

# Bioprospecting Elicitation with Gamma Irradiation Combine with Chitosan to Enhance, Yield Production, Bioactive Secondary Metabolites and Antioxidant Activity for Saffron

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**Abstract:** Saffron (*Crocus sativus L.*) corms were subjected to gamma irradiation doses; 0,5,15,25 Gy ( $G_{1-4}$ ) then were planted as annual crop in complete randomized split block design with three replicates. The resultant plants 3 months aged and every month up tile before flowering, were vegetatively sprayed with chitosan solution; 0,50,100,200 mg/L concentrations. Data ( $C_{1-4}$ ) for growth and yield traits were recorded and subjected to statistic ANOVA, revealed that individual  $G_2$ ,  $G_3$  as well as  $C_2$ ,  $C_3$ ,  $C_4$  performed positive significant impact on growth traits (plant height, flowers fresh weight, number of flowers, fresh and dry weight of flowers  $m^2$ ) that cause to significant positive impacts in yield traits (main saffron stigmas yield /ha, second medicine – therapeutic metabolites (crocin, picrocrocin, safronal), antioxidant activity and secondary by – product, corms yield/ ha) whilst,  $G_4$  attained significant negative impacts in growth and yield traits. Combine applications ( $G_2 C_2$ ,  $G_2 C_3$ ,  $G_2 C_4$ ), ( $G_3 C_2$ ,  $G_3 C_3$ ,  $G_3 C_4$ ) achieved significant synergistic positive impacts on growth and yield traits. Aside ( $G_4 C_2$ ,  $G_4 C_3$ ,  $G_4 C_4$ ) actuated only significant positive impacts on growth and yield traits and quality, that warrant  $C_{2-4}$  overcoming significant impacts for  $G_4$ . Overall, results strongly manifest G and /or C reliable strategy, in vivo to enhance main saffron stigmas yield, secondary metabolite (crocin, picrocrocin, Safronal) and antioxidant activity for saffron plants.

**Keywords:** Saffron, Gamma Irradiation, Chitosan, Elicitation

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## 1. Introduction

The global demands for medicinal plant products has increased in the last two decades leading to over exploitation and sometimes extinction of endogenous medicinal plants. Plant biotechnology offers an efficient approach to enhance compounds biosynthesis of essential and/or bioactive compounds in plants. Since, the side effects of chemical drugs and the human tendency to make greater use of natural products in order to keep their health as well as problems of modern medicinal system caused more attention of human to medicinal plants [1].

Saffron (*Crocus sativus L.*) is perennial herb belong to the Iridaceae family and is cultivated principally in Iran, Azerbaijan, France, Greece, India, Italy, Spain, China, Morocco, Turkey, Egypt and Mexico [2]. It is the oldest expensive crop of aromatic food and medicinal plants [3-6].

Saffron plant species of great economic importance [7]. Saffron stigmas is an important spice that is widely used for its aroma, day properties

Systems, in liver, its anti-depressant, anxiolytic and anti-neoplastic properties as well as its potential use as a functional or neutrino [8-10] Saffron has a potential role in disease cure and prevention via modulation of antioxidants, anti-inflammatory, antidiuretic anti-epileptic, neuroprotective, anti-obesity, anti-aging in cosmetics [11, 12].

Saffron, the dried stigmas is rich in apocarotenoids that include crocins (the main coloured compounds), safronal (the volatile oil responsible for characteristic smell and aroma) and picrocrocin (the main substance responsible for the better taste in saffron [13]. Saffron and its components have been used in traditional medicine as anti-aphrodisiac, anti asthmatic, anti-spasmodic, expectorant, stomach oilmen's and for smoothing menstruation [14]. Recent studies

have revealed anti-carcinogenic, anti-mutagenic, antitumor [15, 16]. The therapeutic properties of saffron including activity on a nervous and cardiovascular [17].

Low gamma irradiation doses could stimulate biochemical and physiological processes such as stimulated growth [18] increasing biomass and dry biomass [18, 19] and enhanced secondary metabolites that have potential importance particularly on human health [20-22].

Elicitation is one of few strategies that enhanced formation and accumulation of secondary metabolites [23, 24]. Elicitors, defined as molecules that stimulate defense or stress induced responses in plants [25]. It can be considered a potential strategy in plant protection and biological control [26, 27]. Elicitors were classified as physical elicitor [27-29], abiotic elicitors [30] and biotic elicitors [31]. Elicitors modify secondary metabolites and subsequently the activity of medicinal and aromatic plants [32]. However, the commercial viability and logistics of elicitor application are debatable [1].

Chitosan, a biopolymer derived from the acetylation of chitin, is one of the polysaccharides obtained from crab and shrimp shells as a waste product of the fishing industry [33]. It has attracted tremendous attention due to its biological properties including biocompatibility, non-toxicity and biodegradability [34]. It has been widely applied in the field of agriculture, environment, pharmaceutical, medicine and industrial food processing [35]. It has significant diverse uses in several fields of life, i.e. plant sciences [36], in cosmetics and biochemical industries [37], biotic elicitor to improve quantitative and qualitative production of agronomic and horticulture crops in addition, it improved secondary metabolites of medicinal and aromatic plants [38]. Chitosan is considered a potent biotic elicitor; in enhancing tolerance of plants to biotic and abiotic stresses, crop plants, shows strong resistance to microbial diseases and insecticidal against various plant pests and nematode effects [39].

Several reports indicated that chitosan positively affected plant growth and development, yield attributes, physiological activities, i.e. ion uptake and transport and transpiration rate [40-42].

Also, chitosan has been used to control the release of inorganic fertilizers [43].

Allied to the fact that has been mentioned herein before, the present study aimed to evaluate the biological valorization potential of physical (gamma irradiation) and biotic (chitosan) elicitors to enhance saffron yield production, quality and promoting health antioxidant activity.

## 2. Material and Methods

### 2.1. Elicitor

Physical elicitor, gamma irradiation doses 0, 10, 25, 50 Gy ( $G_{1-4}$ ) at dose rate 1.4 KGy/h. Biotic elicitor, chitosan solution at 0, 50, 100, 200 mg/L ( $C_{1-4}$ ) concentrations.

### 2.2. Execute Field Experiment

At 15 August (2017), saffron corms that were subjected to  $G_{1-4}$  gamma ray doses were directly planted in the field (sandy soil) as an annual crop, in plots 2x2m consisting of 5 rows 40 cm apart, 2m long and 10cm inter spacing (25 plants/m<sup>2</sup>). The layout of the field experiment in a split complete block design was conducted with three replicates. The resultant plants, at 3 months and every month up to start of flowering, were vegetatively sprayed with  $C_{1-4}$  chitosan concentrations. Low water quality (shallow well water, 900 ppm) was used for irrigation as well as fertilization with NPK 30: 20: 15 Kg/ha.

### 2.3. Biometric Growth Traits

Mean plant height, cm (PHcm) for 5 represented at random plants were recorded at starting flowering period. Total number of flowers/m<sup>2</sup> (NFL/m<sup>2</sup>), total flowers fresh weight, Kg/m<sup>2</sup> (FLFW/Kg/m<sup>2</sup>), and dry flowers weight/m<sup>2</sup> (FLDW, G/M<sup>2</sup>).

### 2.4. Yield Traits

#### 2.4.1. Main Saffron Stigma Yield

Stigmas are the main saffron yield, the flowers are harvested in the next autumn (August 2018), manually picked flowers early in the morning (during flowering period that last up to 15 days); placed in baskets, when the corolla separated from the sepals by opening the corolla cutting the stigmas with the fingers below the branching where the style changes corolla from red to yellow. Stigma yield/plot were dried in oven at 35°C in less time until moisture reduced to 12% then dry weight stigmas/m<sup>2</sup> were recorded elicited treatments as main stigmas. Saffron yield/m<sup>2</sup> (ST Y, g/m<sup>2</sup>). Also, daughter corms yield/m<sup>2</sup> (DCMY, g/m<sup>2</sup>) were recorded as by-product saffron yield.

#### 2.4.2. Bioactive Secondary Metabolites

The amount of crocin, picrocrocin and safranal, mg/g dry weight; CR, PI, SA, respectively were determined according to [44]. Briefly, 500mg of the powdered dry saffron stigmas was transferred into 1000ml volumetric flask and 900ml of distilled water was added, after stirring with an electromagnetic agitator for one hour at room temperature. The solution was made up to 1000ml with distilled water and filtered. The extract was diluted (1:10) with distilled water then the extracts were directly analyzed by spectrophotometer. The amount of CR, PI and SA as absorbance of 1% aqueous solution of dried saffron stigmas at 440, 330 and 257, respectively.

#### 2.4.3. Antioxidant Activity Determination

DPPH radical scavenging activity was determined according to the method of [45]. The ethanol extract (2ml; 10mg/25ml) of each dry stigma was mixed with 2ml of DPPH-free radical solution (0.25mg/25mlx4). The mixture was incubated in the dark for approximately 30min; the absorbance of the resultant mixture was measured at 517nm at room temperature by using UV-visible spectrophotometer. The radical scavenging activity was calculated as percentage

of DPPH discoloration using following equation; % scavenging activity = [(absorbance control – Absorbance of the test sample)/ absorbance of the control] / Absorbance of control X 1000.

### 2.5. Statistical Analysis

All recorded data were statistically analysis for ANOVA and then statistical significant differences between mean elicitation treatments were assessed by the calculated least significant (LSD) at 1% significant level.

## 3. Results

Statistical ANOVA revealed that both gamma irradiation doses and chitosan concentrations alone as well as their combination actuated significantly growth and yield traits.

### 3.1. Growth Traits

#### 3.1.1. Plant Height (PH, Cm)

Table 1, Figure 1 showed that G<sub>2</sub>, G<sub>3</sub> increased significantly up to 121, 118% of control (42-5cm) whereas G<sub>4</sub> inhibited (PH) significantly by 86% of control. C<sub>2</sub>, C<sub>3</sub>, C<sub>4</sub> resulted in significant increment (PH) up to 123, 127, 132% of control. Combined application G<sub>2</sub> C<sub>2</sub>, G<sub>2</sub> C<sub>3</sub>, G<sub>2</sub> C<sub>4</sub> performed significant synergistic positive impact up to 129, 134, 139 % of control that were exceeded for G<sub>3</sub>C<sub>2</sub>, G<sub>3</sub>C<sub>3</sub>, G<sub>3</sub>C<sub>4</sub> of 125,131, 134% of control. Aside G<sub>4</sub>C<sub>2</sub>, G<sub>4</sub>C<sub>3</sub>, G<sub>4</sub>C<sub>4</sub> increased (PH) significantly by 118,122,127% of control and overcoming G<sub>4</sub> alone.

#### 3.1.2. Flowers Weigh Kg/m<sup>2</sup>FLW, Kg/m<sup>2</sup>

Table 1, Figure 2, G<sub>2</sub>, G<sub>3</sub> resulted in significant positive impact 112, 108% of control (4.21 Kg/m<sup>2</sup>) whereas, G<sub>4</sub> declined significantly LFW/m<sup>2</sup> down to 95% of control. C<sub>2</sub>, C<sub>3</sub>, C<sub>4</sub> led to significant increase up to 115, 118, 122% of control. Combination application G<sub>2</sub>C<sub>2</sub>, G<sub>3</sub>C<sub>3</sub>, G<sub>3</sub>C<sub>4</sub> actuated significant synergistic positive impact up to 121,129,137% of control that exceeded for G<sub>3</sub>C<sub>2</sub>, G<sub>3</sub>C<sub>3</sub>, G<sub>3</sub>C<sub>4</sub> 117,120,125% of control. At that G<sub>4</sub>C<sub>2</sub>, G<sub>4</sub>C<sub>3</sub>, G<sub>4</sub>C<sub>4</sub> led to only significant increment up to 112,115,117% of control and overcoming G<sub>4</sub> alone.

#### 3.1.3. Number of Flowers /NFL/m<sup>2</sup>

Table 1, Figure 3, G<sub>2</sub>, G<sub>3</sub> achieved significant positive impact up to 115,111% of control (420) while G<sub>4</sub> decline significant to 92% of control. C<sub>2</sub>, C<sub>3</sub>, C<sub>4</sub> invoked significant increment up to 117,119,121% of control. Combined application; G<sub>2</sub>C<sub>2</sub>, G<sub>2</sub>C<sub>3</sub>, G<sub>2</sub>C<sub>4</sub> resulted in significant synergistic positive impacts up to 124, 130, 135 % of control and exceeded G<sub>3</sub>C<sub>2</sub>, G<sub>3</sub>C<sub>3</sub>, G<sub>3</sub>C<sub>4</sub>that led to 119; 123,128% of control. Aside G<sub>4</sub>C<sub>2</sub>, G<sub>4</sub>C<sub>3</sub>, G<sub>4</sub>C<sub>4</sub> actuated only significant increment up to 108, 113, 116% of control and overcoming G<sub>4</sub> alone application.

#### 3.1.4. Flower Dry Weight FLDW, g/m<sup>2</sup>

Table 1, Figure 4, G<sub>2</sub>, G<sub>3</sub> performed significant increment up to 125, 117% of control (0.359/m<sup>2</sup>) while G<sub>4</sub> decreased significantly to 91% of control. While C<sub>2</sub>, G<sub>3</sub>, C<sub>4</sub> actuated significant increment up to 142; 155, 162% of control. Meanwhile combined applications G<sub>2</sub>C<sub>2</sub>, G<sub>2</sub>C<sub>3</sub>, G<sub>2</sub>C<sub>4</sub> resulted in significant synergistic positive impact up to 167, 185, 302% of control that exceeded G<sub>3</sub>C<sub>2</sub>, G<sub>3</sub>C<sub>3</sub>, G<sub>3</sub>C<sub>4</sub> that led to 150, 171, 191 % of control while G<sub>4</sub>C<sub>2</sub>, G<sub>4</sub>C<sub>3</sub> G<sub>4</sub>C<sub>4</sub> increased significantly up to 132, 145, 151% of control and overcoming G<sub>4</sub> alone application.

Table 1. Saffron growth traits in response to gamma irradiation and chitosan elicitors application.

G	C	PH, cm	FLFW, Kg/m <sup>2</sup>	NF/m <sup>2</sup>	FLFDW, g/m <sup>2</sup>
1	1	42.5 (100)	4.21 (100)	420.7 (100)	0.350 (100)
2	1	51.4 (121)	4.72 (112)	483.8 (115)	2.438 (125)
3	1	50.2 (118)	4.55 (108)	457.00 (111)	0.410 (117)
4	1	36.6 (86)	4.00 (95)	387.00 (92)	0.319 (91)
1	2	52.3 (123)	4.84 (115)	491.2 (117)	0.497 (142)
1	3	54.0 (127)	4.97 (118)	499.8 (119)	0.543 (155)
1	4	56.1 (132)	5.14 (122)	508.2 (121)	0.567 (162)
2	2	54.8 (129)	5.10 (121)	521.67 (124)	0.585 (167)
2	3	57.0 (134)	5.43 (129)	546.91 (130)	0.648 (185)
2	4	59.1 (139)	5.77 (137)	568.00 (135)	0.711 (302)
3	2	53.1 (125)	4.93 (117)	428.69 (119)	0.525 (150)
3	3	55.7 (131)	5.05 (120)	517.46 (123)	0.599 (171)
3	4	57.0 (134)	5.26 (125)	538.50 (128)	0.669 (191)
4	2	50.2 (118)	4.72 (112)	454.36 (108)	0.462 (132)
4	3	51.9 (122)	4.84 (115)	475.39 (113)	0.508 (145)
4	4	54.0 (127)	4.93 (117)	488.01 (116)	0.529 (151)
LSD/%		1.4	0.15	4.3	0.012

G<sub>1</sub> -4 (0,5,15,25 Gy, respectively).

C-4 (0,50,100,200mg/L chitosan, respectively).

Values between parenthesis were percent of control.

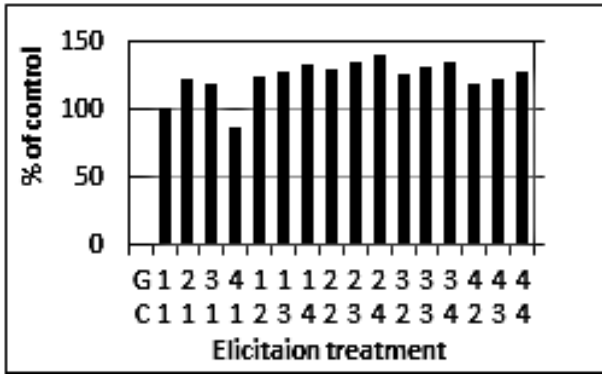


Figure 1. Plant height, cm.

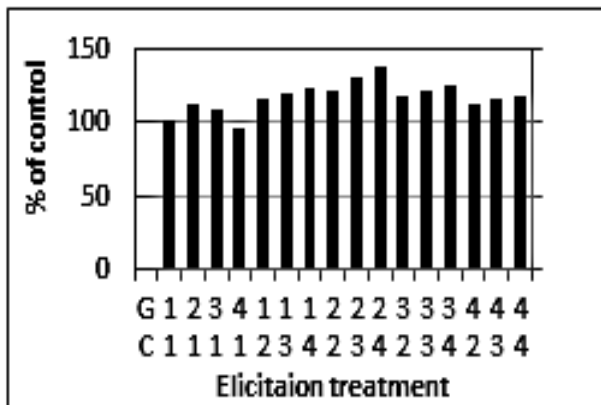


Figure 2. Flowers fresh weight, Kg/m².

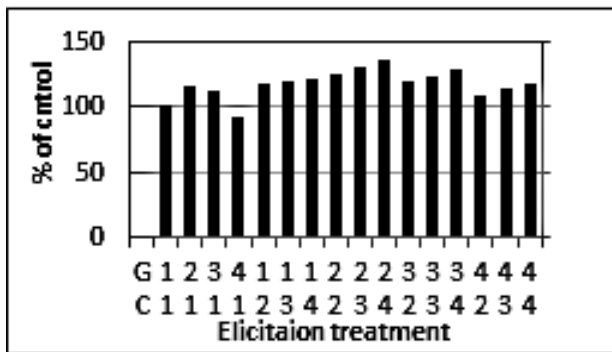


Figure 3. Number of flowers/m².

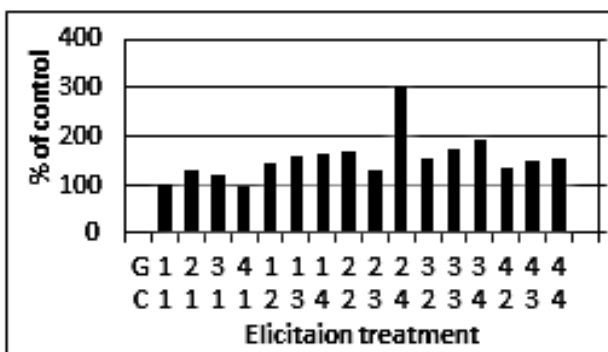


Figure 4. Flower dry weight, g/m².

### 3.2. Yield Traits

#### 3.2.1. Main stigmas Yield, g/m² (STY, g/m²)

STYg/m² were multiplied by 10000m² to produce main saffron yield per hectare. Table 2 and Figure 5 showed, G<sub>2</sub>, G<sub>3</sub> acted significant increase STY, g/m² (and also per hectare) up to 113,110% of control (0.270g/m² = 2.7kg/ha) while G<sub>4</sub> reduced STY for 97% of control whereas, C<sub>2</sub>, C<sub>3</sub>, C<sub>4</sub> resulted in significant increment up to 115, 121, 125% of control. G integrated C application led to significant synergistic positive impact up to 123, 128,135% of control for G<sub>2</sub>C<sub>2</sub>, G<sub>2</sub>C<sub>3</sub>, G<sub>2</sub>C<sub>4</sub>, respectively > G<sub>3</sub>C<sub>2</sub>, G<sub>3</sub>C<sub>3</sub>, G<sub>3</sub>C<sub>4</sub> that were 118,124,130% of control meanwhile, G<sub>4</sub>C<sub>2</sub>, G<sub>4</sub>C<sub>3</sub>, G<sub>4</sub>C<sub>4</sub> invoked only significant increment up to 110,115,119% of control aside overcoming G<sub>4</sub> alone application.

#### 3.2.2. Second Bioactive Metabolites

##### i. Crocin, mg/g Stigmas (CR, mg/g.ST)

Table 2 and Figure 6, cleared, that G<sub>2</sub>, G<sub>3</sub> achieved significant increase up to 191, 175% of control (52.27 mg/g) while G<sub>4</sub> reduced CR content to 95% of control. C<sub>2</sub>, C<sub>3</sub>, C<sub>4</sub> also resulted in significant increase up to 210, 231, 245% of control. Combination applications performed significant synergistic positive impacts up to 225, 280, 295% of control for G<sub>2</sub>C<sub>2</sub>, G<sub>2</sub>C<sub>3</sub>, G<sub>2</sub>C<sub>4</sub>, respectively that > 190, 220, 232 percent of control for G<sub>3</sub>C<sub>2</sub>, G<sub>3</sub>C<sub>3</sub>, G<sub>3</sub>C<sub>4</sub>. Whereas G<sub>4</sub>C<sub>2</sub>, G<sub>4</sub>C<sub>3</sub>, G<sub>4</sub>C<sub>4</sub> resulted in only significant increment up to 165,185,191% of control and overcoming G<sub>4</sub> at alone application.

##### ii. Picrocrocin, mg/g stigmas (PRmg/g ST)

Table 2 and Figure 7, express G<sub>2</sub>, G<sub>3</sub> raised significantly PI, mg/g ST up to 175, 156% of control, otherwise G<sub>4</sub> reduced PI content for 91% of control. C<sub>2</sub>, C<sub>3</sub>, C<sub>4</sub> actuated significant upraise up to 182, 194, 215 % control. Integration G and C applications imply significant synergistic incrementally up to 250, 276, 302% of control for G<sub>2</sub>C<sub>2</sub>, G<sub>2</sub>C<sub>3</sub>, G<sub>2</sub>C<sub>4</sub>, respectively that were > G<sub>3</sub>C<sub>2</sub>, G<sub>3</sub>C<sub>3</sub>, G<sub>3</sub>C<sub>4</sub> which achieved 195, 204, 220 % of control respectively. Aside, G<sub>4</sub>C<sub>2</sub>, G<sub>4</sub>C<sub>3</sub>, G<sub>4</sub>C<sub>4</sub> exceeded significantly up to 141, 182, 195 % of control beside rectify actuation of G<sub>4</sub> alone application.

##### iii. Saffronal mg/g stigmas (SA, mg/g ST)

Table 2 and Figure 8 declared SAmg/g increased significantly in response to G<sub>2</sub>, G<sub>3</sub> up to 208, 190% of control (0.230 mg / g ST; on the contrary G<sub>4</sub> cause to decrease SA to 85% of control. C<sub>2</sub>, C<sub>3</sub>, C<sub>4</sub> attained significant incrementally SA up to 230, 242, 255% of control. Combined G and C application achieved to significant synergistic incrementally PA up to 250, 277, 300% of control for G<sub>2</sub>C<sub>2</sub>, G<sub>2</sub>C<sub>3</sub>, G<sub>2</sub>C<sub>4</sub>, respectively that were above G<sub>3</sub>C<sub>2</sub>, G<sub>3</sub>C<sub>3</sub>, G<sub>3</sub>C<sub>4</sub>. Concerning G<sub>4</sub>C<sub>2</sub>, G<sub>4</sub>C<sub>3</sub>, G<sub>4</sub>C<sub>4</sub> applications, performed only significant increment SA up to 157, 183, 193% of control and rectify G<sub>4</sub> alone application.

##### iv. Antioxidant Activity (AOA)

Table 2 and Figure 9, explained that G<sub>2</sub>, G<sub>3</sub> actuated significant augment (AOA) up to 183, 152% of control (30). C<sub>2</sub>, C<sub>3</sub>, C<sub>4</sub> achieved significant increment AOA up to 200,

217, 233 % of control. (G) interacted with (C) application achieved significant synergistic increase AOA up to 250, 277, 300% of control for G<sub>2</sub>C<sub>2</sub>, G<sub>2</sub>C<sub>3</sub>, G<sub>2</sub>C<sub>4</sub>, respectively which over that of G<sub>3</sub>C<sub>2</sub>, G<sub>3</sub>C<sub>3</sub>, G<sub>3</sub>C<sub>4</sub> led to 187, 213, 227% of control, respectively. Meanwhile, G<sub>4</sub>C<sub>2</sub>, G<sub>4</sub>, C<sub>3</sub>, G<sub>4</sub>C<sub>4</sub> acted significant augment up to 157, 183, 193% of control beside overcoming G<sub>4</sub> alone application.

**3.2.3. Daughter Saffron Corms Yield g/m<sup>2</sup> (DCMy, g/m<sup>2</sup>)**

Table 2 and figure 10 declared G<sub>2</sub>, G<sub>3</sub> acted significant appraise DCM up to 125, 117%, of control (80.5g./m<sup>2</sup>=

810kg/ha), on the contrary G<sub>4</sub> resulted in significant reduction DCM for 97% of control. C<sub>2</sub>, C<sub>3</sub>, C<sub>4</sub> invoked significant augment up to 128, 132, 138% of control GXC interacted applications; G<sub>2</sub>C<sub>2</sub>, G<sub>2</sub>C<sub>3</sub>, G<sub>2</sub>C<sub>4</sub> achieved significant synergistic increment up to 131, 135, 142 % of control that transient G<sub>3</sub>C<sub>2</sub>, G<sub>3</sub>C<sub>3</sub>, G<sub>3</sub>C<sub>4</sub> application values 130, 133,135 % of control Meanwhile, G<sub>4</sub>C<sub>2</sub>, G<sub>4</sub>C<sub>3</sub>, G<sub>4</sub>C<sub>4</sub> actuated significant increment by 121, 125, 130% of control, aside rectiyed G<sub>4</sub> application.

**Table 2.** Main saffron stigmas yield, bio action secondary mthabolite, antioxidant activities and by- product dangther corms in response to gamma irradiation and chitosan elicitors application.

G	C	STYg/m <sup>2</sup>	CR mg./g.ST	PImg/g.ST	SA mg/gST	AOA%	DSCY, g/m <sup>2</sup>
1	1	0.270 (100)	52.27 (100)	35.15 (100)	0.230 (100)	30 (100)	80.5 (100)
2	1	0.305 (113)	99.48 (191)	61.51 (175)	0.478 (208)	55 (183)	100.6 (125)
3	1	0.297 (110)	91.47 (175)	54.84 (156)	0.437 (190)	50 (152)	94.2 (117)
4	1	0.262 (97)	49.66 (95)	31.99 (91)	0.272 (85)	28 (93)	78.1 (97)
1	2	0.311 (115)	109.77 (210)	63.97 (182)	0.529 (230)	60 (200)	103.0 (128)
1	3	0.327 (121)	120.74 (231)	68.19 (194)	0.557 (242)	65 (217)	106.3 (132)
1	4	0.338 (125)	128.06 (245)	75.57 (215)	0.587 (255)	70 (233)	111.1 (138)
2	2	0.332 (123)	117.61 (225)	87.88 (250)	0.679 (295)	75 (250)	105.6 (131)
2	3	0.346 (128)	144.36 (280)	97.02 (276)	0.741 (322)	83 (277)	108.7 (135)
2	4	0.365 (135)	154.20 (295)	106.15 (302)	0.881 (383)	90 (300)	114.3 (142)
3	2	0.319 (118)	99.31 (190)	68.54 (195)	0.554 (241)	56 (187)	104.7 (130)
3	3	0.335 (124)	115.00 (220)	71.71 (204)	0.598 (260)	64 (213)	107.1 (133)
3	4	0.351 (130)	121.27 (232)	77.33 (220)	0.669 (291)	68 (227)	108.7 (135)
4	2	0.297 (110)	86.25 (165)	49.56 (141)	0.405 (176)	47 (157)	97.4 (121)
4	3	0.311 (115)	96.70 (185)	63.97 (182)	0.518 (225)	55 (183)	100.6 (125)
4	4	0.321 (119)	99.84 (191)	68.54 (195)	0.557 (242)	58 (193)	104.7 (130)
LSD1%		0.003	0.52	0.35	0.021	2	0.6

G<sub>1</sub> -4 (0,5,15,25 Gy, respectively).

C<sub>1</sub>-4 (0,50,100,200mg/L chitosan, respectively).

Values between parenthesis were percent of control.

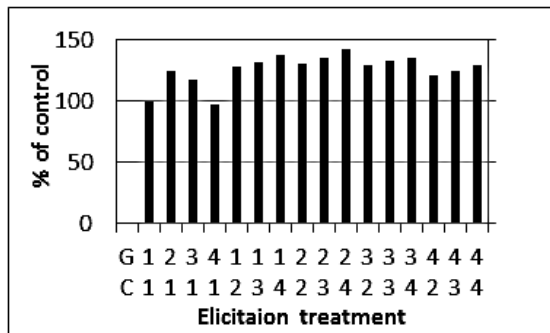


Figure 5. Stigma yield, g/m<sup>2</sup>.

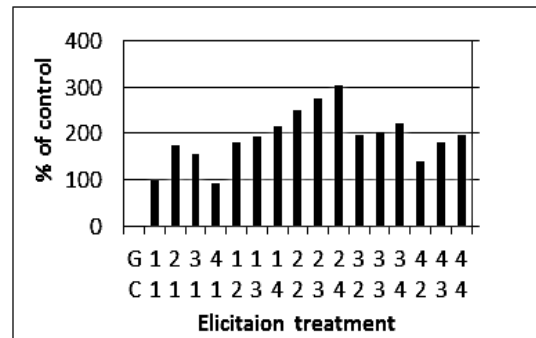


Figure 7. Picrocin, mg/g.stigmas.

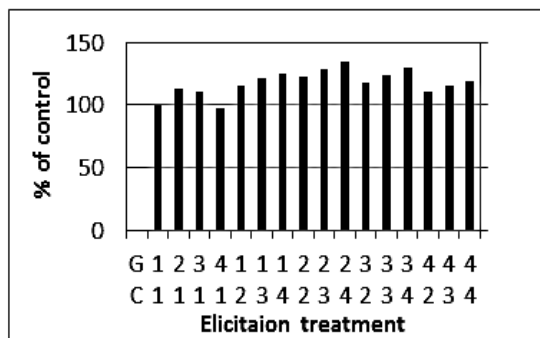


Figure 6. Crocin, mg/stigmas.

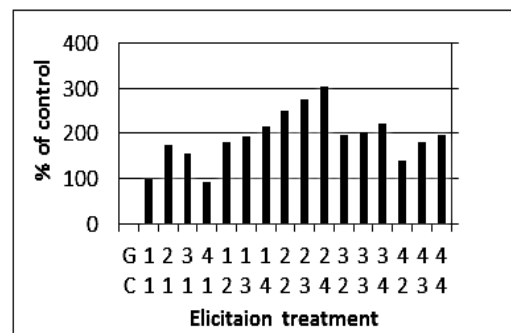


Figure 8. Safronal, mg/g.stigm.

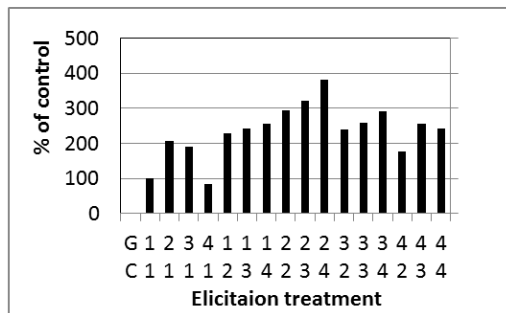


Figure 9. Antioxidant activity.

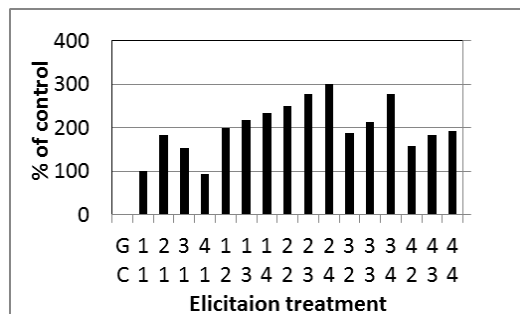


Figure 10. Daughter saffron corms yield g/m<sup>2</sup>.

## 4. Discussion

The results were attributed to gamma irradiation that stimulates biochemical and physiological processes that lead to promoting growth, yield and/or quality (18-21). While chitosan, also activated physiological processes and achieved strong resistance to microbial diseases and insecticidal against various plant pests which in turn improved growth and yield traits (38,40-42). Integrated G<sub>2</sub>, G<sub>3</sub> with C<sub>2</sub>, C<sub>3</sub>, C<sub>4</sub> performed significant synergistic enhancement of growth, yield, bioactive secondary metabolites and anti-oxidant activity that exceeded G<sub>2,3</sub> and C<sub>2,3,4</sub> individually (23-27).

## 5. Conclusion

Précis results strongly suggest that in vivo application with gamma irradiation and/or chitosan, as physical and biotic elicitor, could be considered a reliable strategy to significantly enhance yield and quality for saffron (*Crocus sativus* L.).

## Conflict Case

There is no conflict with any person concerning this manuscript.

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