Demand Online Statistics package program. Means were compared using Duncan's multiple comparison tests for traits in which the difference between treatments was significant. The tests were performed at the α=0.05 significance level (Düzgünes et al. 1987).

4. Results and Discussion

The effects of beneficial bacteria on the development and flowering of the carnation plant are given in Table 3.

Table 3- The effects of bacterial strains on the development and flowering of the carnation plant

Applications	*Bud's first bloom time (days)***	*Full bloom time (days)***	*Time from planting to first harvest (days)***	*Number of flower buds (pieces)***	*Number of petals (pcs) ns*	*Flower diameter (mm)**	*Node number (piece) ns*	*Internode length (cm)ns*	*Petiole thickness (mm)**	*Flower stem length (cm)***	*Branch weight (g) ns*
Control	118.42 bc	120.07 bc	121.62 bc	3.91 b	48.73	43.84 ab	9.42	5.48	3.01 ab	50.86 bc	31.95
KF29A	129.86 ab	131.51 b	134.10 ab	4.44 ab	54.44	38.16 b	8.91	5.80	3.27 ab	53.31 ab	31.99
KF31B	131.35 ab	132.69 b	133.90 ab	4.38 ab	50.62	44.41 ab	9.57	5.41	3.01 ab	51.03 bc	30.01
KF3B	127.71 ab	132.91 b	139.42 ab	4.31 ab	45.00	43.40 ab	9.13	4.77	3.39 a	44.97 d	31.77
KF5A	147.60 a	152.97 a	154.67 a	3.66 b	49.66	46.32 a	9.18	6.23	3.17 ab	47.93 cd	29.51
KF63C	103.38 c	103.74 c	106.28 c	3.84 b	47.02	46.73 a	9.20	5.62	2.72 b	49.54 bcd	34.74
TV126C	135.68 ab	137.13 ab	139.55 ab	4.77 a	51.80	42.32 ab	9.82	5.60	3.13 ab	56.33 a	32.98
F values	11.06	15.47	9.80	5.28	2.63	3.81	2.16	1.25	3.07	13.91	0.80

KF29A: *Enterobacter ludwigii*; KF31B: *Pseudomanas fluorescens*; KF3B: *Paenarthrobacter nitroguaiacolicus*; KF5A: *Pseudomonas* sp. Strain VG242B; KF63C: *Paenibacillus xylanilyticus*; TV126C: *Pseudoalteromonas tetraodonis*
ns: no significant, *: significant at 5%, **: significant at 1%

The differences between the averages denoted by the same letter in the same column are not significant.

4.1. Bud's first bloom time (days)

The effects of beneficial bacteria applications on the first flowering period of the carnation plant were found to be statistically significant at the P<0.01 level. The earliest bud opening time was observed in the application of KF63C bacteria (103.38 days), and the latest bud opening time (147.6 days) was obtained in the application of KF5A bacteria (Figure 9). The buds of the control group carnations bloomed for the first time in 118.42 days. Control buds bloomed up to 15 days later than some KF63C bacteria-inoculated carnations. Earlier blooming between 9-29 days than other bacterial treatments showed that bacterial strains affected the flowering times. As it is known, phosphorus is an element that affects flowering in plants. As seen in the results obtained in the research, the application of KF63C bacteria, which has a versatile effect, had a greater effect than other bacteria and the control application and provided the earliest flowering on the plant (Ali et al. 2012; Gunjal & Glick 2023). The application of KF63C bacteria has produced a very important result, especially in terms of early flowering, bringing the product to the market early and selling it at a higher price.

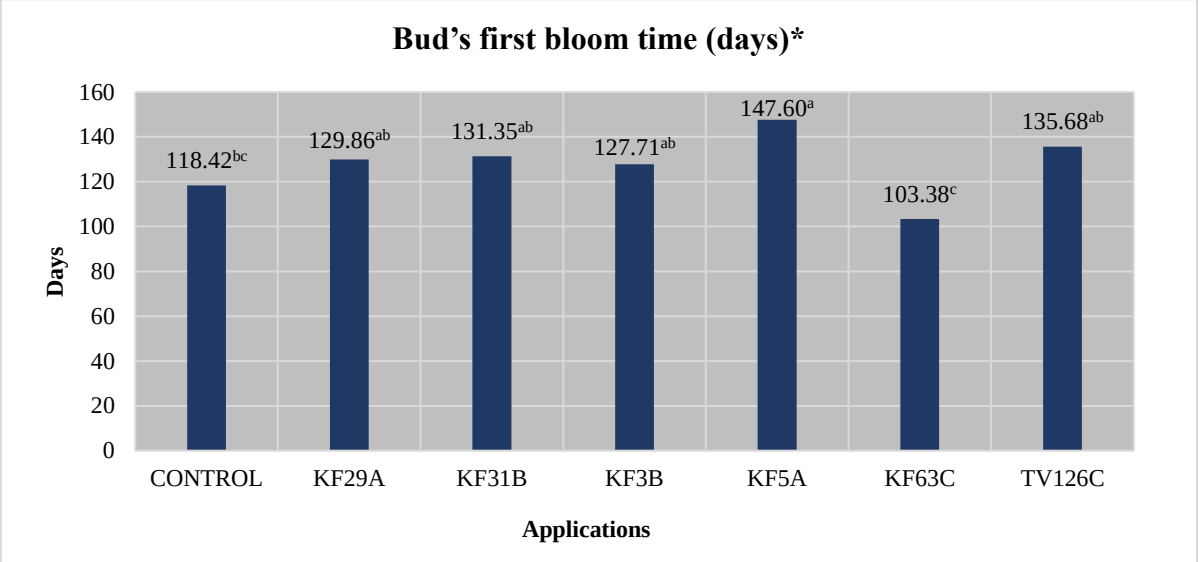

Figure 9- Time taken for the first flowering of carnation buds (days), *: The differences between applications statistically are significant at P<0.01

4.2. Full Bloom time (days)

The effects of beneficial bacteria applications on the full flowering period of the carnation plant were found to be statistically significant (P<0.01). The earliest full flowering period (103.74 days) was observed in the application of KF63C bacteria, and the latest full flowering period (152.97 days) was reached with the application of KF5A bacteria (Figure 10). The data obtained showed parallelism with the first blooming times of the buds. Control carnations reached full bloom in a longer time than carnations inoculated with only KF63C bacteria and reached this stage in a shorter time than carnations treated with other bacteria.

Phosphorus is an element that affects flowering in plants. As seen in the results obtained in the research, in the application of KF5A bacteria, the phosphate solubilization feature showed more effect than other bacteria and caused the full flowering time at the latest. In flower production, late production is as important as early production in terms of market advantage. Especially as the amount of product offered decreases, product prices increase. For this reason, it is thought that the application of KF5A bacteria may cause late full flowering, resulting in product supply at high prices and therefore high economic returns. Flower supply to the market late in cold storage etc. Providing it by taking precautions is very difficult and costly due to energy, storage space, labor and other reasons (Güneş & Babadağ 2022).

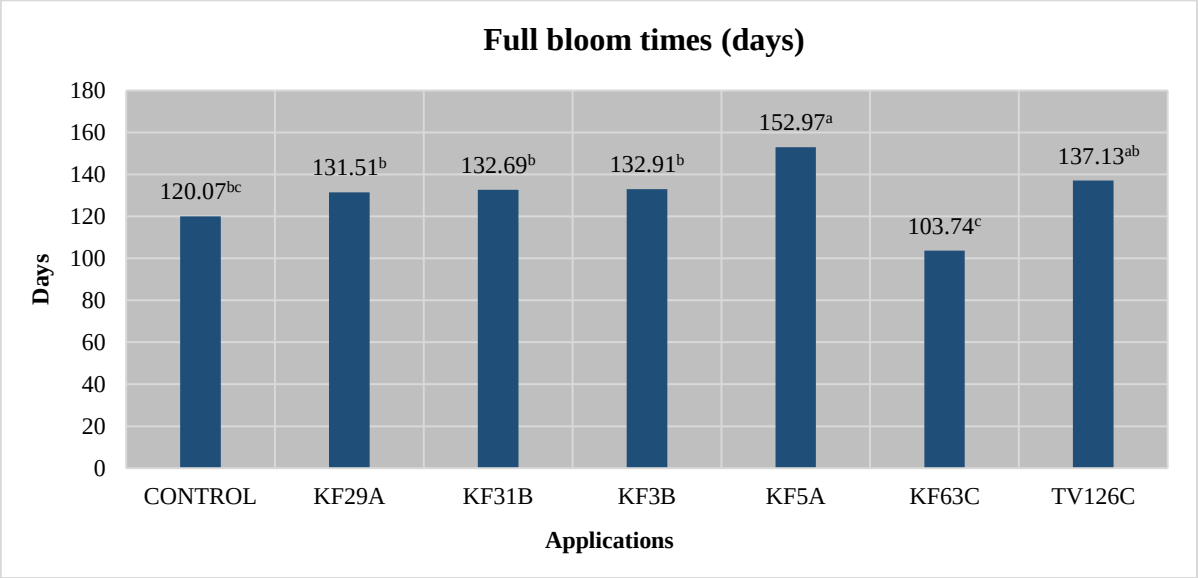

Figure 10- Time taken for full bloom of carnation buds (days), *: The differences between applications statistically are significant at P<0.01

4.3. Time from planting to first harvest (days)

The effects of bacteria applications on the period from planting to harvest in the carnation plant were found to be statistically significant (P<0.01). The time from planting to the first harvest was reached by the application of KF63C at the earliest (106.28 days) and at the latest (154.67 days) with the application of KF5A bacteria (Figure 11). Most of the bacteria applied in our study with carnations caused a delay in harvest time. Of these, only one strain of bacteria (KF63C) provided 15 days of earliness compared to the control group. While KF29A, KF31B, and KF3B bacterial strains caused the flowers to be harvested at almost the same time, TV126C bacterial strain was effective in the harvest for approximately 5 days after these three strains.

In terms of the effect of planting time on the first harvest time, the earliest harvest time among the applications was obtained from the application of KF63C bacteria, which has versatile properties. This result reveals that bacteria with versatile properties are more advantageous in terms of their mechanism of action than bacteria that have a unidirectional effect or have a weak effect in terms of some properties (Çığ et al. 2017; Glick 2020).

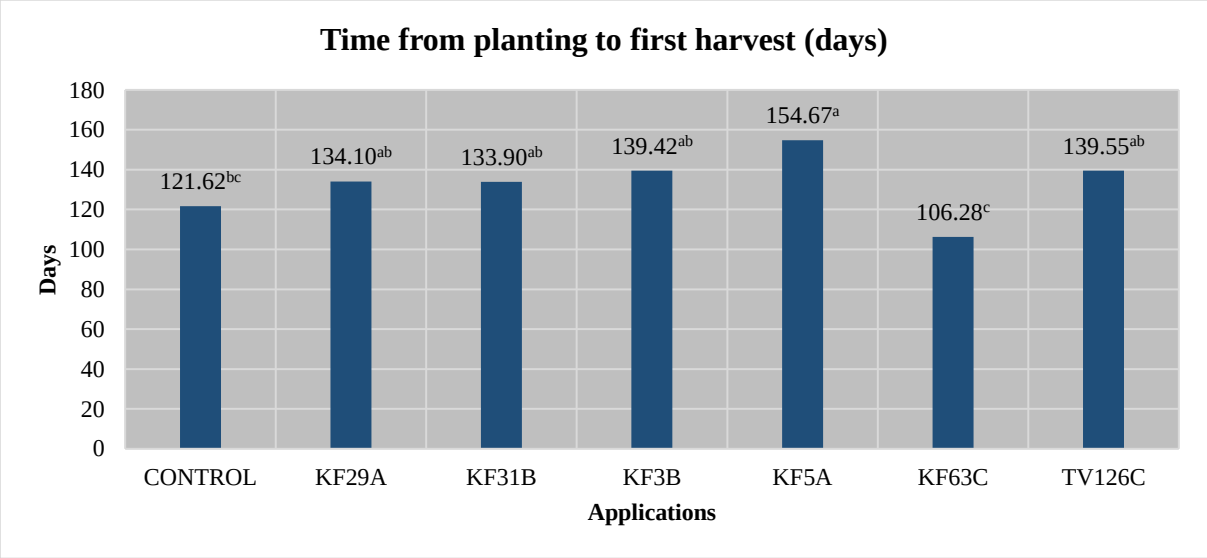

Figure 11- Time spent by carnation seedlings from planting to harvest (days), *: The differences between applications statistically are significant at P<0.01

4.4. Number of flower buds (pieces)

The effects of beneficial bacteria applications on the number of flower buds in carnation were found to be statistically significant (P<0.01). The highest number of flower buds (4.77 pieces) was seen with the application of TV126C bacteria, and the lowest number of flower buds (3.66 units) was reached with the application of KF5A bacteria (Figure 12). The number of buds in the control group carnations was determined as 3.91, and it was observed that this number was more bud formation than the carnations in which KF5A and KF63C bacterial strains were inoculated.

In terms of the number of flower buds, lower values were obtained in KF5A and KF63C applications compared to the control. Although quantitatively lower values are obtained in bacterial inoculations, qualitatively higher quality flower formation can be observed (Ali et al. 2012; Gunjal & Glick 2023). However, obtaining higher values in other bacterial applications compared to the control showed that alternative bacterial strains can be used in cultivation. This data also showed that different properties of bacteria can be used to serve different purposes (Glick 2020).

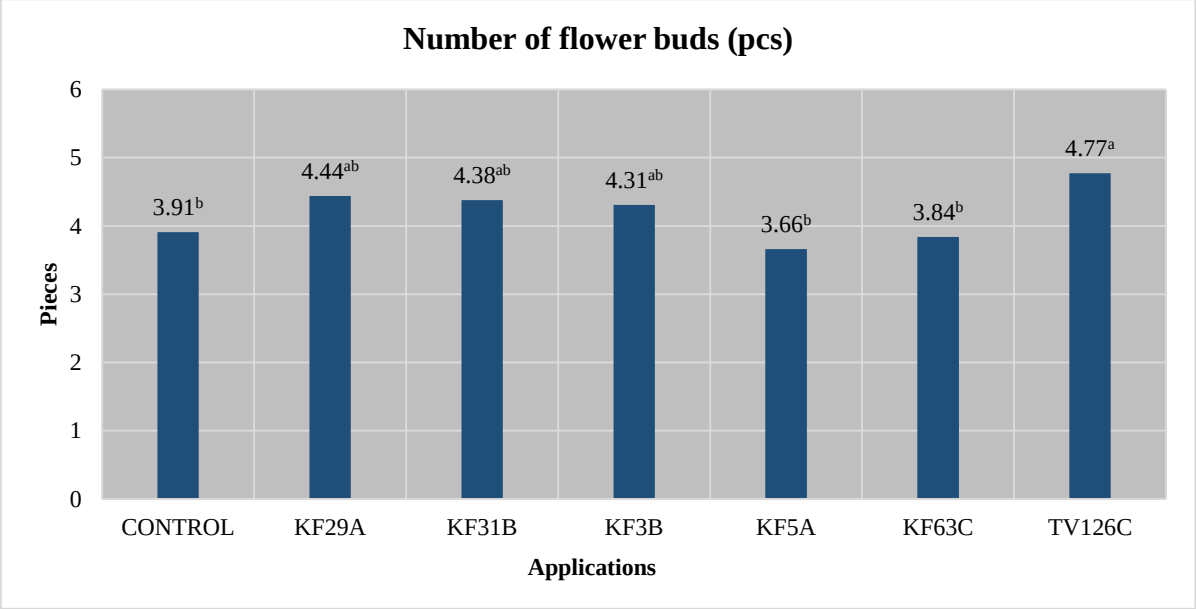

Figure12- The number of flower buds on the carnation plant (pieces), *: The differences between applications statistically are significant at P<0.01

4.5. Number of petals (pieces)

The effects of bacterial applications on the number of petals in the carnation plant were found to be statistically insignificant (Figure 13). The highest number of petals (54.44 units) was observed in the application of KF29A bacteria, and the lowest value of the number of petals (45 units) was obtained in the inoculation of KF3B bacteria. The number of petals in the flowers of the carnations grown in the control group was found to be higher than that of the carnations inoculated with KF63C and KF3B bacteria, and lower than those of other applications. The fullness of the flowers causes the carnations to be showier. Therefore, the difference in the lowest and highest number of petals is important for the showiness of the flowers.

It is thought that the KF29A bacteria, which has a strong phosphate solubilizing activity and gives the highest values in terms of petal number, ranks first due to the effect of phosphorus on flowering and flower quality. However, TV126C bacteria, which has strong nitrogen fix and weak phosphate solubilization activities, ranks second due to its bidirectional effect (Erman et al. 2024). With this research, it can be concluded that strong nitrogen activity has a positive effect on flower quality, even if the phosphorus effect is weak (Çığ et al. 2021).

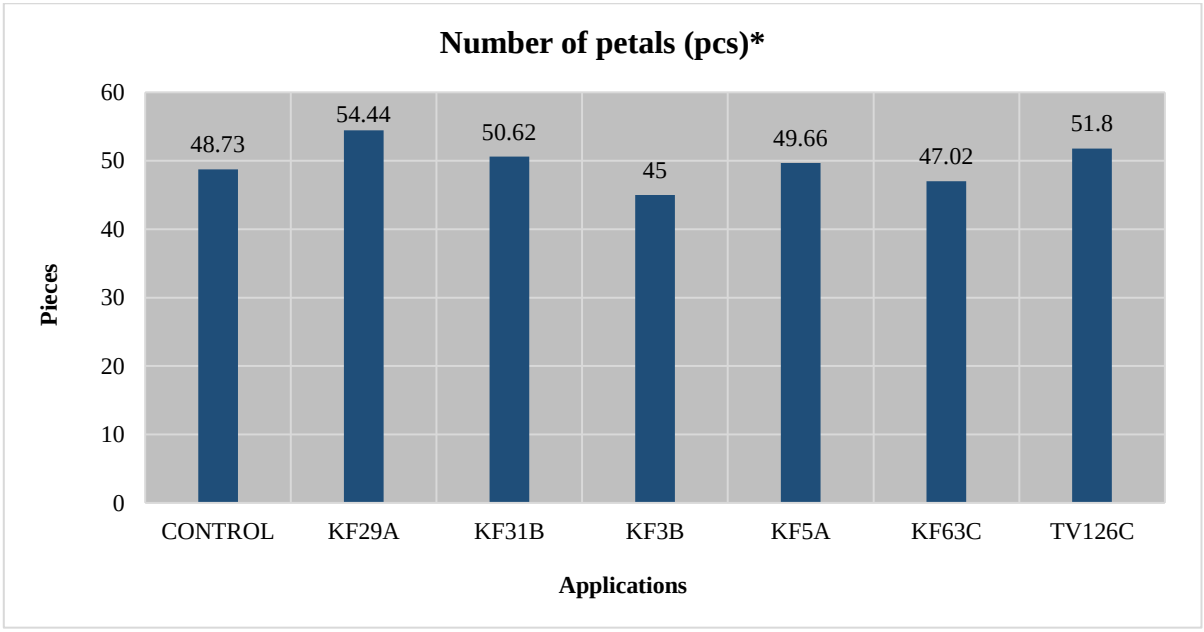

Figure 13- Number of petals (pieces) of carnation flowers, *: The differences between applications statistically are not significant

4.6. Flower diameter (mm)

The effects of bacterial applications on flower diameter length in carnation plants were found to be statistically significant (P<0.05). The highest flower diameter value was seen in the application of KF63C at 46.73 mm and KF5A at 46.32 mm and was statistically included in the same group. The lowest flower diameter value was found in the application of KF29A bacteria with 38.16 mm (Figure 14). The diameters of the control group carnation flowers were found to be higher than those of the carnations inoculated with KF29A, KF3B, and TV126C bacteria. It is seen that bacterial inoculations affect the diameter of carnation flowers at different levels. There is a difference of approximately 8 mm between the lowest and highest values between bacterial strains inoculations. Since flower size is also important in terms of flower quality, applications that have a positive effect on this parameter should be carefully evaluated.

KF63C, the bacterial application in which the highest value in terms of flower diameter is obtained, stands out with its versatile feature. It is thought that such bacteria cause improvements in flower quality because they contribute to resistance to nutrition and stress conditions with their nitrogen fixing, phosphate solubilization, siderophore production and ACC deaminase properties.

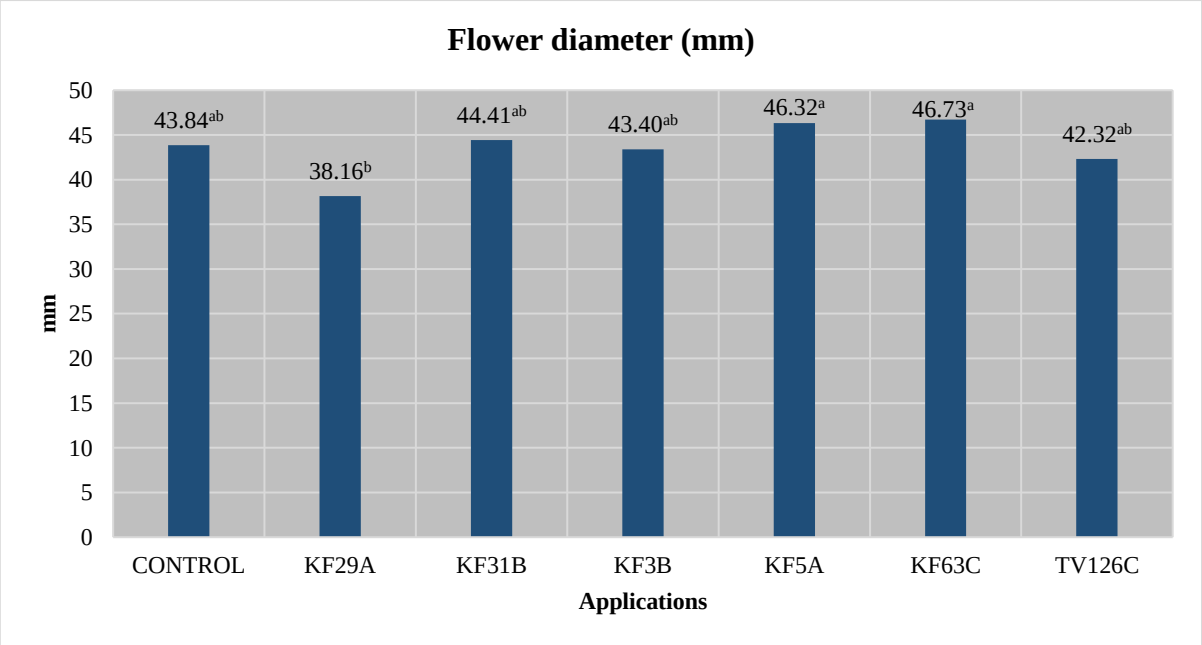

Figure14- Diameter of carnation flowers (mm), *: The differences between applications statistically are significant at P<0.05

4.7. Node number (pieces)

The effects of bacterial applications on the number of nodes in the carnation plant were found to be statistically insignificant. The number of nodes on the carnation branches of the control group was determined as 9.42. As a result of the applications in which bacterial inoculations were made, the number of nodes varied between 8.91 and 9.82, and the lowest and highest values were obtained in the inoculations of KF29A and TV126C bacterial strains, respectively. After TV126C and KF31B bacterial strains, the highest internode control group was determined in carnations (Figure 15).

TV126C, the bacterial application that gives the highest value in terms of number of branches, stands out with its strong nitrogen fixation and weak phosphate production. This dual feature reveals that nitrogen, in particular, encourages plant vegetative development, is effective in increasing the number of plant nodes and therefore in length, and is effective in flowering due to its phosphate solubilizing activity, although it is weak (Sonkurt & Çığ 2019). As a result of this research, selection of bacteria with dual activity and especially strong nitrogen fixation activity may contribute to cultivation for tall height and multiple flower setting.

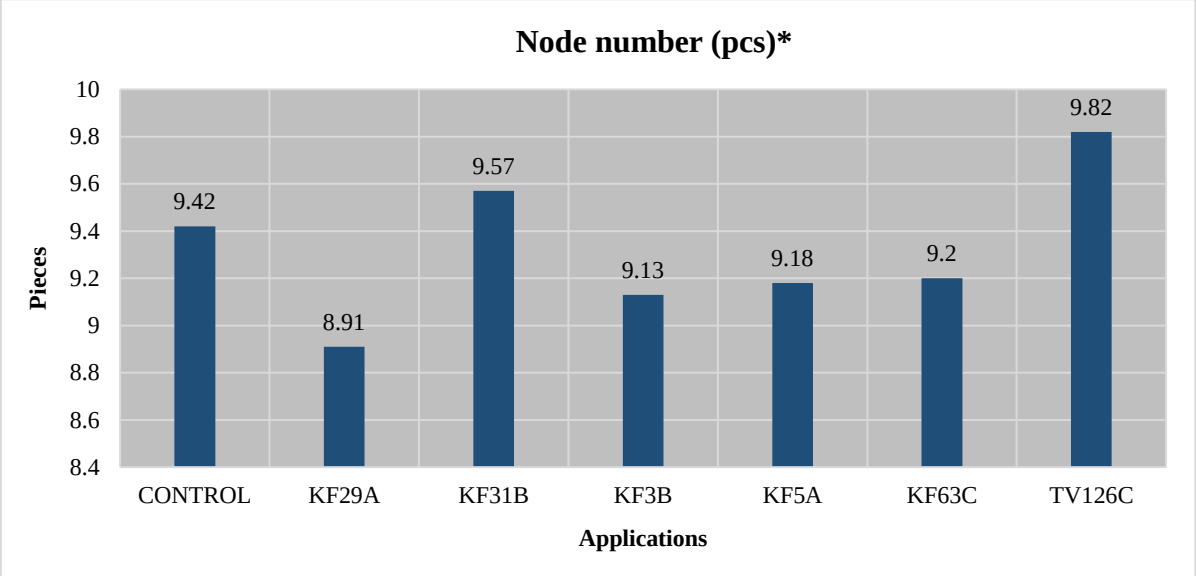

Figure 15- Node number of carnation plants (pieces), *: The differences between applications statistically are not significant

4.8. Internode length (cm)

The effects of bacterial applications on the number of nodes in the carnation plant were found to be statistically insignificant. The highest internode length was observed in the application of KF5A bacteria and 6.23 cm was obtained. The lowest internode length value was found to be 4.77 cm, and it was detected in the application of KF3B bacteria (Figure 16). The effect of the bacteria applied in our study on the internode length was not statistically significant. The internode length of the control group carnation plants was higher than the carnations in which KF3B and KF31B bacteria inoculations were made.

In the data obtained in the research, it is thought that the highest internode length was achieved with the application of KF5A bacteria, which has nitrogen-fixation and strong phosphate-solubilizing activity, and this result was achieved with the positive contribution of the dual synergistic effect. Similarly, it is thought that the partially superior effect of the KF5A bacterial species over other bacteria with dual properties may be due to its ability to combat other organisms in the soil and its greater effectiveness in plant-bacteria compatibility.

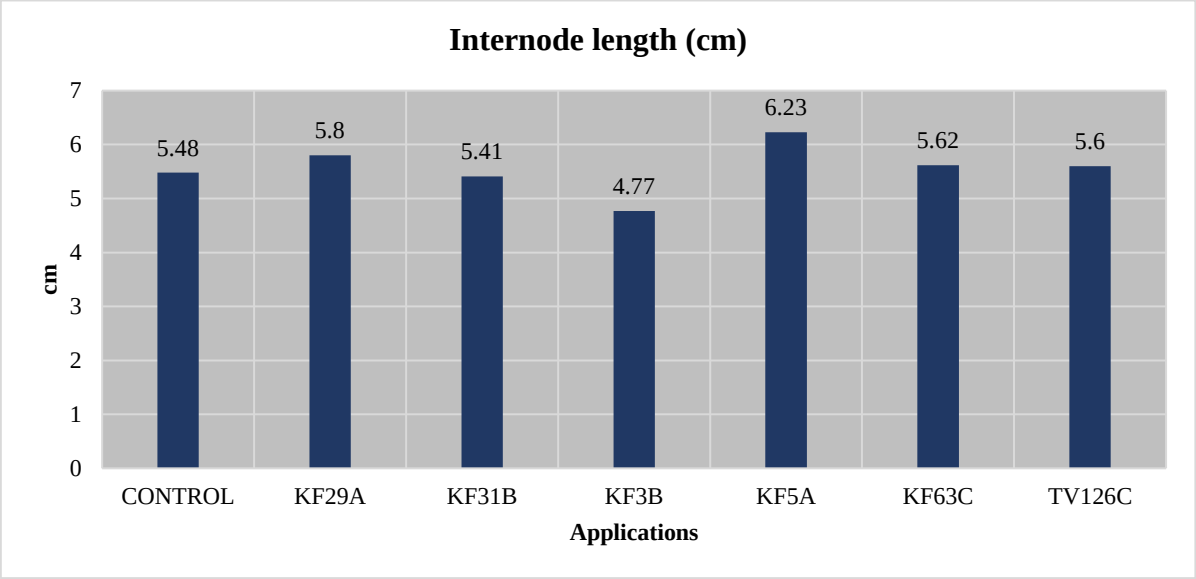

Figure 16- Internode length in carnation plant (cm), *: The differences between applications statistically are not significant

4.9. Flower stem thickness (mm)

The effects of bacterial applications on the flower stem thickness of the carnation plant were found to be statistically significant ($P<0.05$). The highest flower stalk thickness was determined as 3.39 mm and was determined in the application of KF3B bacteria. The lowest petiole thickness was 2.72 mm and was obtained with the application of KF63C bacteria (Figure 17).

It is thought that the main reason why KF3B bacteria show the highest value in terms of flower stalk thickness is due to its very strong ACC deaminase activity, unlike other bacteria. As it is known, ACC deaminase is effective by reducing the ethylene concentration in flowering, which is the generative period, and is known to extend this period (Söğüt & Çığ 2019). Thus, it is thought to have an effect on flower quality and therefore the thickness of the flower stem.

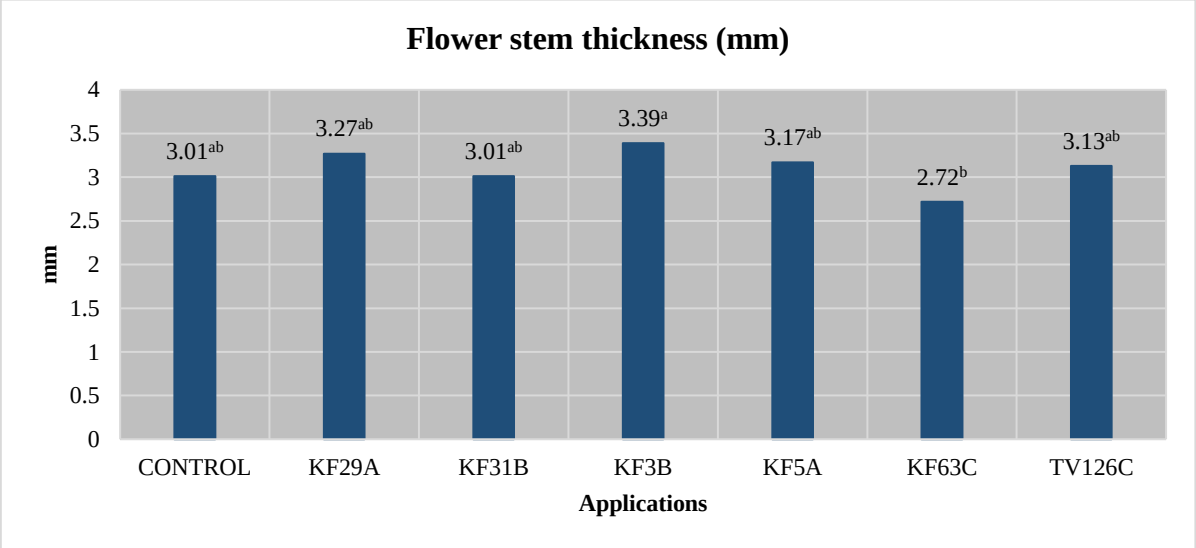

Figure 17- Flower stem thickness of the carnation plant (mm), *: The differences between applications statistically are significant at P<0.05

4.10. Flower stem length (cm)

The effects of bacterial applications on the flower stem length of the carnation plant were found to be statistically significant (P<0.01). The highest stem length (56.33 cm) was observed in TV126C bacteria inoculation, and the lowest stem length (44.97 cm) was determined in KF3B bacteria inoculation (Figure 18). Flower stem length is an important cut flower criterion for use in a vase. Although it is related to the various feature, it can be kept under control with applications or its length can be increased. In our study, the stem length of the control group carnations was found to be 50.86 cm, and it was recorded as higher than the stems of the carnations inoculated with KF3B, KF5A, and KF63C bacteria. These bacterial strains had a reducing effect of around 6 cm on the stem length of the control group plants. On the other hand, KF29A, KF31B, and TV126C bacterial inoculations increased stem length compared to control plants.

As a result of the study, it is thought that the main reason for obtaining the highest flower stem length with TV126C application is the high nitrogen amount resulting from its strong nitrogen fixation feature and therefore high vegetative development. In this way, an increase in height and therefore a higher flower stem length is achieved.

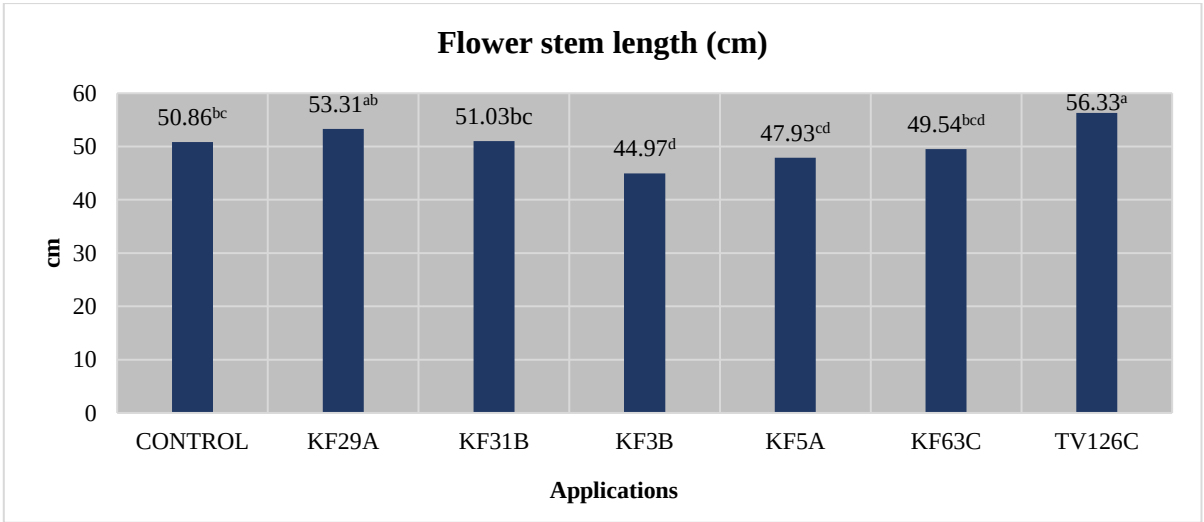

Figure 18- The length of the flower stalk of the carnation plant (cm), *: The differences between applications statistically are significant at P<0.01

4.11. Branch weight (g)

The effects of bacterial applications on the branch weight of the carnation plant were found to be statistically insignificant. The highest branch weight value (34.74 g) was observed with the application of KF63C bacteria, and the lowest branch weight value (29.51 g) was obtained with the application of KF5A bacteria (Figure 19). The average branch weight value of the control group carnations was found to be higher than the branch weight of the carnations inoculated with KF3B, KF31B, and KF5A bacteria. This creates the idea that some of these bacterial strains do not cause an increase in the branch weight and do not have a positive effect in this direction.

It is thought that the main reason why the highest value in terms of branch weight was obtained from the KF63C application is that it is a bacterial strain that is effective in terms of nitrogen fixation, ACC deaminase and siderophore production, as well as its strong phosphate solubilization activity. Likewise, it has been concluded that the main reason for obtaining higher values than other applications with similar features is high-level plant-bacteria interaction. Studies have shown that the exudates secreted by plants are used more effectively by some bacteria and affect some properties of the plant more (Glick 2020).

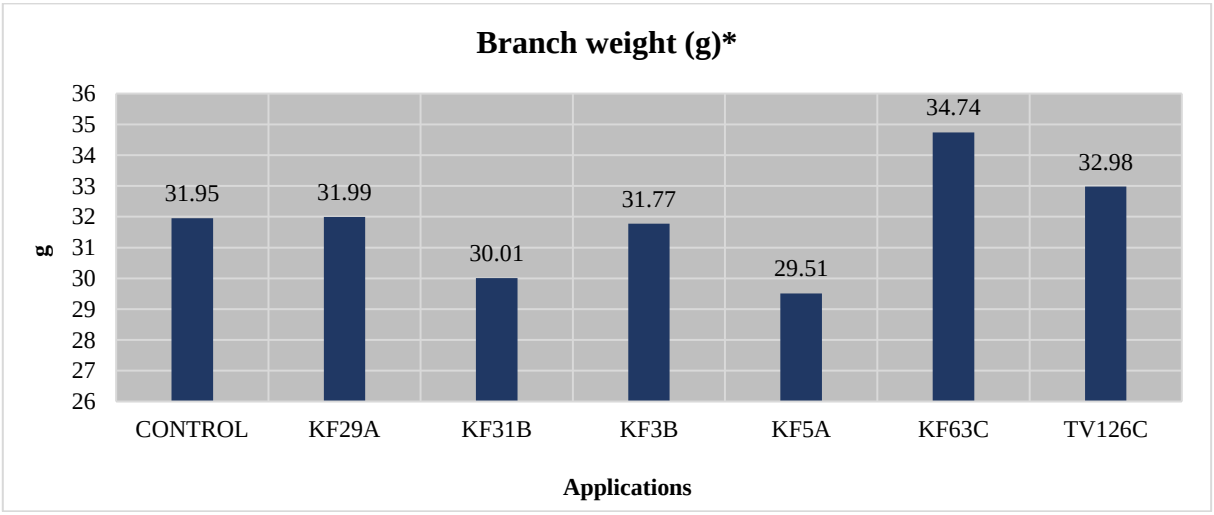

Figure 19- Branch weight of carnation plant (g), *: The differences between applications statistically are not significant

In the previous bacterial inoculation studies on ornamental plants, the results related to flowering were generally found to affect the beginning of flowering, harvest time, and plant growth parameters. In studies with tulips (*Tulipa gesneriana* L. cv. 'Clear Mater') in Faisalabad, *Burkholderia phytofirmans* (PsJN), T2 *Bacillus* sp. (MN-54), T3 *Enterobacter* isolates were used as foliar fertilizer. The results showed that the tulips responded well to bacterial treatments and significant improvements were observed in morphological traits and other quality criteria. During flowering, bacteria caused a delay of a maximum of 6 days and a minimum of 4 days compared to control treatments (Bashir et al. 2019). In the treatment of *Gladiolus grandiflorus* cv. White Friendship variety with *Rhizobium*, Phosphorous Solubilizing Bacteria (PSB), *Azotobacter* and *Azospirillum* biofertilizers, the control group plants emerged in the latest time. The highest values of plant height, the number of lamps per spike, branch length, and branch fresh weight were obtained in bacterial applications. Flower emergence time was determined early in control group plants (Ali et al. 2014). Bacterial applications caused late blooming. There is a partial similarity to these studies mentioned in our study. With the effect of bacteria, the beginning of flowering and harvesting time was detected earlier or later than the control, and this period even varied among bacteria.

In a field study in Egypt, three different biofertilizers were applied to the *Dahlia pinnata* var. Moonlight plant as single, double and triple applications. The highest flowering time was obtained with BİOgen (nitrogen fixation) + Phosphorein (phosphate solvent) + Active Dry Yeast (dry yeast) applications (Manoly & Nasr 2008). Increasing positive effects were determined in applications where bacteria were added.

In this study, the effects of biostimulants and biofertilizers on the growth, flowering, and quality of *Gladiolus grandiflorus* L. cv. American Beauty was determined under greenhouse conditions. Four biostimulants and three biofertilizers were applied. The results were obtained that the application of biostimulants and biofertilizers to the soil during planting and two months after planting will give higher vegetative growth, flowering, and quality parameters in gladiolus (Pansuriya et al. 2018).

In this study, the effect of organic fertilizers and biofertilizers on the growth and flowering of two standard carnations (*Dianthus caryophyllus* L.) Raggio-de-Sole and Murcia, when Raggio-de-Sole carnation variety is grown in sand + soil + vermicompost (1:1:1) When grown in (v/v) + inorganic fertilizers + biofertilizers @ 2 g/plant (*Azospirillum* and phosphate-solubilizing microorganisms), maximum plant height, the number of flowers, stem length, flower size, early flowering, the maximum percentage of class A flowers and vase life was obtained. (Bhalla et al. 2007). In our study, the effect of bacteria on

spray carnations is seen differently on these parameters. Some bacteria had an increasing effect on the investigated properties compared to the control group plants and some had a decreasing effect. It is thought that this may be due to the difference in bacterial breeds and carnation cultivars.

In the study conducted on the cultivation of six types of spray carnations in Bolu province, the plants came in 220 days from planting to harvest, and the flower stem length was 64.10-75.60 cm, the flower diameter was 42.08-51.25 mm, the flower stem thickness was 5.08-6.49 mm, the number of knots was 10.00-11.40 pieces, the branch weight was 45.73-73.67 g, and the number of buds varied between 2.70-5.00 pieces (Karadeniz et al. 2020). When the time from planting to harvest is evaluated, it can be said that the carnations inoculated with bacteria are exposed to much warmer conditions and the daylight because they are grown in Antalya, and it can be said that they are harvested in almost half the time of this study in Bolu. As it is understood from our study, other characteristics of the carnations in which bacteria were inoculated, except for the number of flower buds (bud), were below the range of values obtained by the researchers. Here, it was concluded that the number of buds in spray carnations is based on the cultivar trait, but other traits may be caused by the growing area and climate, and bacteria are not encouraging for these traits.

In our study, the bacterial strains inoculated to the root crown in the planting area of carnations were selected considering their specific characteristics, as shown in Table 3. The bacteria applied in many studies were generally selected from the same strains. In our study, it was tested in field and greenhouse conditions and its superiority was determined and was used by selecting from strains that are not frequently encountered in other studies.

Bacteria such as *Azospirillum, Acetobacter, Herbaspirillum, Azotobacter,* and *Azoarcus*, as root bacteria and plant growth-promoting bacteria, independent the nitrogen in the atmosphere that cannot be used by plants in the soil and turn it into a usable form (Enez 2022). The bacterial groups that solibilize phosphate best are the *Pseudomonas, Bacillus*, and *Rhizobium* families (Seshadri et al. 2000; Antoun 2002). Root bacteria synthesize ACC (1-amino-cyclopropone-1carboxylic acid) deaminase, keeping the ethylene level in the plant low, thus contributing to plant growth (Wang et al. 2000). According to Carrillo-Castañeda et al. (2005), chemical compounds produced by microorganisms around plant roots (in the rhizosphere) increase the presence and uptake of some essential minerals such as iron (Erdem 2013). Hydroxamate and catecholate siderophores produced by rhizospheric bacteria are used by plants. It has been reported that siderophores produced by *Pseudomonas* bind the necessary Fe-III, preventing spore formation of fungal pathogens and eliminating the disease (Montesinos et al. 2002). It has been reported that especially *Azotobacter* and *Pseudomonas* bacteria can be used in agricultural applications to increase product, quality, and yield, to make arid, industrially contaminated soils more suitable for agriculture due to salinity, and in biotechnological studies such as biological control against some plant pathogens (Cornelis & Matthijs 2007; Couillerot et al. 2009). Some bacterial species such as *Bacillus, Pseudomonas, Arthrobacter, Serratia*, and *Stenotrophomonas* stimulate plant growth by promoting volatile organic compounds (Seymen et al. 2019).

Bacteria families that contribute to the development and yield of cultivated plants can be expressed as *Artrobacter, Azoarcus, Azospirillum, Azotobacter, Bacillus, Burkholderia, Enterobacter, Klebsiella, Pseudomonas, Serratia* and *Rhizobia* (Burdman et al. 2000). Recent studies have shown that plant size and flower number and plant quality increase with different PGPR applications in ornamental plants grown in greenhouses and exposed to abiotic stress (Flores et al. 2007; Nordstedt et al. 2020). Positive effects on yield were observed when geranium (*Pelargonium graveolens* L. Herit) plant was inoculated with *Bacillus subtilis* (MA-2) and *Pseudomonas fluorescens* (MA-4) bacteria (Mishra et al. 2010). Plant growth, nitrogen content, and root colonization were increased when *Amaranthus paniculatus* and *Eleusine coracana* plants were inoculated with *Pseudomonas corrugata, P. corrugata* 1, *P. corrugata* 7, and *Azotobacter chroococcum* (Pandey et al. 1999). The highest rooting rate was achieved with the use of *Bacillus megaterium* and *Pseudomonas fluorescens* bacteria in *Rosa canina* cuttings (Kınık 2014). *Bacillus subtilis* bacteria increased root formation and *Pseudomonas putida* bacteria increased the number of leaves in *Ficus benjamina* L. (Sezen et al. 2014). In a study investigating the effects of three different *P. putida* strains and their mixtures on two poinsettia (*Euphorbia pulcherrima*) cultivars, an increase in plant growth and anthocyanin pigmentation was found. The effect of *P. putida* on cyathia number, root volume, number of leaves, and leaf area was significantly affected compared to the control. In addition, *P. putida* played an important role in the coloration of the bracts (Zulueta-Rodriguez et al. 2014). *Rosa damascena* Mill. F6 (*Pseudomonas fluorescens*), LSI19 (*Rhizobium leguminosarum*), and LC4 (*Vibrios vulnificus*) bacterial isolates were effective on shoot and root development, root growth, root length, and root fresh and dry weight parameters in cuttings of the plant (Tariq et al. 2016).

As can be seen from Table 1, KF29A: *Enterobacter ludwigii*, KF31B, *Pseudomanas fluorescens*; KF3B: *Paenarthrobacter nitroguaiacolicus*, KF5A: *Pseudomonas* sp. Strain VG242B and KF63C: *Paenibacillus xylanilyticus* bacteria are all sufficient in terms of nitrogen fixation, while TV126C: *Pseudoalteromonas tetraodonis* bacteria is stronger than the others. Phosphate solubilizing properties of bacteria strains KF3B: *Paenarthrobacter nitroguajacolicus* and TV126C: *Pseudoalteromonas tetraodonis* are absent or weak, respectively. Phosphate solubilizing abilities of other bacterial strains are better (++) than these two strains. Bacteria with ACC deaminase synthesizing power were evaluated as KF3B *Paenarthrobacter nitroguajacolicus* (very good/+++), KF63C: *Paenibacillus xylanilyticus* (moderately good/++), and TV126C *Pseudoalteromonas tetraodonis* (good/+). Except for TV126C: *Pseudoalteromonas tetraodonis* bacteria, which do not perform in the production of siderophores, the performance of KF5A group bacteria strains is good in this feature.

KF63C: *Paenibacillus xylanilyticus* bacterial strain was the bacterial strain that shortened the bud opening, full flowering, and harvest time, and increased the flower diameter and branch weight the most compared to other applications. Since this bacterial strain is strong in terms of nitrogen fixation, phosphate solubilizing, ACC deaminase synthesis, and siderophore production, it provided earliness in our carnation variety. On the other hand, KF5A: *Pseudomonas* sp. strain VG242B, on the other hand, was the most effective bacterial strain in internode length, as it can fix nitrogen, solubilize phosphate and produce siderophores. It was determined that the plants inoculated with KF31B: *Pseudomonas fluorescens* performed better than the plants treated with some control group bacteria based on the parameters examined, considering that this bacterial strain has good nitrogen fixing, phosphate solubilizing, and sidereophore production properties. The best development was recorded in the number of flower buds and nodes and the length of the flower stalk in carnations infected with TV126C: *Pseudoalteromonas tetraodonis* bacteria strain.

5. Conclusions

While some bacteria have positive effects on some parameters, some bacteria have lower effects both among themselves and compared to the control group plants. Generally, bacteria have had positive effects on the carnation plant.

Bacterial inoculation at each stage of flowering appears to cause a delay of about 10-30 days. Of these, only one bacterial strain (KF63C) provided earliness. Bacterial applications that cause late flowering according to the emergence times of some diseases and pests may be advantageous. In addition, 10-30 days of late flowering can give us an advantage that we are continuing while the product is finished in the market.

New alternatives are sought to minimize the high cost and destructive effect of inorganic fertilizers. To fill this gap, there is a growing demand for the use of organic fertilizers that have the least impact on nature. In light of this increasing demand and commercial input, researches are continuing to reduce costs in many plants. In this study, beneficial bacteria were shown to improve the development and flower quality of the carnation plant.

As seen in previous studies, bacterial application with chemical fertilizers can increase plant growth and flowering. Considering the pollution and high cost of chemical fertilizers that are used too much, it is necessary to increase the use of bacteria and to carry out more studies in this area. Biological fertilization should be adopted as an alternative way of fertilization for carnation plants.

As it is understood from this study, although the carnation plants affected by the inoculated bacteria showed better growth compared to the control group plants and conformed to the cut flower criteria, it should not be forgotten that PGPR will have a positive effect if the appropriate environment and plants are selected, as Çığ et al. (2017) stated.

Acknowledgements

This study was produced from the MSc thesis of the first author. We thank Flora City in Aksu District of Antalya Province in Türkiye for allowing the use of the greenhouse where the study was conducted.

Data availability: Data are available on request due to privacy or other restrictions.
Conflicts of Interest: No conflict of interest was declared by the authors.
Authorship Contributions: Concept: A.Ç., Design: A.Ç., Data Collection or Processing: E.Y.T., Analysis or Interpretation: E.Y.T., A.Ç., N.T., Literature Search: E.Y.T., A.Ç.. Writing: E.Y.T., A.Ç.

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