Research Article

Effect of Corm Size, Row Spacing and Plant Distance on the Flower Production of Saffron (*Crocus sativus* L.) Under Rainfed Conditions of Quetta, Balochistan

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Abstract | Saffron flower production and stigma yield with respect to corms size and spacing were determined by conducting two experiments at Pakistan Agricultural Research Council (PARC) center based at Quetta, Pakistan, during 2015-2016. The experiments were carried out under rainfed Conditions using Randomized Complete Block Design (RCBD). In the one experiment, three treatments (T1 to T3) of corm sizes viz: large (8-11 g), medium (5-7 g) and small (less than 4 g) were used. In the second experiment, five treatments (T1 to T5) of row to row and plant to plant distance/spacing viz: T1= 15 cm, T2= 20 cm, T3= 25 cm, T4= 30 cm and T5= 35 cm were used. The results indicated that only large corms produced the flowers while medium and small corms failed to produce flowers. Large corms emerged out in less (46) days, survived higher (95.33 %) with a maximum leaf height (25.32 cm), higher number of leaves/plant (7.26) and higher number of daughter corms (6.33). However, the saffron stigma fresh and dry weight produced by large corms was recorded as 0.0354 and 0.0075g, respectively. Further results indicated no significant differences among the parameters recorded for the row to row and plant to plant spacing experiment except for total flower production where the highest production (162.3) was recorded in 15 cm spacing. The results also indicated that corms with a weight of 8-11 g are suitable for sowing while below 8 g are not suitable for flower production of saffron.

Received | April 02, 2021; **Accepted** | December 19, 2022; **Published** | May 04, 2023
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Citation | Ahmed, M.F., A. Hanan and S. Ahmed. 2023. Effect of corm size, row spacing and plant distance on the flower production of saffron (*Crocus sativus* L.) under rainfed conditions of Quetta, Balochistan. *Sarhad Journal of Agriculture,* 39(2): 396-405.
DOI | https://dx.doi.org/10.17582/journal.sja/2023/39.2.396.405
Keywords | Crocin, Perennial, Propagation, Stigma and Triploid

Introduction

Saffron (*Crocus sativus* L.) is a perennial crop, belongs to family Iridaceae which produces flowers in autumn season (Fernandez, 2004). It is a perennial stemless plant having sterile flowers so it is propagated by daughter corms only (Hosseinzadeh *et al*., 2009). It is a well-known spice and is famous as one of the oldest and an expensive spice in the world. It is gathered by picking up the red dried stigma out of the flower of *Crocus sativus* (Ghorbani and Koocheki, 2006). Although the origin of cultivation of saffron has been found in Iran, parts of Turkey and Greece but now is being grown efficiently in Spain, Italy,

France, Switzerland, Israel, Balochistan (Pakistan), Azerbaijan, China, Egypt, United Arab Emirates, Japan, Afghanistan, Morocco, Iraq. Currently, it has been reported that saffron can be profitably cultivated in Australia, Mexico, New Zealand and Argentina (Jalali-Heravi *et al*., 2010; Bakhtavari *et al*., 2011). Nevertheless, the countries which share the major portion in the production of saffron are Iran, Spain, Turkey, Morocco, Greece, India, Kashmir, Switzerland and Australia. Among these saffron producing countries Iran captures the top position by producing about more than 90% of the world's total production (Fernandez, 2004). The world's total production of dried saffron is estimated to around 250-300 tons annually (Kafi *et al*., 2018). Saffron is the most expensive herb spice in the world, once sold as near as the cost of gold as one kg of this spice costs around 1000 to 2000 US dollars (Kafi *et al*., 2018). According to Schmidt *et al*. (2007) in order to obtain a single kilogram of saffron nearly 150,000 to 220,000 good quality flowers are required. In another study, Kafi *et al*. (2018) determined that about 250,000 to 300,000 flowers are needed to collect one kilogram of saffron stigma. Its cultivation is therefore of great economic significance.

In saffron cultivation, among other factors the important factor is the selection of appropriate corms upon which the flowering capacity of the plant relies very greatly. Results of a 5-years experimental study on the effects of corm size on its capability to regenerate and produce flower surmised that both flower's yield and corm production depend on the initial corm size during planting (Kaushal and Upudhyaya, 2002). The propagation season of saffron corms begins in July and continues till mid of September. Nonetheless, some studies recommend that the early spring is the best suitable season for achieving vigorous growth of saffron (Naderi *et al*., 2012). Saffron initiates flowering in almost mid of October and depending upon the temperature, continues till the end of November (Nehvi and Salwee, 2010).

Several studies also demonstrated that spacing usually has a positive effect on the saffron stigma yield. Behnia and Mokhtarian (2010) investigated the effects of line spacing on saffron yield and determined the maximum yield of saffron has been obtained when 10 corms were sown with 30 cm spacing between rows. The closer densities of saffron plantation had increased the yield for successive three years plantation (Kochaki *et al*., 2012). The corms sown at an inter-row distance of 20 cm and intra-row of 10 cm would increase the yield of saffron for one to two years while during the third year these become congested and become stunted so necessary to be harvested and resown separately (Ait-Oubahov and El-Otmani,1999). Similarly, Andabjadid *et al*. (2015) stated that by keeping a distance of 7-10 cm between corms appeared to be the most suitable row spacing for getting higher yield of flowers and corms.

The chemical constituents of saffron for decades have been studied and reviewed. Saffron consists of nearly150 volatile and aroma-secreting compounds, comprehensive of 40-50 volatile compounds with strong odour (Srivastava *et al*., 2010). However, the color of saffron can be attributed to crocin while aroma and bitter taste to picrocrocin and safranal (De Juan *et al*., 2009).

In Pakistan, saffron cultivation has been reported by several authors such as Coskun *et al*. (2017), Bashir *et al*. (2019) and Khalili *et al*. (2020). However, no published work based on scientific data has been undertaken in the region regarding its cultivation on a large-scale basis. Nevertheless. preliminary studies on its cultivation in Quetta, Dasht and Mastung districts of Balochistan has given promising results, which shows potential of saffron in the area (personal information). Geographically, Balochistan is the largest province of the Pakistan, consisting of about 44% of its land area (Tareen *et al*., 2010). Balochistan is located in the South-Western part of Pakistan and located between the North latitudes of 24°-53° and 32°-05° and the East Longitudes of 60°-52° and 72°-18° (Hughes, 1977).

Although the climatic conditions of most parts of Balochistan, particularly, highlands are favorable e for the growth of saffron, nonetheless, not much scientific and consolidated effort has been undertaken to make it a successful story in the country. Till now. not much scientific data on its cultivation practices has been gathered in Pakistan; therefore, experiments were carried out on different corm sizes of saffron to determine whether different corm sizes can have a significant impact on the development of saffron flowering and stigma production. Moreover, experiment was also undertaken on the impacts of planting saffron corms in different spaces between plants and rows. on the yield and production of

saffron flowers and stigma. The information collected would enhance the knowledge for the improvement of growth and yield of saffron in suitable parts of Pakistan.

Materials and Methods

Plant material

To evaluate the yield of saffron (stigma) with respect to flowers and other related parameters for saffron production, two experiments were designed; one on effects of different corm sizes and the other on the effects of row to row and plant to plant distance. In both the experiments, a local variety of saffron was used, which was collected from the saffron farm located in Mustang district. These bulbs were planted at the then Arid Zone Research Centre (AZRC) Quetta during 2004-05 where these bulbs were repeatedly replanted and increased for a few years. In the first experiment, corms of three different sizes (large, medium and small) of saffron corms were used while in the second experiment (spacing experiment), saffron corms of similar size and weight were used. Before conduction of the experiment, the experimental block was divided into different plots and soil samples were taken from each plot for soil analysis (Table 1) while weather data of the months from sowing to harvest of flowers and bulbs were collected (Table 2).

Table 1: *Soil analysis of the experimental area.*

Depth of soil 0-15 cm	Nos. of samples			
Parameters	S-1	S-2	S-3	Average
pH (Alkaline-Acidic)	7.9	7.8	7.9	7.9
EC (dS m-1)	1.39	1.22	1.25	1.28
OM (%)	0.6	0.3	0.2	0.36
Nitrite (%)	0.003	0.001	0.001	0.0018
SOC (%)	0.34	0.17	0.11	0.20
Depth of soil 15-30 cm	F-1	F-2	F-3	Average
pH (Alkaline-Acidic)	7.9	7.8	8	7.9
EC (dS m-1)	1.17	1.18	1.24	1.19
OM (%)	0.2	0.5	0.1	0.26
Nitrite (%)	0.0001	0.0025	0.0005	0.0010
SOC (%)	0.11	0.29	0.05	0.15

Design of experiments

Both the experiments were carried out in a randomized complete block design (RCBD) and replicated three times. The sowing of bulbs was done on 7th September, 2015., In the first experiment, three treatments (T) i.e., bulb sizes were set. The treatments were: (1) 8-11 g (2-2.5 cm in dia.), (2) 5-7 g (≤ 2 cm dia.) and (3) < 4g (< 1 cm dia.). Each replication contained 6 lines (4-meter-long) with a total size of 6m^2 (1.5X4 m) plot in each replication. In this experiment, a distance of 25×25 cm for both plants and rows were maintained in each treatment with 140 bulbs in each replication.

Table 2: *Weather data of experimental area at BARDC, Quetta during 2015-16.*

Months	Relative humidity (RH)(%)	Temperature (°C)		Rainfall (mm)
		Min	Max	
August, 2015	11.09	20.3	35.52	0
September, 2015	12.73	14.90	31.02	0
October, 2015	14.32	13.91	27.49	1.52
November, 2015	21.1	5.53	21.34	25.11
December, 2015	20.45	0.53	14.76	1.77
January, 2016	20.35	1.8	16.22	53.12
February, 2016	17.75	1.1	16.64	0
March, 2016	24.83	8.13	19.80	69.49
April, 2016	15.46	12.30	26.32	1.26
May, 2016	11.48	18.30	33.88	4.06
June, 2016	8.75	16.43	28.32	23.86

In the second experiment, there were five treatments (T1 to T5) on the plant to plant and row to row distance. The treatments were; T1= 15 cm, T2= 20 cm, T3= 25 cm, T4= 30 cm and T5= 35 inches that made a combination of 15×15, 20×20, 25×25, 30×30 and 35×35 distance for both rows and plants. This experiment was consisted of uniform sized saffron bulbs (2 to 2.5 cm dia.) and with a weight of 8-11 g. The experiment was also conducted in randomized complete block design and replicated three times. The plot size of each replication was kept at 6m^2.In this way, 495, 140, 96, 65 and 44 bulbs were planted from T1 toT5, respectively and total 840 bulbs were planted in this experiment. The sowing of corms was done on 7th September, 2015. Data were collected when saffron bulbs started to emerge out of the soil.

Data collection

The data were collected on different parameters such as how many days taken by bulbs to emerge, percentage of survival of corms, leaf height (cm), number of leaves plants^{-1}, flowering days, weight of fresh and dry flowers (g), weight of fresh and dry stigma (g), weight of fresh and dry stamens, fresh and dry length of stigma (mm), total flowering period and

flower yield/ha. When flowers began to bloom from the corm leaves, these were collected early before sunrise and were taken to the laboratory for weighing, drying, and stigma and stamens separation from the flowers. The fresh weight of flowers and stigma and stamens was recorded in grams on a digital electronic balance machine daily. Later, separated stigma and stamens were dried under the shade in the laboratory. When both stigma and stamens became fully dried their dry weight was also recorded.

Antibacterial activity

Methodology: The air dried and powered stigma and stamens of saffron (1g) were extracted with ethanol using the rotary apparatus. The extract (100 mg/ml) was evaporated and then extracted in ethanol, and then kept in small sterile bottles under refrigerated conditions until used. The stigma and stamen sample extracts were separately tested for antibacterial activity. Two strains of bacteria including gram positive, *Staphylococcus aureous* and gram-negative, *Escherichia coli* ATCC 25922 were used for tests. The pure strains were obtained and identified from the Institute of Microbiology, Agriculture University Faisalabad, Pakistan. Using agar solution (Lab-Lemco' powder 1.0 gm/lit., Yeast extract 2.0 gm/lit., Peptone 5 gm/lit., Sodium chloride 5.0 gm/lit., Agar 15 gm/lit.) (Oxoid, Hampshire, UK) at 37 °C the bacterial strains were cultured overnight. The extracts were tested using disc diffusion method for their antibacterial activity. Microorganisms were tested in 100 µL of suspension on nutrient agar medium containing 10^7 colonies-forming units (CFU/mL) of bacterial cells. The saffron samples were loaded in the wells developed in the agar plate which was inoculated with the tested microorganisms previously. As a negative control the well without samples were used. A rifimpacin (Oxoid, Hampshire, UK) (30 µg/well) were used as a positive reference for bacteria, to relate the sensitivity of isolate in analyzed microbial species. After two hours at 4 °C and 18 h at 37 °C plates were incubated for bacteria strains. The evaluation of antibacterial activity was measured the basis of growth size of inhibition zones in millimeter comparing to control.

Results and Discussion

Impact of bulb size on the production of saffron flowers

Days to emergence: The results demonstrated that saffron bulb sizes significantly affected the days to emergence of saffron bulbs (F= 16; P ≤ 0.004). The large sized bulbs took the shortest days (46) in emergence whereas, the highest days (50) were acquired by small sized bulbs in emergence (Figure 1). Likewise, survival (%) of different size of bulbs significantly affected by corm sizes (F= 64.03; P ≤ 0.0001). The higher (95.33%) survival (%) was recorded in large sized bulbs whereas, the lower (76.67%) survival (%) was recorded in small sized bulbs (Figure 2).

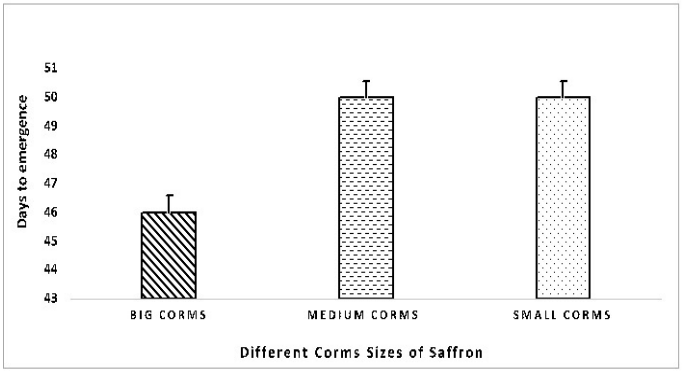

Figure 1: *Effect of bulb size on days to emergence of saffron.*

Figure 2: *Effect of bulb size on survival percentage of saffron bulbs.*

Plant height

The results indicated that bulb size had significantly affected the plant height of saffron (F= 13.51; P ≤ 0.006). The plant height significantly improved with the enlargement in bulb size. The plants height of large sized bulbs was observed to be the highest while the lowest was recorded in small sized bulbs with mean values of 25.32, 21.98 and 19.50 cm leaves height for large, medium and small sized bulbs, respectively (Figure 3).

Number of leaves per plant

Different corm sizes significantly affected the number of leaves/plant (F= 309.02; P ≤ 0.001). The mean maximum number of leaves/plants was recorded by large sized corms (7.3 leaves/plant) followed by medium (4.3 leaves/plant) and the minimum number by small sized corms (2.6 leaves/plant). Increased size of corm increased the number of leaves (Figure 4).

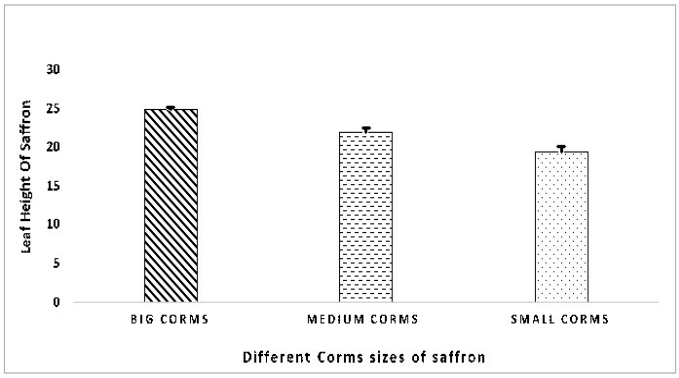

Figure 3: *Effect of corm size on Plant height of saffron (Correct) Leaf height may be changed with plant height.*

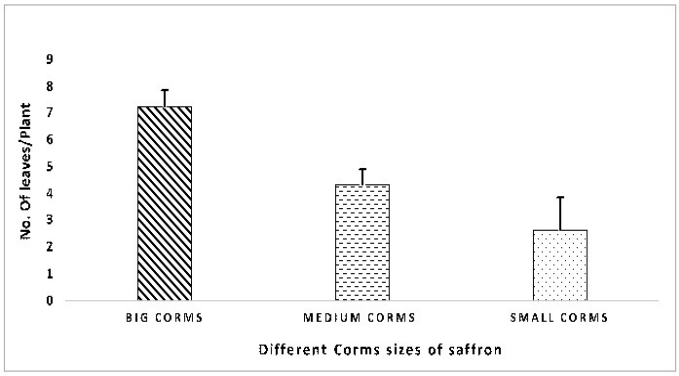

Figure 4: *Effect of bulb size on saffron No. leaves plant*$^{-1}$.

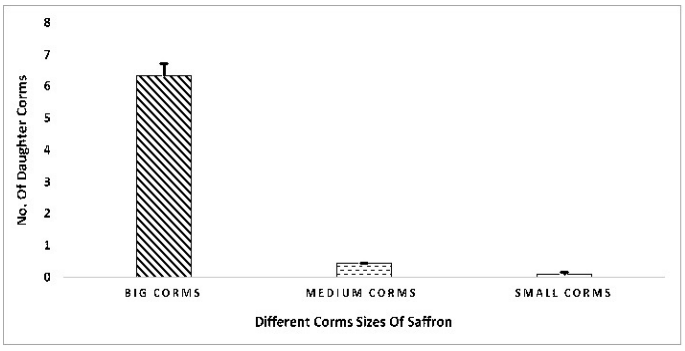

Figure 5: *Effect of bulb size on saffron No. of daughter corms.*

Number of daughter corms

The number of daughter corms improved significantly with the improvement in bulb size (F= 45.29; P ≤ 0.0001). The mean value demonstrated that highest number of daughter corms (6.33) was produced by large sized bulbs, which was significantly higher than the medium and small sized bulbs whereas, the lowest number of daughter corms was produced by small sized bulbs (0.101) (Figure 5).

Different flower yield components

The data record of corm size showed that only large sized bulbs produced flowers while medium and small sized bulbs could not produce flowers, resulting in failure to produce saffron stigma and stamens. Consequently, data could not be analyzed as only large corms data is available. However, the total flowering period of large size corms was recorded as 21 days with 60.67% corms bloomed with the overall yield of flowers (272.67), a single flower fresh weight (0.417 gm) and dry weight (0.0469 gm.), fresh (0.0354 gm) and dry stigma weight (0.0075 gm), a single fresh (24.492 mm) and dry stigma size (16.095 mm) and fresh (0.033 gm) and dry stamen weight (0.0093 gm).

Antibacterial activity

The results showed that *C. sativus* stigmas and stamens can be a possible source of new antimicrobial agents. The stigma and stamen of saffron were screened for their *in vitro* antibacterial activity against the two strains of bacteria i.e., gram positive, *S. aureous* and gram negative, *E. coli* by the disc diffusion method and the results are given in Table 1. The antibacterial activities results were recorded as zone of inhibition in mm for all the samples. The Antibacterial activity of stamen was lower than that of stigma extracts. The Sample stigma 3 showed the strong antibacterial activity against *E. coli* while sample stamen 5 and stamen 6 exhibited strong activity against *S. aureous*. The stigma extract showed a maximum zone inhibition against *S. aureous* while the positive standard drug control Rifimpacin antibiotic very strongly inhibited the growth of *E. coli*. (Table 3).

Table 3: *Antibacterial activity of saffron stigma and stamen extracts against bacterial strains.*

Serial #	Sample name	Antibacterial activity	
		E. coli **(Zone of inhibition in mm)**	***S. aureus*** **(Zone of inhibition in mm)**
1	stigma 1	21	26
2	stigma 2	18	27
3	stigma 3	26	24
4	stamen 1	23	15
5	stamen 2	19	28
6	stamen 3	18	29
7	Rifimpacin	40	35

DPPH (organic chemical compound 2, 2-diphenyl-1-picryl hydrazyl) scavenging activity

The antioxidant activity of saffron stigma and stamen were evaluated by using DPPH radical scavenging assay. Results demonstrated that the extracts of saffron stamen showed the highest radical scavenging activity (67.76%). The scavenging activity of saffron stigma extracts with values of (64.62, 67.13, 61.10%) and stamen extracts values of (63.88, 67.75, 64.65%, respectively) (Table 4).

Table 4: *Antioxidant biological activities like TPC, TFC and DPPH of saffron stigma and stamen extracts.*

S. No.	Sample name	Antioxidant assays		
		2,2-diphenyl-1-picrylhydrazyl (DPPH) (% of DPPH)	Total phenolic content (TPC) (mg/g)	Total flavonoid content (TFC)(mg/g)
1	stigma 1	64.62	36.82	1.88
2	stigma 2	67.13	43.05	4.14
3	stigma 3	61.10	25.96	2.14
4	stamen 1	63.88	61.41	3.01
5	stamen 2	67.75	59.50	2.52
6	stamen 3	64.65	41.05	3.05

Table 5: *Mean±SD of different traits of saffron plant under row to row and plant to plant distance.*

Treatments (Row/ Plant distance) (cm)	Days to emergence	survival % of corms	Plant height (cm)	No. leaves/ plant	Days to flowering
15	45.00±1.00	97.00±0.43	22.52 ± 1.98	7.20±0.36	74.33±4.16
20	47.00±1.00	92.00±1.37	24.77± 2.27	7.40±0.53	75.33±2.89
25	46.00±1.00	89.63±0.64	24.07± 3.08	7.30±0.62	71.67±0.57
30	41.67±2.52	99.07±1.60	20.00± 2.18	6.70±0.34	65.33±3.51
35	29.70±2.57	80.10±5.29	20.40±1.85	4.37±3.80	48.70±4.22

Determination of total phenolic content (TPC) and total flavonoid contents (TFC)

The phenolic and flavonoid contents of stigma and stamen methanolic extracts of saffron are shown in Table 2. The stigma and stamen showed different phenolic and flavonoid contents. The three stigma extracts showed phenolic contents (36.82, 43.05, 25.96, mg GAE/g extract, respectively). The phenolic contents of stamen were (61.41, 59.50, 41.05 mg GAE/g extract, respectively). The highest phenolic contents were found in methanolic extract of stamen (61.41 mg GAE/g extract). Similarly, the stigma and stamen methanolic extracts showed different flavonoid contents. The three stigma extracts showed flavonoid contents (1.88, 4.14, 2.14, mg/g extract, respectively). The flavonoid contents of stamen were (3.01, 2.52, 3.05 mg/g extract, respectively). The highest flavonoid contents were obtained in methanolic extract of stigma (4.14 mg/g). The results of the antioxidant biological activities like TPC, TFC and DPPH were presented in Table 4.

Experiment 2

Effect of row and plant spacing on saffron flower yield/ha during 1st year: To investigate the impact of row and plant spacing on the flowers yield of saffron under rainfed conditions of Quetta, Balochistan, various parameters such as emergence time, percentage of bulb's survival, plant height, number of leavesplant^{-1}, days before flowering, a single flower fresh and dry weight, stigma and stamens' fresh and dry weight, total flowering period and number of daughter corms and total production of flowers were recorded. The results showed non-significant effects of plant and row spacing for all parameters except saffron flowers production, which was significantly higher in bulbs sown with a distance of 15 cm (F= 44.22; P ≤ 0.001; CV= 23.40).

The present study indicated that emergence time of bulbs was significantly affected by different bulb sizes of saffron. The large sized corms were observed to be emerged out of the soil earlier than medium and small sized corms. According to Sadeghi (2010) that the bulbs with a weight of 4 to10 grams have a significant impact on the percentage of buds which can increase the production healthy plants and in turn increased flower production.

Our study also indicated that the flower production significantly increased with the increase in corms sizes from 8 to 11 g while flower production significantly decreased with the decrease in corm size, which ultimately in reduction in the overall yield of saffron. Small sized corms (less than 8 g) couldn't produce flowers. These results suggest that large corms possess more nutrition needed for the growth of saffron flowering. These findings are in agreement with the findings of (Kaushal and Upadhyay, 2002) who recorded that the production of flowers has positive

Table 6: *Mean±SD of different traits of saffron flower under row to row and plant to plant distance.*

Treatments (Row/Plant distance) (cm)	Flower fresh weight (g)	Flower dry weight (g)	Stigma fresh weight (g)	Stigma dry weight (g)	Stigma size fresh (mm)
15	0.481±0.057	0.054±0.008	0.040±0.003	0.008±0.001	25.12±0.449
20	0.431±0.095	0.045±0.007	0.035±0.002	0.007±0.001	24.15±0.771
25	0.407±0.022	0.041±0.008	0.030±0.011	0.006±0.001	24.33±0.440
30	0.280±0.041	0.133±0.173	0.021±0.002	0.006±0.001	23.56±1.299
35	0.428±0.100	0.020±0.018	0.024±0.017	0.005±0.002	14.67±12.71

Table 7: *Mean±SD of different traits of saffron plant under row to row and plant to plant distance.*

Treatments (Row/Plant distance) (cm)	Stigma size dry(mm)	Stamen fresh weight (g)	Stamen dry weight (g)	Saffron flowers yield plot^{-1}	No. of daughter corms/corm
15	16.98±0.57	0.037±0.005	0.0109±0.0015	162.3±25.8	5.00±3.00
20	15.88±0.72	0.032±0.003	0.0092±0.0013	58.00±14.11	6.33± 1.155
25	15.59±0.40	0.031±0.011	0.0078±0.0015	52.67±8.33	6.67± 1.155
30	17.52±0.51	0.018±0.001	0.0061±0.0012	37.00±9.54	5.00± 1.73
35	10.66±0.27	0.018±0.016	0.0048±0.0042	11.33±10.26	5.00±2.65

correlation with the initial size of bulbs on the time of sowing. Andabjadid *et al.* (2015) also described that large sized bulb produce large flowers which ultimately increase the weight and length of stigma, thereby increasing the saffron yield. Mashayekhi *et al.* (2007) also got the similar results and determined that large sized bulbs have a positive influence on the production of saffron flowers but not on stigma weight.

According to (Iqbal *et al.*, 2012) increasing the weight of saffron bulbs have significantly produced more flowers as well as a greater number of baby bulbs. This suggests a significant positive correlation between the bulb size and baby bulbs production, production of flowers and yield of saffron stigma.

In the present study, the results for survival percentage of corms showed a significant difference among different corm sizes. According to Mashayekhi *et al.* (2007) saffron corm survival percentage and development are directly related to initial corm weight. Only 26% survival was achieved increase in corms with less than one gram while more than 88% survival in corms having weight more than one gram.

The flowering period (from first flowering day to last flowering day) was also significantly influenced by the bulb size and plant to plant distance. The large corms started flowering earlier in 56 days and flowering period lasted for 21 days. However, the other corm sizes failed to produce flowers. Similar results were also reported by Cavusoglu *et al.* (2009) that larger corms blooms earlier and their harvest time lasts longer than the smaller ones.

In the second experiment i.e., plant and row spacing, no significant difference was observed in days to emergence, plant height and number of leaves (Table 5). The earliest days to emergence (47.00 days), plant height (24.77 cm) and number of leaves plant^{-1} (7.4) were observed in 20 cm inches spacing. However, saffron bulbs planted with a space of 15 cm started blooming earlier in 55 days and lasted for 18 days followed by 20 cm inches spacing which bloomed in 59 days and lasted for 17 days (Table 5). However, Bhande *et al.* (2015) reported a maximum leaf or plant height in wider spacing of 45 × 15 cm in gladiolus plant.

According to Andabjadid *et al.* (2015) the flower yield increased with the increase in the space between the rows. In this case, the higher flower yields were recorded in 30 and 40 cm row to row distance as compared to 50 cm (Table 7). Similarly, Bhande *et al.* (2015) recorded that 5 days earlier sprouting of corms was observed when the bulbs were sown closer to each other.

The current study also recorded a significantly higher flower production, higher weight of fresh and dried flowers, and higher weight of fresh and dried stigma and stamens in 15 cm spacing (Table 6). The significantly higher flower production may be

attributed to a greater number of bulbs planted in a plot with 15 cm spacing which increased the plant density, thereby increasing the production of stigma and stamens. Similarly, Gresta *et al.* (2009) determined that the increased density of saffron corms has a positive effect on the number of flowers and the yield of saffron stigma.

According to the two years' study conducted by Andabjadid *et al.* (2015) the effects of corm size and plant to plant and row spacing were substantial on the weight of fresh and dried flowers and stigma. This suggests that plant spacing can be effective for getting higher yield of saffron.

The analysis of variance for total flowering period exhibited no significant effect of planting saffron bulbs in different plant and row spacing. However, lengthier flowering period was recorded in 15 cm spacing. Similarly, Rostami and Mohammadi (2013) reported that the crowded density of bulbs not only induces early flowering but also the lengthier flowering period. The non-significant results obtained from different parameters indicating that maintaining space between saffron bulbs seems unlikely to be effective in the first year of plantation.

In this study, saffron stigma and stamen extracts were also assessed against bacteria such as gram positive, *S. aureous* and gram negative, *E. coli*. Standard antibiotics was used for comparison of antibacterial activity. Results indicated that stigma extracts showed the maximum zone inhibition ranged from 24-27 mm against *S. aureous*. The positive standard drug control (Ampicillin antibiotic) very strongly inhibited the growth of *E. coli.* Similar kind of findings were also recorded by Okmen *et al.* (2016). They reported that *C. sativus* extracts have potential antibacterial activities against *S. aureus.*

The antioxidant activity of saffron stigma and stamen were assessed by applying the DPPH radical scavenging assay. Results indicated that the methanolic extracts of saffron stamen showed the highest radical scavenging activity (67.76%). Wali *et al.* (2020) reported that *Acacia accuminata* and *C. sativus* can be utilized for the improvement of numerous ingredients used as the antioxidants. In the current, the total phenolic and flavonoid contents of stigma and stamen methanolic extracts of saffron were assessed. The stigma and stamen showed different phenolic and flavonoid contents. The maximum phenolic contents were obtained in methanolic extract of stamen (61.41 mg GAE/g extract, respectively). Similarly, the highest flavonoid contents were obtained in methanolic extract of stigma (4.14 mg/g). These findings are in conformity with the finding of Rahaiee *et al.* (2015). They reported that saffron methanolic extract reported 80% effect on TPC of saffron as compared to other solvent types. The results of the study gave an indication that *C. sativus* stigma and stamens can be a possible source of new antimicrobial agents.

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The use of large corm (8-11 g) has produced the maximum number of flowers, higher stigma and stamen yield, and higher of daughter corms during the very first year of planting, suggesting that one should be more careful in corm selection while planting saffron. The plantation of corms with less space i.e., 15 cm space between row and plant resulted in the higher number of flowers which increased the stigma production, which suggests that corms should be planted closer for stigma production.

Conclusions and Recommendations

In conclusion, the climatic conditions of Balochistan were found to be suitable for the healthier growth and yield. The bulbs with a weight of 8 g or more produced flowers while below 8 g failed to produce flowers. Moreover, larger bulbs showed significantly better survival and attained a higher plant length which ultimately increased the saffron production. While, spacing between the bulbs and rows could not affect the most of the parameters except planting corms in 15 cm space.

Therefore, corms with a weigh of 8 g and sowing them in a space of 15 cm between plants and between rows produced the higher flower production, resulting in the higher production of saffron.

Novelty Statement

This study provides the sufficient and new information about the introduction and cultivation of saffron and its importance in Balochistan, Pakistan

Author's Contribution

Mahpara Fida Ahmed: Collected data and wrote the paper.

Abdul Hanan: Supervised and analyzed the data.
Sher Ahmed: Designed the experiments and provided the research materials such as bulbs collection.

Conflict of interest
The authors have declared no conflict of interest.

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