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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 vegitatively 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 ANDVA, 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_2C_3, G_2C_4), (G_3C_2, G_3C_3, G_3C_4) achieved significant synergistic positive impacts on growth and yield traits. Aside (G_4C_2, G_4C_3, G_4C_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, pircorcrocin, Safronal) and antioxidant activity for saffron plants.

Keywords: Saffron, Gamma Irradiation, Chitosan, Elicitation

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 plans [1].

Saffron (*Crocus sativus L.*) is perennial herb belong to the Iridaceous 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, neroprotictive, anti-obesity, anti-aging in cosmetics [11, 12].

Saffron, the dried stigmas is rich in aposcarotenoids that include crocins (the main coloured compounds), saffronal (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]. Recant studies

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

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

Elicitation is one of few strategy that enhanced formation and accumulation secondary metabolites [23, 24]. Elicitors, are define as molecule that stimulate defense or stress induced response 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 the acetylation of chitin, is one of polysaccharides, obtained from crab and shrimp shells as a west produced by fishing industry [33]. It has attracted tremendous attention due to its biological properties including biocompatibility, non, toxicity and biodegrade ability [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 metabolite of medicinal and aromatic plants [38]. Chitosan is considered as potent biotic elicitor; in enhancing tolerance of plants to biotic and abiotic responses crop plants, chows strong resistant to microbial diseases and insecticidal against various plant bests 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, the faith of 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 irritation doses 0,10,25,50 Gy (G_{1-4}) at dose rate 1.4 KGy/h. Biotic elicitor, chitosan solution at 0, 50,100,200mg/L (C_{1-4}) concentrations.

2.2. Execute Field Experiment

At 15 August (2017), saffron corms that were subjected for G_{1-4} gamma ray doses were directly planted in the field (sandy soil) as annual crop, in plots 2x2m consisted 5 Rows 40 Cm apart 2m long and 10cm inter spacing (25 plant /m^2). The layout of the field experiment in split complete block designee were conducted with three replicates. The resultant plants. at 3 months and every month up tile start flowering, were vegitatively sprayed with C_{1-4} chitosan concentrations. low water quality (Shallow well water, 900 ppm.) were used for irrigation as well fustigation 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 flower/ m^2 (NFL/m^2), total flowers fresh weight, Kg/m2 (FLFW/Kg/m^2). and dry flowers Weight/m^2 (FLDW, G/M^2).

2.4. Yield Trails

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 15 days); placed in baskets, when the corolla separated from the Sepals by open the corolla cutting the stigmas with the fingers below the branching where the style changes corolla from red to yellow. Stigmas yield/plot were dried in oven at 35°C in less time until moisture reduced to 12% then dray weight stigmas /m^2 were recorded elicited treatments as main stigmas. Saffron yield/ m^2 (5T Y, g/m^2). Also, daughter corms yield/m^2 (DCMY, g/m^2) were recorded as by- product saffron yield.

2.4.2. Bioactive Secondary Metabolites

The amount, of crocin, picrocrocin and Safronal, mg/g dry weight; CR, PI, SA, respectively were determined according [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 mode up to 1000ml. with distilled water and filtered. The extract was diluted (1:10) with distilled water then the extracts were directly analyzed 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, stigmas was mixed with 2ml of DPPH- free radical solution (0.25mg/25mlx4). The mixture was incubated in the dark was 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 ANUVA and then statistical significant differences between mean elicitation treatments were assessed by the calculated least significant (LSD) at 1% significant level.

3. Results

Statistical ANONA 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_2, G_3 increased significantly up to 121, 118% of control (42-5cm) whereas G_4 inhibited (PH) significantly by 86% of control. C_2, C_3, C_4 resulted in significant increment (PH) up to 123, 127, 132% of control. Combined application G_2 C_2, G_2 C_3, GC_4 performed significant synergistic positive impact up to 129, 134, 139 % of control that were exceeded for G_3C_2, G_3C_3, G_3C_4 of 125,131, 134% of control. Aside G_4C_2, C_4C_3, G_4C_4 increased (PH) significantly by 118,122,127% of control and overcoming G4 alone.

3.1.2. Flowers Weigh Kg/m^{2}FLW, Kg/m^2

Table 1, Figure 2, G_2, G_3 resulted in significant positive impact 112, 108% of control (4.21 Kg/m^2) whereas, G_4 declined significantly LFW/m^2 down to 95% of control. C_2, C_3, C_4 led to significant increase up to 115, 118, 122% of control. Combination application G_2C_2, G_3C_3, G_3C_4 actuated significant synergistic positive impact up to 121,129,137% of control that exceeded for G_3C_2, G_3C_3, G_3C_4 117,120,125% of control. At that G_4C_2, G_4C_3, G_4C_4 led to only significant increment up to 112,115,117% of control and overcoming G_4 alone.

3.1.3. Number of Flowers / NFL /m^2

Table 1, Figure 3, G_2, G_3 achieved significant positive impact up to 115,111% of control (420) while G_4 decline significant to 92% of control. C_2, C_3, C_4 invoked significant increment up to 117,119,121% of control. Combined application; G_2C_2, G_2C_3, G_2C_4 resulted in significant synergistic positive impacts up to 124, 130, 135 % of control and exceeded G_3C_2, G_3C_3, G_3C_4that led to 119; 123,128% of control. Aside G_4C_2, G_4C_3, G_4C_4 actuated only significant increment up to 108, 113, 116% of control and overcoming G_4 alone application.

3.1.4. Flower Dry Weight FLDW, g./m^2

Table 1, Figure 4, G_2, G_3 performed significant increment up to 125, 117% of control (0.359/m^2) while G_4 decreased significantly to 91% of control. While C_2, G_3, C_4 actuated significant increment up to 142; 155, 162% of control. Meanwhile combined applications G_2C_2, G_2C_3, G_2C_4 resulted in significant synergistic positive impact up to 167, 185, 302% of control that exceeded G_3C_2, G_3C_3, G_3C_4 that led to 150, 171, 191 % of control while G_4C_2, G_4C_3 GC_4 increased significantly up to 132, 145, 151% of control and overcoming G_4 alone application.

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

G	C	PH, cm		FLFW, Kg/m^2		NF/m^2		FLFDW, g/m^2	
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_1 -4 (0,5,15,25 Gy, respectively).
C-4 (0,50,100,200mg/L chitosan, respectively).
Values between parenthesis were percent of control.

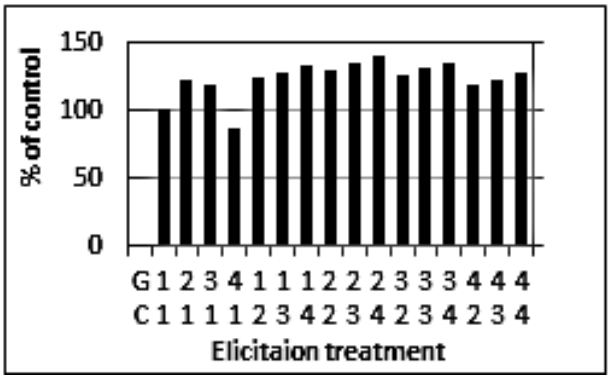

Figure 1. *Plant height, cm.*

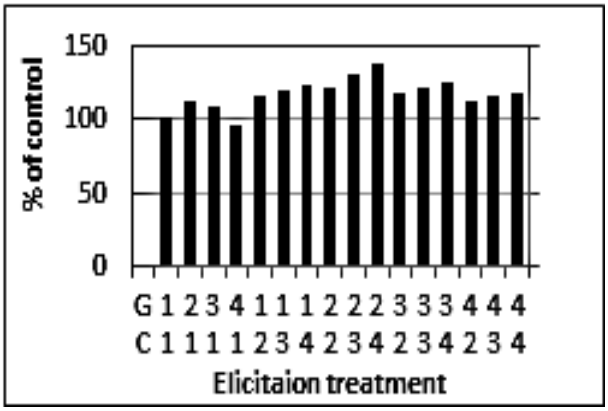

Figure 2. *Flowers fresh weight, Kg/m^2.*

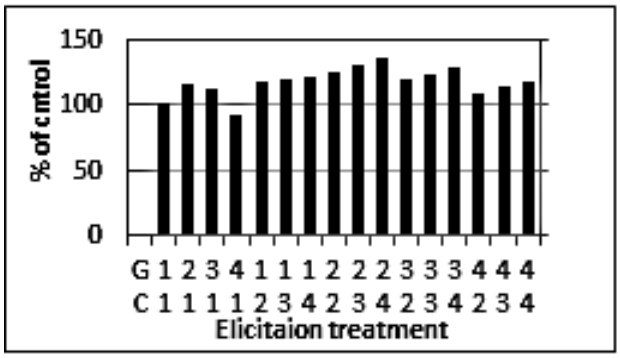

Figure 3. *Number of flowers/m^2.*

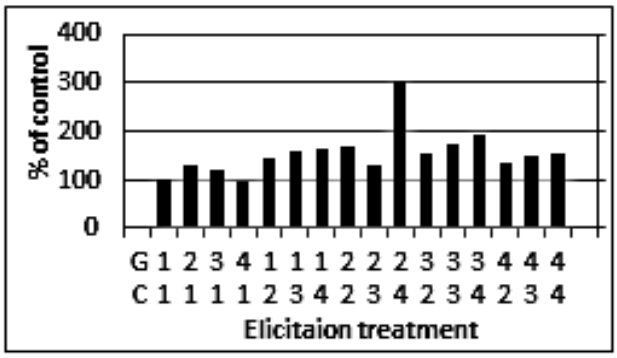

Figure 4. *Flower dry weight, g/m^2.*

3.2. Yield Traits

3.2.1. Main stigmas Yield, g/m^2 (STY, g/m^2)

STYg/m^2 were multiplied by 10000m^2 to produce main saffron yield per hectare. Table 2 and Figure 5 showed, G_2, G_3 acted significant increase STy, g/m^2 (and also per hectare) up to 113,110% of control (0.270g/m^2 = 2.7kg/ha) while G_4 reduced STY for 97% of control whereas, C_2, C_3, C_4 resulted in significant increment up to 115, 121, 125% of control. G integrated C application led to significant synergistic positive impact up to123, 128,135% of control for G_2C_2, G_2C_3, G_2C_4, respectively > G_3C_2, G_3C_3, G_3C_4 that were 118,124,130% of control meanwhile, G_4C_2, G_4C_3, G_4C_4 invoked only significant increment up to 110,115,119% of control aside overcoming G4 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_2, G_3 achieved significant increase up to 191, 175% of control (52.27 mg/g) while G_4 reduced CR content to 95% of control. C_2, C_3, C_4 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_2C_2, G_2C_3, G_2C_4, respectively that > 190, 220, 232 percent of control for G_3C_2, G_3C_3 G_3C_4. Whereas G_4C_2, G_4C_3, G_4C_4 resulted in only significant increment up to 165,185,191% of control and overcoming G_4 at alone application.

ii. Pirocrocin, mg/g stigmas (PRmg/g ST)

Table 2 and Figure 7, express G_2, G_3 raised significantly PI, mg/g ST up to 175, 156% of control, otherwise G_4 reduced PI content for 91% of control. C_2, C_3, C_4 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_2C_2, G_2C_3, G_2C_4, respectively that were > G_3C_2, G_3C_3, G_3C_4 which achieved 195, 204, 220 % of control respectively. Aside, G_4C_2, G_4C_3, G_4C_4 excessed significantly up to 141, 182, 195 % of control beside rectify actuation of G_4 alone application.

iii. Safronal mg/g stigmas (SA, mg/g ST)

Table 2 and Figure 8 declared SAmg/g increased significantly in response to G_2, G_3 up to 208, 190% of control (0.230 mg / g ST; on the contrary G_4 cause to decrease SA to 85% of control. C_2, C_3, C_4 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_2C_2, G_2C_3, G_2C_4, respectively that were above G_3C_2, G_3C_3, G_3C_4. Concerning G_4C_2, G_4C_3, G_4C_4 applications, performed only significant increment SA up to 157, 183, 193% of control and rectify G_4 alone application.

iv. Antioxidant Activity (AOA)

Table 2 and Figure 9, explained that G_2, G_3 actuated significant augment (AOA) up to 183, 152% of control (30). C_2, C_3, C_4 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_2C_2, G_2C_3, G_2C_4, respectively which over that of G_3C_2, G_3C_3, G_3C_4 led to 187, 213, 227% of control, respectively. Meanwhile, G_4C_2, G_4, C_3, G_4C_4 acted significant augment up to 157, 183, 193% of control beside overcoming G_4 alone application.

3.2.3. Daughter Saffron Corms Yield g/m² (DCMy, g/m²)

Table 2 and figure 10 declared G_2, G_3 acted significant appraise DCM up to 125, 117%, of control (80.5g./m2= 810kg/ha), on the contrary G_4 resulted in significant reduction DCM for 97% of control. C_2, C_3, C_4 invoked significant augment up to 128, 132, 138% of control GXC interacted applications; G_2C_2, G_2C_3, G_2C_4 achieved significant synergistic increment up to 131, 135, 142 % of control that transient G_3C_2, G_3C_3, G_3C_4 application values 130, 133,135 % of control Meanwhile, G_4C_2, G_4C_3, G_4C_4 actuated significant increment by 121, 125, 130% of control, aside rectiyed G_4 application.

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

G	C	STYg/m²		CR mg./g.ST		PImg/g.ST		SA mg/gST		AOA%		DSCY, g/m²	
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_1 -4 (0,5,15,25 Gy, respectively).
C_1-4 (0,50,100,200mg/L chitosan, respectively).
Values between parenthesis were percent of control.

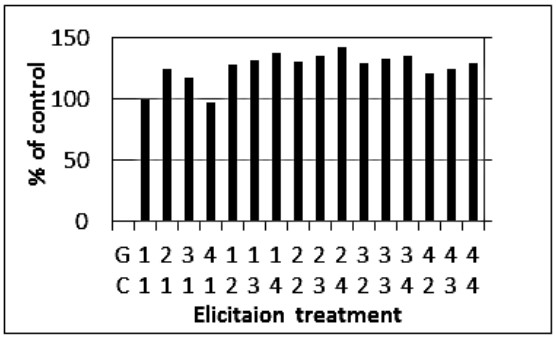

Figure 5. *Stigma yield, g/m².*

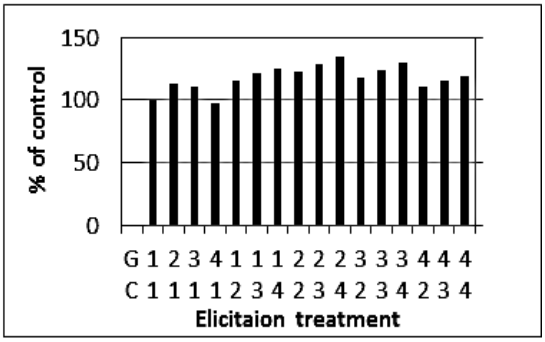

Figure 6. *Crocin, mg/stigmas.*

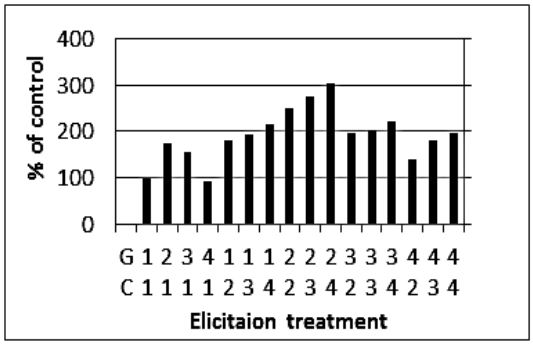

Figure 7. *Pircrocin, mg/g.stigmas.*

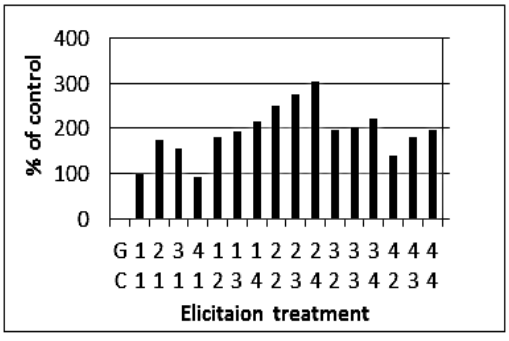

Figure 8. *Safronal, mg/g.stigm.*

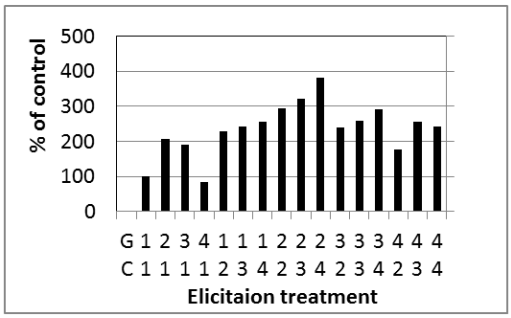

Figure 9. *Antioxidant activity.*

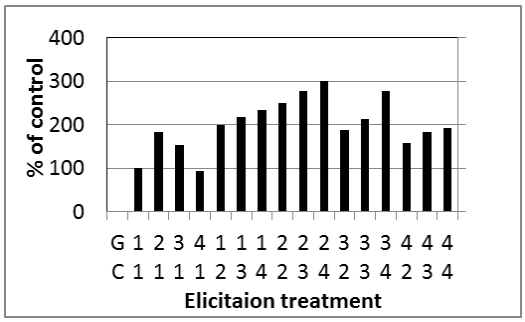

Figure 10. *Daughter saffron corms yield g/m^2.*

4. Discussion

The results Were attributed to, gamma irradiation that Stimulate biochemical and physiological processes that lead in 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 tern improved growth and yield traits (38,40-42) Integrated G_2, G_3 With C_2, C_3, C_4 Performed Significant Synergistic enhancement growth, yield, bioactive secondary metabolites and anti-oxidant activity that exceeded $G_{2,3}$ an $C_{2,3,4}$ 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 reliable Strategy to Significant enhancement yield and quality for Saffron (Crocus sativus L.).

Conflict Case

There is no conflict with any person concerning this manuscript.

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