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Evaluating the Effects of *Bacillus subtilis* Treatment and Planting Depth on Saffron (*Crocus sativus* L.) Production in a Green Roof System

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Abstract. **Given the current urbanization context and rising interest in green roof systems, growing a high-value crop such as saffron crocus in green roof medium could be an opportunity to use the benefits of both the crop and the green roof system; the drainage, aeration, and sand-like texture of green roof media make it suited for saffron production, and the saffron market price could make green roof production commercially viable. Various factors, including plant diseases and planting depth, could affect saffron production. Therefore, this research was conducted to evaluate the effects of planting depth and biofungicide treatments using *Bacillus subtilis* on saffron production in a green roof system. A completely randomized factorial block design was used with planting depth (10 cm and 15 cm) and *B. subtilis* strain QST 713 biofungicide treatments (an untreated control, 15.6×10^9 cfu/L, and 31.2×10^9 cfu/L) as independent variables. In 2019, fresh flower yield, fresh stigma yield, and dry stigma yield were calculated during harvesting, and additional data on flower number, tepal length and width, stigma length, and harvest time were collected in 2020. All variables were analyzed using analysis of variance (ANOVA) with planting depth and biofungicide treatments as fixed effects using R. Fresh stigma yield and dry stigma yield were higher in the 10-cm planting depth in 2019. Results were opposite in 2020: flower number, fresh flower yield, fresh stigma yield, dry stigma yield, and harvest time were higher in the 15-cm planting depth than the 10-cm planting depth. *B. subtilis* treatments did not affect any studied variable in 2020, but in 2019, the higher level of fungicide treatment resulted in lower fresh flower yield and dry stigma yield. There was no effect of biofungicide treatment and planting depth on tepal length, tepal width, and stigma length in both years. This study showed that growing saffron crocus on green roofs is feasible and even resulted in higher yield than field production in many saffron-producing regions and countries. In addition, results indicated that shallow planting might be suitable for annual production, whereas deeper planting could be ideal for perennial production based on the objective. Our findings demonstrated the feasibility of saffron production in the green roof system and suggest further research to develop best management practices.**

As of 2019, ∼55% of the world's population lived in urban areas, and the urban population in the United States was 80% [United Nations Department of Economic and Social Affairs (UN DESA) 2018]. According to UN DESA (2018) projections, the world's urban population could reach 60% by 2030 and 68% by 2050. The use of green roofs could be an innovative technique that helps to grow plants in rooftops, which otherwise remain unused. The green roof is a technology that uses an engineered media that is light in weight and has the capacity to hold the nutrients and water required for plant growth (Ampim et al. 2010; Whittinghill and Starry 2016). Besides using unused space, growing plants on rooftops helps restore lost green space, helps to alleviate urban heat islands, and reduces urban stormwater impacts (Ackerman et al. 2014; Whittinghill and Rowe 2012; Whittinghill and Starry 2016). Green roofs have also been viewed as a method of helping to fulfill local food demand and reduce the energy required for food transportation (Ackerman et al. 2014; Whittinghill and Rowe 2012; Whittinghill and Starry 2016). The potential of agricultural green roofs to contribute to an urban food supply is still being explored and crops best suited to the green roof environment are still being identified. Saffron crocus is one high-value crop with potential for production in a green roof system.

Saffron is known for its color, unique flavor, and aroma, associated with different phytochemical constituents: crocins, picrocrocin, and safranal, respectively (Guclu et al. 2020; Pandita 2021). Saffron is mainly used for culinary purposes (Betti and Schmidt 2007), although it also has several medicinal properties (Bhargava 2011; Razak et al. 2017). Saffron has been a part of the dyeing, cosmetic, and flavoring industries as well (Basker and Negbi 1983; Mzabri et al. 2019). At present, saffron crocus is cultivated in several countries; Iran, Afghanistan, India, and Spain are the top four producers (Golmohammadi 2019). The world's total saffron production was 475 tons in 2018, with Iran alone contributing 85.2% of production, or 402 tons (Golmohammadi 2019). Iran is the leading saffron exporting country (International Trade Center 2020), which presents some political obstacles for western saffron importing countries like the United States. Saffron imports to the United States are also increasing. The United States imported saffron with a value of 15.3 M USD in 2019, mostly from Spain (92.3%) (US Census Bureau 2020). This highlights a considerable opportunity for domestic saffron production by small-scale and urban farmers who are seeking high-value crops to maximize their profit.

The use of novel production technologies like green roofs could enable saffron production in urban areas and solve problems and obstacles in saffron production. Green roof media have water retention capacity within the range of 45% to 65% to avoid water logging at the plant root zone [Forschungsgesellschaft Landschaftsentwicklung Landschaftsbau (FLL) 2002]. According to FLL (2002), green roof media have an air-filled porosity greater than 10% (FLL 2002). Growing saffron crocus in green roof media could avoid precipitation-induced corm rot problems because of the good drainage and aeration of green roof media (Friedrich 2005). Intensive green roof media are made up of two or more components, with silt and clay content limited to 20% (Friedrich 2005) and clay content to 3% to 10% by mass (FLL 2002), making the texture similar to sandy soil. Growing saffron crocus in sandy soil with a low clay content produces higher stigma yield along with higher and larger corm production (Gresta et al. 2010). Too much precipitation and soil moisture are conducive to the growth of pathogenic fungi (*Fusarium* sp., *Penicillium* sp., *Rhizoctonia* sp., etc.), which can cause diseases like corm and root rot (Gresta et al. 2008). This is one of the major causes of reduced saffron yield in some of the saffron-producing countries (Jan and Baba 2018).

In addition to cultivating saffron crocus in well-draining soils, biofungicides may be useful in combating corm rot problems. Research has shown that biofungicide treatment of saffron crocus corms using *Bacillus subtilis* L. can reduce corm rot, which also increases the quality and quantity of saffron produced (Gupta et al. 2020; Kumar 2018; Sharaf-Eldin et al. 2008). *B. subtilis*, a plant growth–promoting rhizobacteria (PGPR), promotes plant growth and development through suppressing plant diseases, improving nutrient uptake, and producing a bio-stimulant (Blake et al. 2020; Hashem et al. 2019; Saharan and Nehra 2011). Several strains of *B. subtilis* were found to promote plant growth: B4 (Park et al. 2013), CKT1 (Walia et al. 2014), and BEB-13bs (Mena-Violante and Olalde-Portugal 2007). Saffron crocus corms can be dipped in the solution of *B. subtilis* (1×10^9 cfu/mL) for 30 min before planting (Gupta et al. 2020; Kumar 2018). Growing media can also be treated with *B. subtilis* (3 mg, 3×10^9 cfu, in 1 L of growing medium) (Prisa 2020). *B. subtilis* may not only help to mitigate corm rot problems but also to promote saffron crocus growth (Akinrinlola et al. 2018).

Choosing an appropriate planting depth might increase saffron production and quality. Planting depth in saffron crocus can affect saffron production and quality by affecting corm growth and development along with leaf and flower size and number (De Juan et al. 2009; Galavi et al. 2008; Kumar et al. 2012; Negbi et al. 1989). Generally, saffron crocus is planted at depths of 8 to 20 cm (Deo 2003; Gresta et al. 2008); however, planting depth depends on purpose and planting methods. For example, saffron crocus corms are planted deeper when grown as a perennial crop than when grown as an annual crop (Gresta et al. 2008). Planting too shallow may expose corms to extremely hot temperatures during summer and freezing temperatures during winter, negatively affecting saffron crocus growth and development (Kumar et al. 2008). Planting corms too deep may decrease the number of daughter corms formed, affecting flower production in the following year (Negbi et al. 1989). The depth of green roof planting media depends on the objective, type of plants being supported, and the ability of the roof to hold the weight (Ampim et al. 2010). Extensive roofs have depths less than 15 cm, and intensive roofs have depths greater than 20 cm (FLL 2002). Both extensive and intensive media depths could support saffron crocus planted in green roof systems using the standard planting depths (10–20 cm) of in-ground field systems (Alonso Díaz-Marta et al. 2006). Nonetheless, information on the effects of planting depth on saffron production in green roof systems is not available to the best of our knowledge.

The production of saffron on green roofs could also help alleviate some of the issues that rooftop vegetable production faces. High installation and maintenance costs are the major barriers to adoption of green roof technologies for crop production (Whittinghill and Rowe 2012). Inclusion of a crop like saffron crocus with a high market price, 6000–18,000 $/kg based on the quality (Skinner et al. 2018), could help rooftop farmers recover that cost faster than production of vegetables alone. The increased cost of labor on a rooftop, which otherwise can be reduced through mechanization in field production, is also a substantial concern for food production. Because most saffron production practices are done manually, there is no increased cost of production on a rooftop, unlike some other crops. Saffron crocus does not need high maintenance, including irrigation and fertilization (Koocheki et al. 2020; Shahandeh 2020), compared with commonly grown vegetable crops like lettuce and tomato. This could decrease the chance of nutrient runoff, a major problem of food production in green roof systems (Whittinghill and Starry 2016; Whittinghill et al. 2016). Saffron crocus flower blooms most heavily in the fall when many food crops have finished production and they remain dormant during summer. Therefore, saffron and vegetable production are not mutually exclusive, and the saffron crocus may take up nutrients that would otherwise be lost from the agricultural roof over the winter. These factors could make saffron crocus an ideal candidate for production in green roof media.

Results from this study will help determine the feasibility of saffron production in green roof systems. Comparison of effects of different levels of biofungicide treatment and planting depths on saffron production will help to determine the best way to treat corm rot and optimal planting depth in green roof media. Performance at shallower depths will also help determine how widely rooftop saffron production could be implemented on existing roofs using this technology. Findings from this research provide initial steps for developing best management practices for producing high-quality saffron in this system.

Material and Methods

This research was conducted at the Herald R. Benson Research and Demonstration Farm at Kentucky State University in Frankfort, KY [USDA plant hardiness zone 6b (USDA Plant Hardiness Zone Map 2012)], starting in Sep 2019. A completely randomized factorial block design was used for the research, with two planting depth treatments (10 cm and 15 cm) and three biofungicide treatments (untreated control, 15.6×10^9 cfu/L, and 31.2×10^9 cfu/L) as the independent variables. There were six treatment combinations and four replicates of each treatment in each of four blocks (from east to west) to reduce the effect of shading from nearby trees and farm structures, making a total of 96 experimental units or green roof modules. The green roof modules (Weston Solutions, Inc., Glastonbury, CT, USA) used were 60.96 cm × 60.96 cm × 20.32 cm, made up of high molecular weight polyethylene (Weston Solutions 2019). Green roof modules were filled with intensive green roof media from GreenGrid® Materials (Weston Solutions, Inc.) to a depth of 20 cm and laid on the ground over landscape fabric. The components and some physical and chemical properties of green roof media used are presented in Table 1. Twenty-four organic saffron crocus corms (Roco saffron, Voorhout, Netherlands) were planted in each module in four rows, with a planting density of 64 corms/m^2. Biofungicide, Serenade® garden fungicide, containing *B. subtilis* strain QST 713 (Serenade Garden Disease Control Concentrate; AgraQuest, Inc., Davis, CA, USA), which has a bacterial spore count of 1×10^9 cfu/mL, was used for corm treatment. Corms receiving fungicide treatments were dipped in a solution of biofungicide and water at the appropriate concentration (15.6×10^9 cfu/L or 31.2×10^9 cfu/L) for 30 min before planting. Jobe's organic fertilizer (4N–4P–4K) (Jobe's Easy Gardener Products, Franklin Avenue, TX, USA) was applied as topdressing at a rate of 11 kg/ha nitrogen as per the recommendation of Alonso Díaz-Marta (2006) at planting and again in Sep 2020. The biofungicide and fertilizer used were listed in the Organic Materials Review Institute.

Data collection and analysis. The final emergence of saffron crocus shoots was recorded in 2020 at the end of the harvesting

Table 1. Initial physical properties of the green roof media properties provided by the manufacturer (GreenGrid, as supplied by Turf and Soil Diagnostics).

Component	Analysis result
Passing US sieve (mm)	
Gravel (9.53) (%)	99.7
Gravel (3.175) (%)	67.9
Gravel (2.0) (%)	44.3
Very Coarse (1.0) (%)	27.6
Medium (0.25) (%)	16.9
Very Fine (0.063) (%)	13.7
Total Sand (2.0–0.063 mm) (%)	30.5
Silt (%)	8.4
Clay (%)	5.4
Bulk density (g/cm^3)	0.89
Air-filled porosity (%)	11
Maximum water retention (%)	52
Water permeability (mm/min)	19.6
pH	6.5
Conductivity (mmhos/cm)	1.4
Organic matter (%)	9.8

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season on 19 Nov 2020. Opened saffron crocus flowers (flowers with visible stigma with even slightly separated tepals) were picked by hand every day when flowering occurred. Flower harvesting season ranged from 6 Nov to 12 Dec in 2019 and 21 Oct to 12 Nov in 2020. The amount of time taken to harvest each plot, referred to as harvest time, was recorded during each harvest. Harvested flowers were processed on the day of harvest whenever possible. In 2020, a combination of factors such as weather, harvest volume, and personnel limitation because of COVID-19 restrictions prevented the same day processing, and thus flowers were stored overnight in a refrigerator at ~4 °C and processed next day for the 21 and 23 Oct harvests. Fresh flower yield and fresh stigma yield of the total harvest were recorded for each experimental unit at every harvest. In 2019, the red stigma and yellow style of each saffron crocus flower were separated carefully by hand. In 2020, the yellow and white style was separated from the stigma just below the joint of the three red-colored stigma threads. During processing, tepal width, tepal length, and the longest stigma length of the first 10 flowers of each plot were also measured in 2020. For tepal length, measurements were taken from the tip of the tepal to the base of the tepal. Tepal width was considered the widest width of tepal, and stigma length was the length from the top of the red part down to the joint of the three red stigma threads (Supplemental Fig. 1). Stigma threads were dried in a dehydrator at 57.2 °C (135 °F) for 30 min, and the dry yield of stigma was measured. Weather data such as average air temperature and total precipitation data were obtained for the Franklin County Mesonet weather station located at the Harold R. Benson Research and Demonstration Farm.

Statistical analyses were performed using R (The R Project for Statistical Computing, Vienna, Austria). Non-normal data were transformed using log10 (fresh flower yield in 2019) and square root (fresh stigma yield in 2019) transformations. All variables (final emergence, tepal width, tepal length, stigma length, flower number, fresh flower yield, fresh stigma yield, dry stigma yield, and harvest time) were analyzed using Type II sum of square ANOVA with planting depth and biofungicide treatments as factors. Comparisons between growing years were not possible because of changes in methodology. Significant differences among treatments were separated using least-square means post hoc test with an alpha level of 0.05.

Results

Weather conditions. In the first growing season of saffron production, from planting through flowering (Sep to Dec 2019), total precipitation was 50.4 cm, mostly in October (23.2 cm) (Fig. 1). Total precipitation in September (1.04 cm) was lower than the other months. In 2020, total precipitation was 122.57 cm. May, August, and October were the months with the highest precipitation, and September and November received lower precipitation (Fig. 1). In 2019, the average temperature of November was 4.3 °C, with minimum temperature of −12.1 °C, which was the major flowering period of the first growing year. In 2020, the average temperatures of January, February, and March (major vegetative and corm formation stage) were 4.2, 3.4, and 10.4 °C, with minimum temperatures of −11, −11, and −4.2 °C (Fig. 1). During the summer months in 2020, average temperatures in June, July, and August were 22.2, 25.3, and 22.9 °C, and maximum temperatures were 32.4, 33.5, and 31.7 °C, respectively.

Final emergence. Emergence data collection in 2019 was disrupted by an early snow fall, so final emergence data could not be analyzed for that year. However, no variations would be expected in 2019, as this was the first year of planting and corm density is the primary determinant of shoot emergence. There was no significant interaction effect of biofungicide treatment and planting depth (F value = 2.468, P value = 0.067) or main effect of biofungicide treatment (F value = 1.194, P value = 0.309) on the final emergence count of saffron crocus shoots in 2020. Planting depth did have a significant effect on saffron corm shoot emergence. Emergence was greater in the planting depth of 15 cm than 10 cm (Fig. 2).

Flower size. Average tepal width, tepal length, and stigma length in different biofungicide treatments and planting depths are presented in Supplemental Table 1. The average tepal width, tepal length, and stigma length of saffron crocus flowers grown in the green roof system was 17.97 mm, 37.31 mm, and 29.41 mm, respectively (Supplemental Table 2).

Flower production. There were no significant interactions between biofungicide treatment and planting depth for fresh flower yield, fresh stigma yield, and dry stigma yield of saffron crocus in 2019 or 2020 (Supplemental Table 3). Similar results were found for flower number and harvest in 2020 (Supplemental Table 3).

Flower number. There was no significant main effect of biofungicide treatment (F value = 0.645, P value = 0.528) on flower number in 2020 (Fig. 3); however, planting depth affected the mean flower number that year. Higher flower numbers were found in

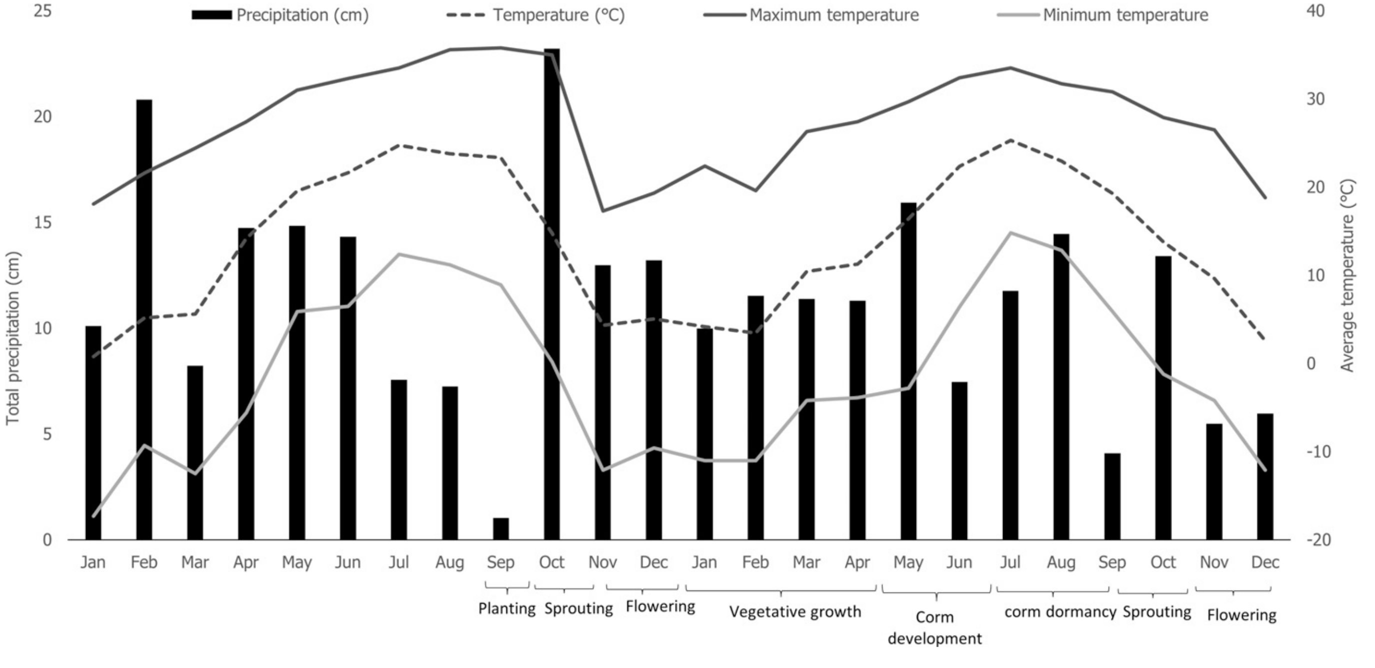

Fig. 1. Observed monthly maximum, minimum, and average temperature, and precipitation in 2019 and 2020 (Kentucky Mesonet 2021) with growth cycle of the corm when planted as a perennial crop shown below the x-axis.

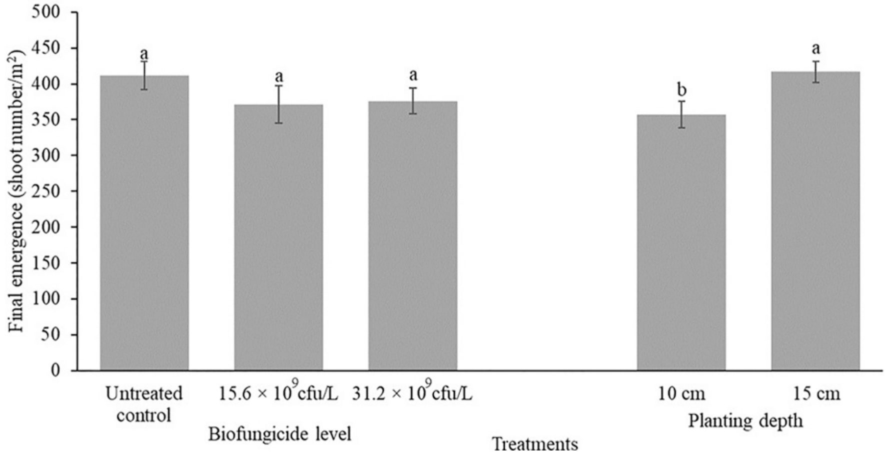

Fig. 2. Mean final emergence for all biofungicide treatments and planting depths in 2020. Letters denote significant differences among treatments at the alpha level of 0.05.

the 15-cm planting depth compared with the 10-cm planting depth (Fig. 3).

Fresh flower yield. No significant effect of biofungicide treatment was seen on fresh flower yield in 2020 (*F* value = 1.526, *P* value = 0.224), but there was a significant effect of biofungicide treatment in 2019. There was a higher mean flower yield in the untreated control and 15.6 × 10^9 cfu/L fungicide treatments than in the 31.2 × 10^9 cfu/L fungicide treatment (Table 2). There was no significant effect of planting depth on flower yield in 2019 (*F* value = 3.004, *P* value = 0.086), although it was significant in 2020 (Table 2). Mean fresh flower yield was higher in the 15-cm planting depth than the 10-cm planting depth.

Fresh stigma yield. Biofungicide treatments had no effect on fresh stigma yield in the study years of 2019 (*F* value = 2.572, *P* value = 0.081) or 2020 (*F* value = 0.552, *P* value = 0.578). However, the main effect of planting depth was significant in both study years, but the results were opposite. The planting depth of 10 cm had a higher fresh stigma yield than the 15-cm planting depth in 2019, whereas the fresh stigma yield was higher in the 15-cm planting depth in 2020 (Table 2).

Dry stigma yield. The main effect of biofungicide treatment was significant in 2019, but not in 2020 (*F* value = 0.254, *P* value = 0.777). The yield of dry stigma was significantly higher in the untreated control and 15.6 × 10^9 cfu/L fungicide treatment than the 31.2 × 109 cfu/L fungicide treatment in 2019 (Table 2). The effect of planting depth on dry stigma had the same pattern as fresh stigma yield (Table 2).

Harvest time. There was no significant effect of biofungicide treatment on harvest time in 2020 (*F* value = 0.602, *P* value = 0.551). Nonetheless, there was a significant effect of planting depth on mean harvest time. The planting depth of 15 cm had a higher harvest time than the planting depth of 10 cm (Fig. 4).

Discussion

The average dry stigma yield of saffron in the green roof system was 0.24 g/m^2 in 2019, and 0.78 g/m^2 in 2020. This yield was higher than the saffron produced in the field condition (0.22 and 0.42 g/m^2 in 2019 and 2020, respectively) in the same location (Poudel P, Whittinghill L, Kobayashi H, Lucas S, unpublished). Saffron yield in the first year was higher than the saffron yield (0.14 g/m^2) found by Gheshm and Brown (2021) in the northeastern United States, despite having lower planting density (64 vs. 150 $corm/m^2$); however, the second-year yield was lower in this study (0.78 vs. 1.35 g/m^2). On comparison of saffron yield to most frequent saffron crocus growing countries, saffron yield in the first year was lower; however, yield in the second year was greater than the saffron yields in 2016 in Iran (0.33 g/m^2), India (0.39 g/m^2), and Greece (0.4 g/m^2), and lower than in Spain (1.4 g/m^2) (Shahnoushi et al. 2020). The average field age in those countries was unknown, but yields likely represent an average across field ages. Generally, saffron yield is lower in the first year but increases gradually for up to 3 to 4 years due to daughter corm formation in subsequent years (Temperini et al. 2009). Therefore, the average yield also depends on the age of the saffron crocus plantings, and even higher yields are possible in the coming years.

Biofungicide treatment. There was no effect of biofungicide treatment on saffron crocus flower growth (tepal width, tepal length, stigma length) or flower production (flower number, fresh flower yield, fresh stigma yield, dry stigma yield, and harvest time) parameters in the second study year (2020) (Table 2). In

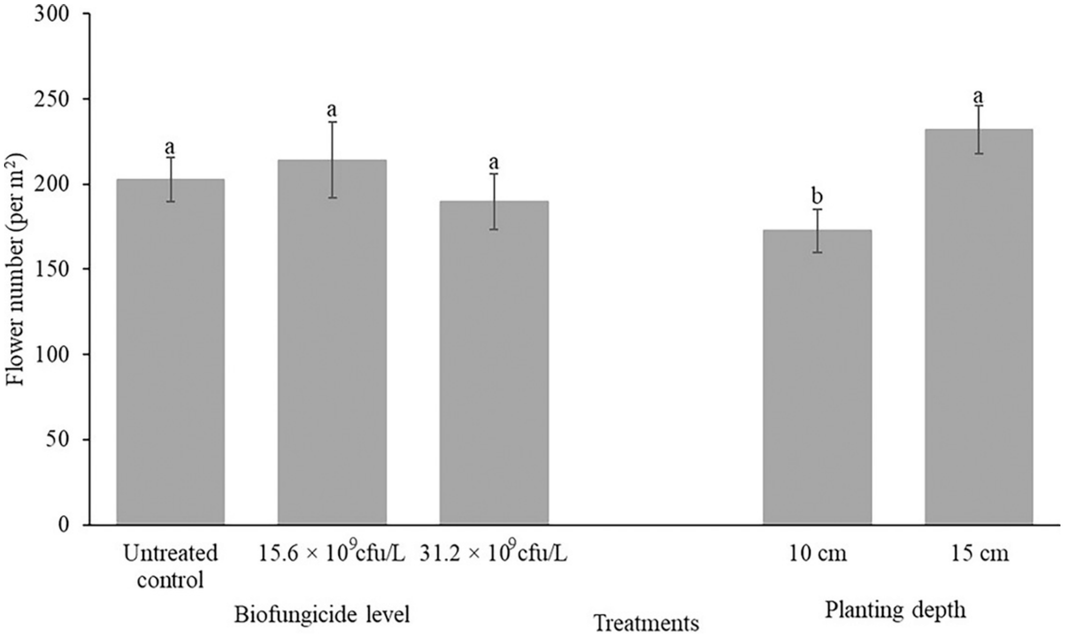

Fig. 3. Mean flower number for all biofungicide treatments and planting depths in 2020. Letters denote significant differences among treatments at the alpha level of 0.05.

Table 2. Mean fresh flower yield, fresh stigma yield, and dry stigma yield for all biofungicide treatments and planting depths in 2019 and 2020. Letters denote significant differences among treatments within a year at the alpha level of 0.05.

	Fresh flower yield (g/m^2)		Fresh stigma yield (g/m^2)		Dry stigma yield (g/m^2)	
Treatments	2019	2020	2019	2020	2019	2020
Biofungicide level						
Untreated control	16.71 a	64.28	1.10	4.68	0.24 a	0.78
15.6 × 10^9 cfu/L	16.57 a	72.14	1.24	5.00	0.29 a	0.80
31.2 × 10^9 cfu/L	9.31 b	58.36	0.99	4.44	0.19 b	0.75
Planting depth						
10 cm	14.37	56.75 b	1.21 a	4.12 b	0.27 a	0.70 b
15 cm	13.97	73.14 a	0.99 b	5.30 a	0.22 b	0.86 a

the first year, higher levels of biofungicide treatment resulted in lower fresh flower yield and dry stigma yield. Because flower buds were already present in the corms before soaking in biofungicide solution and the biofungicide product is intended for use as a foliar spray or soil drench, other "inactive" components of the formulation may have been phytotoxic to the corms at the high concentration, damaging flower buds, resulting in lower saffron production in the first growing year with a higher level of biofungicide treatment. There are several possible reasons that the treatments had no effect after the first year. First, the biofungicide may have had poor or unsuccessful colonization of the rhizosphere. The effect of biofungicide treatments using PGPR on plant growth depends on the successful colonization of the rhizosphere by such bacteria (Blake et al. 2020; Podile et al. 2014). Successful colonization depends on many biotic and abiotic factors, including plant-microbe interactions, the genotype of the plant and bacteria, soil moisture, soil type, pH, organic matter content, and soil temperature (Albareda et al. 2006; Arora et al. 2010; Basu et al. 2021; Benizri et al. 2001). Measuring colonization of bacteria was out of the scope of this study; however, this could help to better understand the result.

Davies and Whitbread (1989) stated that root colonization and growth stimulation have complex relations, so it is hard to predict growth promotion with PGPR applications. This is another possible explanation for lack of effect of biofungicide in this study. In Davies and Whitbread's (1989) study, *Pseudomonas fluorescence* failed to promote radish growth. Similarly, Sharaf-Eldin et al. (2008) did not find a difference in total flower number per corm compared with the untreated control, when *B. subtilis* strain FZB24® was used to treat saffron crocus corms and treat soil after planting. Nevertheless, in the same study, they found higher fresh stigma yield from the first flowers from the *B. subtilis*–treated corms. Sharaf-Eldin et al. (2008) also found lower total fresh stigma yield per corm from *B. subtilis*–treated corms than the untreated control corms. The lower fresh flower yield and dry stigma yield in a higher level of *B. subtilis* compared with the untreated control and lower level of biofungicide treatment in the first growing season (Table 2) was otherwise unsupported by the literature. Several other studies have shown positive effects of *B. subtilis* when applied as a corm treatment (Kumar 2018) on saffron crocus flower number or when *B. subtilis* was applied directly to the growing medium on flower number per corm and stigma length (Prisa 2020), and leaf area per plant and daughter corm weight (Al-Ahmadi et al. 2017).

Planting depth. The similarity of flower yield between the 10-cm and 15-cm planting depths in the first year of harvesting (2019) (Table 2) is supported by Negbi et al. (1989); flowering in saffron crocus is unaffected by planting depth. In contrast, some studies have shown an effect of planting depth on stigma length, flower number, fresh flower, fresh stigma, and dry stigma yield in some shallow planting, which increased production metrics (De Juan et al. 2009; Nazir et al. 2000), and in others deeper planting increased production metrics (Galavi et al. 2008; Yildirim et al. 2017). In this study, planting depth did not influence studied flower growth metrics: tepal width, tepal length, and stigma length (Supplemental Table 1). Nonetheless, flower production metrics were affected by different planting depths. Gresta et al. (2016) also did not find an effect of treatment studied on stigma length, and average stigma length was 29.7 mm, which is similar to stigma length found in this study. However, various studies have found a difference in stigma length among different planting depths. Cardone et al. (2020) found an effect of soil types and properties on stigma length (28.8 to 37.7 mm). Similarly, significant effect of population and growing year (23–35 mm, Baghalian et al. 2010), ecotypes (31–39 mm, Bayat et al. 2016), and chemical and manure application (19–30 mm, Amiri 2008) was found in the stigma length. Amiri (2008) also found a high correlation of stigma length with yield (r = 0.743) compared with the flower number (0.570). Higher fresh stigma yield and dry stigma yield were found in the shallow planting depth (10 cm) than the deeper planting depth in the first growing year; however, results were opposite in the second growing year for flower number, fresh flower yield, fresh stigma yield, and dry stigma yield when grown as a perennial crop. Higher fresh and dry stigma yield from the 10-cm planting depth than the planting depth of 15 cm in the

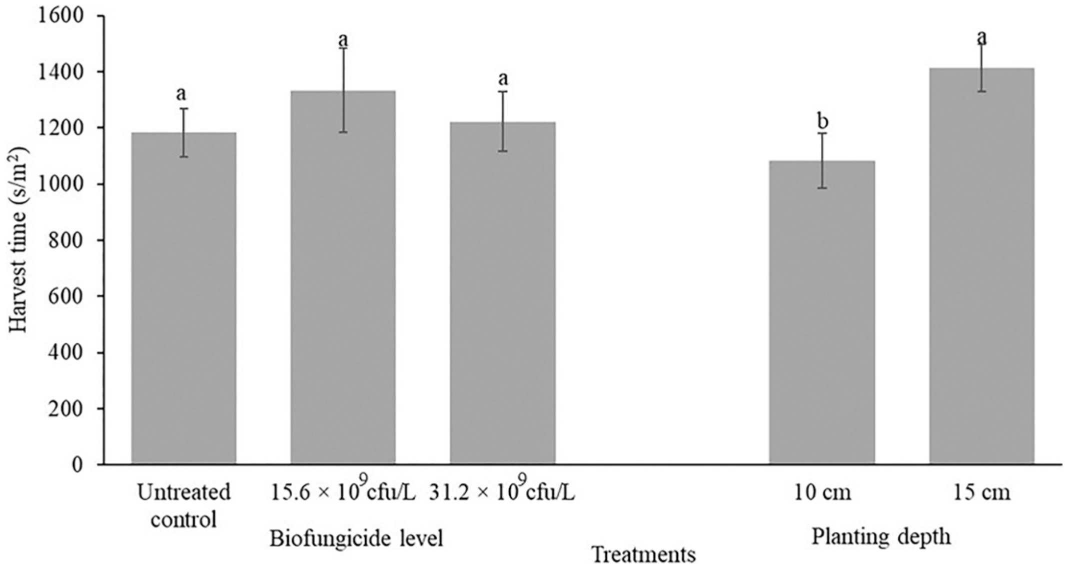

Fig. 4. Mean harvest time of flowers for all biofungicide treatments and planting depths in 2020. Letters denote significant differences among treatments at the alpha level of 0.05.

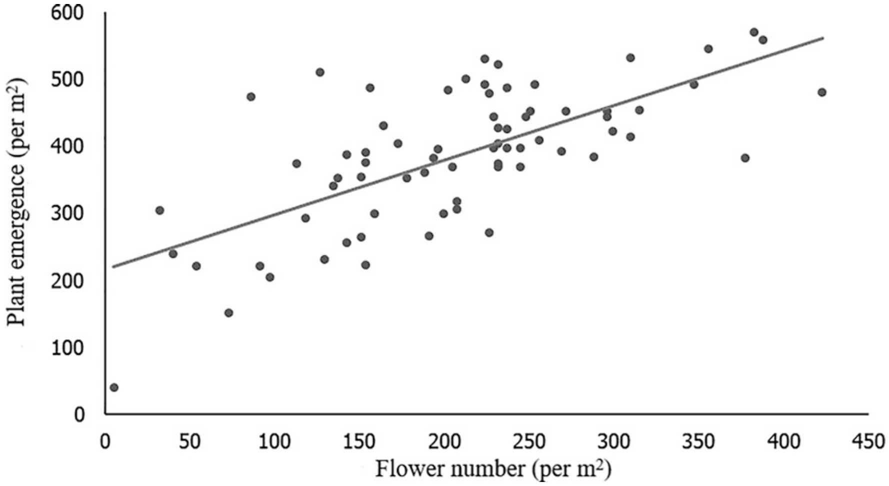

Fig. 5. Correlation of flower numbers with final emergence in 2020.

first growing year was supported by De Juan et al. (2009). They found higher flower numbers and fresh stigma yield from a 10-cm planting depth than a 15-cm planting depth in both the first and second growing seasons. As flower buds were present already in the corms during planting, saffron crocus plants might have used more energy to emerge from the deeper depth compared with the shallow depth, resulting in higher saffron fresh and dry yield in the 10-cm planting depth compared with the 15-cm planting depth in the first growing year. Similar flower yield between the two planting depths could be due to equal flower number and individual flower yield, as flower bud was formed in the corm before planting; however, we did not take flower number data in the first year.

Higher flower numbers, fresh flower yield, fresh stigma yield, and dry stigma yield in the 15-cm planting depth in the second growing season can be linked with higher emergence in 2020 (Fig. 2). Higher shoot emergence also suggests the possibly higher daughter corms formation in the 15-cm planting depth compared with 10 cm. However, we did not measure the daughter corms number data in any year of production. Flower number and final emergence are correlated with each other ($r = 0.69$, Fig. 5). In addition, high fresh yield of flowers from 15 cm was also supported by higher flower number (Fig. 3). This result is supported by the findings of Galavi et al. (2008) and Yildirim et al. (2017). Galavi et al. (2008) found an increase in flower number and stigma length when the planting depth was increased from 10 to 15 cm. Likewise, Yildirim et al. (2017) found an increase in flower number and fresh stigma yield in the second year in a planting depth of 15 cm than in the shallower planting depths (5 cm) and linked the higher flower number with lower variability of temperature at that depth. Shallower planting exposes corms to higher temperatures in summer and freezing temperatures in winter, which could affect saffron crocus growth (Kumar et al. 2008). Corms at shallow depths have a chance of freeze-induced damage with increased electrolyte leakage and decreased corm growth (Koocheki and Seyyedi 2019), which could lead to lower flower numbers, fresh flower yield, fresh stigma yield, and dry stigma yield. Minimum temperatures in Nov 2019, Jan 2020, and Feb 2020 were −12.01, −11, and −11 °C (Kentucky Mesonet 2021), which might have caused freezing injury, affecting plant and corm growth and development in the shallow planting depth. Studying the minimum media temperature at different media depths during the winter period might confirm if there is any chance of freeze injury to the corms planted at shallow depths. This study was carried out at ground level using green roof media, hence growing saffron crocus on the rooftops of buildings in urban areas might reduce the chance of freezing-induced problems in shallow-planted corms because the temperatures on rooftops are higher than at ground level (Griffith and McKee 2000). However, it may expose saffron crocus to higher temperatures during the summer season, as maximum temperatures were 32.4, 33.5, and 31.7 °C in June, July, and August, respectively. Further, saffron crocus corms move up (about 2 cm) toward the soil surface every growing season (Gresta et al. 2008), so shallow planting increases the chance of corm exposure to the cold weather in winter and very hot temperatures in summer each year, which might affect saffron crocus growth (Koocheki and Seyyedi 2019; Kumar et al. 2008). Reduced growth in shallow plantings could lead to lower stigma yields than deeper plantings the next year.

The higher harvest time of saffron crocus from the 15-cm planting depth compared with the 10-cm planting depth in the second growing season (Fig. 4) can be linked with higher flower numbers in the 15-cm planting depth (Fig. 3). Higher fresh and dry stigma yield from the 10-cm planting depth in 2019, but higher flower number, fresh flower yield, fresh stigma yield, dry stigma yield, and harvest time, indicates that the shallow planting at 10 cm might be suitable for annual production practices; however, possible freezing damage on the 10-cm depth could affect the daughter corm production that is harvested in the following summer, sorted out mainly based on size and replanted in the following season (Alonso Díaz-Marta et al. 2006). With possible less freeze damage and higher yield of saffron, deeper planting at 15 cm is suitable for daughter corm production and perennial saffron production. This study also indicated that saffron can be produced in both extensive (growing media depth less than 15 cm) and intensive (growing media depth greater than 15 cm) green roof systems based on the objective. For example, if saffron crocus is intended to be grown as an annual crop and larger saffron yield is expected in the first year, then saffron crocus could be planted in an extensive green roof; nonetheless, daughter corm size might be small with risk of freeze damage during winter. On the other hand, if the objective was to plant saffron crocus as a perennial crop, then intensive green roofs might be a better choice, as they could result in higher yield in coming years despite having lower yield in the first growing year. Nonetheless, economic analysis of saffron production could help to determine the economic sustainability of saffron production in extensive and intensive green roofs.

Conclusion

Growing saffron crocus in green roof media is possible. People especially in an urban area could produce saffron on their rooftop, back yard, etc. using green roof media. Although biofungicide used in this study was not effective on promoting saffron production in green roof production, shallow planting showed increased yield parameters in the first year of planting and deeper planting in the second season. Future research is needed to further explore the economic sustainability of saffron production in green roofs. Similarly, additional research is required to examine how growing saffron crocus in green roofs affects the known benefits of green roofs, including reduction of urban heat island effect and storm water retention. Promoting saffron production in urban areas might help to fulfill some domestic saffron demand, using unproductive spaces like rooftops.

Further research on *B. subtilis* and planting depth on number and size of daughter corm formation would help to better understand the effects of those treatments on saffron production the year after treatment. Moreover, research on other production practices, mother corm size, and planting density suited to green roof saffron production is needed to optimize the production capacity of green roof systems. Along with producing saffron on green roofs, green roof's known benefits, like the ability to retain storm water, mitigating urban heat island effects, and negative affects like nutrient runoff, also should be evaluated for the sustainability of saffron production in green roof systems.

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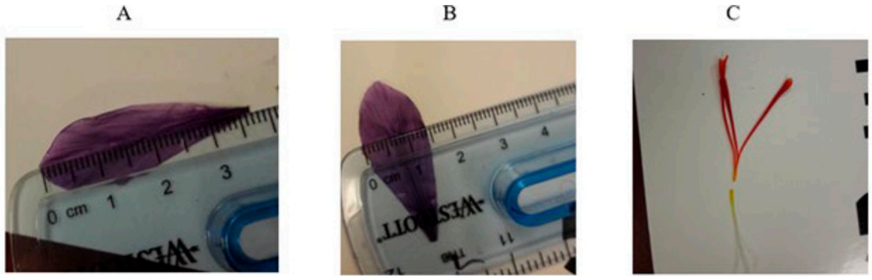

Supplemental Fig. 1. Showing measurement of tepal length (**A**), tepal width (**B**), and stigma length (**C**).

Supplemental Table 1. Analysis of variance results for 2-way interactions between biofungicide treatment and planting depth and main effects for tepal width, tepal length, and stigma length.

Variable	Two-way interaction	Biofungicide treatment	Planting depth
Tepal width	F value = 0.208, P value = 0.812	F value = 0.863, P value = 0.426	F value = 1.205, P value = 0.276
Tepal length	F value = 1.564, P value = 0.215	F value = 0.132, P value = 0.876	F value = 0.184, P value = 0.669
Stigma length	F value = 0.412, P value = 0.664	F value = 1.218, P value = 0.301	F value = 0.037, P value = 0.847

Supplemental Table 2. Mean tepal width, tepal length, and stigma length of saffron crocus flower for all biofungicide treatments and planting depths in 2020.

Treatments	Tepal width (mm)	Tepal length (mm)	Stigma length (mm)
Biofungicide level			
Untreated control	17.77	37.51	29.29
15.6×10^9 cfu/L	18.17	37.84	29.85
31.2×10^9 cfu/L	17.89	37.31	29.10
Planting depth			
10 cm	18.07	37.45	29.44
15 cm	17.80	37.64	29.38

Supplemental Table 3. Analysis of variance results for two-way interactions between biofungicide treatment and planting depth for fresh flower yield, fresh stigma yield, dry stigma yield, flower number, and harvest time.

Yr	Variable	Biofungicide treatment-planting depth interaction
2019	Fresh flower yield	F value = 1.909, P value = 0.153
	Fresh stigma yield	F value = 0.234, P value = 0.791
	Dry stigma yield	F value = 0.357, P value = 0.701
2020	Flower number	F value = 2.658, P value = 0.077
	Fresh flower yield	F value = 2.019, P value = 0.141
	Fresh stigma yield	F value = 0.552, P value = 0.578
	Dry stigma yield	F value = 1.047, P value = 0.356
	Harvest time	F value = 2.168, P value = 0.122