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Practical field Application for Elicitation Efficacy with Gamma Irradiation, Nano- Selenium- Oxide and / or Saccharomyces Servisiae as Elicitors Mediated Amelioration Biomass, Flavonoids, Rutin and Antioxidant capacity of Tartary Buckwheat

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Abstract:- For 3 replicate. Statistical analysis of variance for; grain biomass yield (GBY), rutin leaves content (RLC),total flavonoids content (TFC) and rutin content (RC) for grains as well as total antioxidant capacity (TAOC), revealed that both 3 elicitors (G,S,Y) achieved significant enhancement for these 5 traits aside biotic elicitor surpassed Nano- abiotic elicitor (S) that transient physical elicitor (G).Even more, all combined elicitors, i.e. interactions (GS, GY, SY, GSY). Exhibited synergistic significant increment for all 5 traits. Alongside the highest and best positive effect were performed for(G2S3Y3> S3Y3> G2Y3> G2S3. Over all, the results of this study suggested that Yeas - elicitation integrated with gamma irradiation and for name – selenium oxide coed be considered as cogitative – oriental strategy to significant Synergistically enhance biomass grain yield, flavonoids, rutin, and antioxidant activity in TBW.

***Keywords**:- Buckwheat, Tartary buckwheat, Elicitation, Gamma- irradiation, Nano- Selenium Particles, Flavonoids, Ration, Antioxidant capacity, Biotic electors, Abiotic elicitation, Physical elicitor.*

I. INTRODUCTION

Buckwheat, a very important edible medicinal plant, has been praised as one of green food for humans in the 21 century [1]. Over recent years the close connections, food and health has become increasing important for consumers, food industry and scientists. Thus, healthy, added – value foods containing bioactive compounds are constantly entering the market. Among grain corps, buckwheat (Fagopyrum spp) is gaining momentum because of its content of such healthy compounds [2]. Buckwheat is a minor crop long to the polygonaceae family that can be considered as sustainable crop for its low impute requirement. It has a short growing span [3], it is produced in almost countries where cereals are grown [4], and also the leading continents producers are Europe and Asia [5]. Buckwheat has wide ecological adaptability, is grows well in infertile land, in adverse climatic conditions and without the use of pesticides and fertilizers.

Buckwheat has played an important role in diets around world over the last 8000 years, mainly in Eastern Europe and Asia [6]. It is considered an important pseudocereal owing to its medicinal and agricultural value. It has various allopathic [7] and medicinal; anti- hypertensive [8], anti- allergic [9]; antibacterial [9-12], protective capacities on DNA damage [13]. These properties are attributed to various nutrients such as vitamins, minerals, amino acids, fatty acids, dietary fibers, phenolic compounds and flavonoids [12,14]. Additionally, buckwheat gluten – free and thus provide an important alternative nutritious food for people with celiac disease [15]. Also, buckwheat seeds are important source that can be processed into various food products such as tea, noodles and pastries [10, 11,16] and BW- Seed – sprouts [17-20]. Buckwheat – seed sprouts, germination process, is the only food processing which provides a significant increase the bioactive secondary metabolites; polyphenols, flavonoids and flavonols during the seven days of germination. Therefore, sprouts can be considered good candidate ingredients for functional foods to be used for lowering the risk of various diseases and / or for exerting health – promoting effects in addition to its nutritive value [17-21]; Exceedingly grains, hay or silage buckwheat can be fed to cattle, sheeps, pigs, goats and poultry, it features similar quality to millet forage [22] but with higher, concentration of protein, 14-15% [23,24].

Investigation of the chemical composition and active principles of buckwheat revealed that grains were rich in constuents, which could be used in contemporary phototherapy. The most important constituents in buckwheat are flavonoids [25-29], in particularly rutin [8], [30- 32] which exhibit various health benefits [33– 38]. The antioxidant capacity of Tartary buckwheat grains is 3-4 times higher than that common buckwheat (CBW) grains, the rutin content in TBW grains is approximately 100 tines that in CBW grains [39]. Additionally, the free phenolic content in TBW is 23 to

45 and 25 to 50 folds higher than those in corn and wheat, respectively [40]. rutin extracted from (TBW)have protective and have possible application for treatment of Alzheimer's disease (AD) [35].

The antioxidant potential of BW seeds is determined mainly by phenolic compounds [43]. BW seed contain low molecular weight phenolic, phenolic acids and flavonoids [28,29]. The predominant phenolic compound is rutin, main flavonoids [41,42]. Rutin contribution in BW seeds play a significant role in the antioxidant capacity of BW seeds [30]. These authors demonstrate a strong correlation between rutin content assayed for seeds of various cultivars / accession and their antioxidant capacity (r=0.976). A statistically significant correlation (r=0.987) also reported by [43] who compared rutin content with antioxidant of seeds and other aerial part of BW. For whole CBW seeds, the contribution of the rutin in the antioxidant capacity was estimated at 25%. Elicitation, with physical [44], abiotic [45,46], biotic [47-49] elicitor is one of few strategies that commercial application in the improvement of bioactive secondary metabolites production from plants as well as cell and organ cultures [50-52,3,53].It was, therefore, through of trying to find reliable tool to assure further improvement biomass production and bioactive secondary metabolites, under field and in vitro conditions the present study has also conducted.

II. MATERIAL AND METHODS.

- *Elicitor*

Physical elicitor: Tartary buckwheat (TBW); fagopyrum tatricum, grains (8% moisture) were subjected to 0, 20, 25 GY gamma irradiation doses / at 1.5 KGY (G1-3) / h, abiotic elicitor: Nano- selenium – oxide particles (S1-3) solution at 0, 20, 40ppb concentrations with 0.01% (V/V) solution of Tween – 20 as surfactant. Biotic elicitor: saccharomyces cervisiae (Y) dissolved in water of concentration 0,0.1,1.0% (W/V) with 0.01% (V/V) solution of Tween – 20 as surfactant. (Y1-3)

- *Planting*

At 10 February (2017) gamma irradiated grains were planted in trays contained soil, sand, beat nixed (1: 1: 1 ratio V/V) subsequently established in greenhouse. After 7 days, seedlings were fertilized with nutritive solution; 1.5gm Zn, 0.49gm Cu, 1.2gm. Fe, 1.29mgB, 0.29 gm. Mo/20L water. Seedlings 3 weeks- age were transplanted to the feed (Sandy soil) in plots 4x7m consisted 10 rows, 20cm apart 7m long and 20Cm interspacing (350plant / plot, i.e.12.5 plant / m2 to give target plant population 52500 plant / Fadden.(125000 plant / h.).

The layout of the field experiment in split – split plot design were conducted with three replicates (3R). the main plot for gamma ray treatments (3G), Nano- selenium= particle (3S) sub- main plot and yeast (Y) sub- sub main plot, that form 81 plots3Rx3Gx3NSx3Y). plants were irrigated with brackish shallow well water, 1100ppm and were fertigated at 5 weeks from planting with 1.4: 2 NPK, 450Kg/h., through surface irrigation system.

- *Elicitor application*

Plants that were resulted from seeds subjected to G1-3 as physical elicitor, at 8 weeks age, were foliarly sprayed with S1-3 and Y1-3 elicitor.

- *Biometric field traits*

Just before flowering, at 1st April, 5 reprentative plants, at random, from each plot, leaves samples were undertaken for rutin content. Whereas, at 20 June plants for each plot were harvested and were quick dried through exposed to sunlight and open air until reach humidity of 12% for grains. Then grain yield per plot and per Fadden were quantitated and converted to grain yield / h (GBY).

- *Bioactive secondary metabolite evaluation*

- Extraction

Dry leaves and whole grain sample / plot were extracted by ultrasound assisted extraction (UAE) was used for flavonoid extraction, according to [12], under the following conditions; ethanol concentration 65%, solid: liquid ratio of 1:4 gm/ml, extraction time of 35min. and extraction temperature of 35min and extraction temperature of 65℃.

- Grain Total flavonoids contents (GTFC)

An aliquot, ml of phenolic / extract was mixed with 1.4 ml of solution containing 25g/L sodium nitrate and 50g/L aluminum nitrate. After 6min, 5ml of 1M sodium hydrate solution were added up to a total volume of 25ml with 95% ethanol. The solution was mixed and incubated at room temperature for 10min. the absorbance was read at 510 n m spectrophotometry (54) and the flavonoid content was calculated with a calibration curve of rutin and expressed as mg quercetin equivalents per gram d .w. grains (mg QE /g.d.w.) per gam of buckwheat grains.

- Rutin content in leaves (RLC) and grains rotin content (GRC)

HPLC analysis was monitored at 320 nm and completed using a kromasil 100-5C18 Colum (250mm x 4.6mm 5 μm). Two mobile phase were prepared for mixing: The ratios of methanol: water : acetic acid for mobile phase A and B were (V/V/V) 5: 92.5: : 2.5 and 95: 2.5: 5, respectively. The column was maintained at 35℃ and the flow rate was set at 1.0mL min -1. The injection volume was 20μL. flavonoids were identified by comparing the retention time with those standard compounds and the peak area was calculated to assess the content of rutin (mg/g d .w).

- *Total antioxidant capacity (TAOC):*

The DPPH radical scavenging activity was tested by the method of [55] Briefly, various treatments under investigation,. DPPH solution was also prepared by dissolving 6.0mg. of DPPH in 100ml methanol then, 1.0ml of from treatment was added into the test tube containg 2.0ml of DPPH solution control was prepared by adding 1.0 of methanol to 2ml of DPPH solution. The mixture was shaken vigorously and was left to stand in the dark for 30min. the absorbance of the resulting solution was measured spectrophotometricaly at517nm. The scavenging activity of DPPH radical was calculated using the following equation: Scavenging activity (%)=(1-(A sample at 517nm)/(A control at 517nm))×100

➢ *Statistical analysis:*

The obtained data were subjected to computerize analysis of variance using M- state program. The differences between means were statically tested by the calculated LSD at 1% level.

III. RESULTS

Statistical analysis of variance reveled that both physical (G), Nano – abiotic (S) and biotic (Y) elicitor as well as their interaction (i.e. combinations); GS, GY, SY, GSY; affected significantly on biomass grains yield /h (BGY), leaves rutin content (RLC), grain total flavonoids content (GTFC), rutin – grain content (GRC) rutin leaves content (RLC) and total antioxidant activity whereas the differences between mean treatment were tested according the calculated LSD at 1% level.

➢ *Biomass grain yield, Kg/h. (BGY)*

(G20, 25Gy), (S20, 40ppb), (Y0.1, 1.0 g/L) achieved significant increment (BGY) as percent of control (100)up to (112,110) (121,126), (125,130) respectively (Table 1), that denote (Y) exceed (S) which excel (G) elicitor.wherease, GS, GY, SY and GSY performed significant synergistic enchantment up to (as % of control), 134,137,142,145, for (G20Gy S40ppb), (G20Gy Y1.0g/L), (S40ppb/ Y1.0 g/L), (G20YG S40ppb) respectively as represented in Table (1).and represented in Figure (1) .

➢ *Bioactive compound:*

- *Grain total flavonoids (GTFC):*

(G20GY, G25Gy), (S20ppb, S40ppb), (Y0.1g/L, Y 1.0g/L) led to significant enhancement (GTFC), as % of control up to resulted in (135,120), (1.72, 186), (210, 230), respectively. Whilst; (1.72, 186), (186,190), (210, 230),whereas; GS, GY, SY, GSY led to significant synergistic increment, as % of control: up to 225, 270,290,370 for (G20Gy S40ppb), (G20Gy Y0.1g/L), (S1.0g/L Y1.0g/L), respectively, as listed in Table (2)and Figure(2).

- *Bioactive secondary metabolites:*

Rutin leaves content (RLC):(G20Gy, G25Gy), (S20ppb, S40ppb), (Y0.1g/L, Y1.0g/L) increased RLC up to , as % of control (123,117), (135, 147), (142, 160) respectively (Table 2), that also declared (Y) elicitor surpassed (S) elicitor which exceed (G) elicitor, GS, GY, SY, GSY led to significant synergistic increase (as % of control) up to 173, 182, 186, 200 for (G20Gy, S40ppb), (G20Gy, Y1.0g/L), (S40ppb y1.0g/L), (G20Gy S40ppb y1.09/L), respectively (Table 3and Figure 3).

- *Grains rutin content (GRC):*

GRC at G1, S1, Y1 as control was 1.052% increased significantly in response to (G20, G25), (S20 S40), (Y0.1, Y1.0) applications up to (180, 162) (205, 230), (220, 255) percent of control, respectively. Combined; i.e interactions, GS, GY, SY, GSY led to significant synergistic increase GRC up to ; 375, 290, 365, 425 as present control for (G20S40), (G20Y1.0), (S40Y1.0), (G20S40 Y1.0) application, respectively (Table 4 and Figure 4).

- *Total antioxidant capacity (TAOC):*

TAOC for Buckwheat extract for control was 60%, while (G20GY G25G0) (S20,40ppb), (Y0.1,1.0g./l) exceed significantly up to (80%, 70%), (85%, 95%), (95%, 115%), respectively Table (5) Figure (5). Elicitor combination, GS, GY, SY, GSY achieved synergistic increase TAOC up to 130, 145, 150, 170 for combination application treatment (G20Gy S40ppb), (G20GyY1.0g./l), (S40ppb Y1.0g/L),(G20Gy S40ppbY1.0g/L), respectively as showed Table 5 and represented Figure (5).

IV. DISCUSSION

The results indicated that physical (G) elicitor, Nano abiotic elicitors and biotic (Y) elicitor achieved significant enhancement BGY/h, GTFC, RLC, GRC, TAOC aside (Y) surpassed (S) which excel (G). In combined applications (i.e. interactions), GS,GY, SY, GSY resulted significant synergistic enhancement for these five traits, this were attributed to G,S,Y have beneficial effects on morphological physiological and biochemical characters of plant that led to stimulate growth, upraise biomass yield and enhanced formation and accumulation of bioactive compounds as it has been reported for (G) elicitor [56- 58), (S) elicitor [58- 61],(Y) elicitor [61]. Strong radical scavenging activity due to resultant increment of TAOC and RC, were in agreement with has been investigated previously [62].

These findings were in accordance with that has been reported for G [56-58], [59-61] and Y [52]. Whereas, strong radical scavenging activity was due to resultant increment of TAOC and RC were in agreement with that has been investigated previously [62]. Aside, biotic and abiotic elicitors exert their effects by taking part, either directly or inatirectly in multiple signaling pathways by activating transcription genes involved in biosynthesis for Secondary metabolites through up – regulation or dower – regulation) gene expression[63-68]. Further, elicitors can have beneficial effects on morphological, physiological, biochemical characteristics, enhanced growth, biomass production and quality of the formed secondary metabolites [69-75].

V. CONCLUSION

Overall the results suggested that elicitation under field application, with G, S and / or Y seems to be reliable powerful technological tool to ameliorate GBY, GTFC, RLC, GRC for TBW. Furthermore, results, also, suggested that the expected Hyperporoduction of rutin extracted from leaves owing to high significant rutin content in leaves that were taken from elicited plants, that required for rutin pharmaceutical industries.

G, S,Y.

G,S,Y	1		2		3		LSD 1%
G	1220.2	(100)	1366.2	(112)	1344.7	(110)	12.5
S	1215.3	(100)	1470.1	(121)	1531.4	(126)	18.2
Y	1222.7	(100)	1528.4	(125)	1589.5	(130)	21.7

GS

G/S	S_1		S_2		S_3		LSD 1%
G1	1212.4	(100)	1393.2	(115)	1370.5	(113)	23.2
G2	1370.6	(113)	1539.4	(127)	1624.3	(134)	
G3	1358.9	(112)	1576.6	(139)	1564.8	(129)	

GY

Gyy	y_1		y_2		y_3		LSD1%
G1	1219.4	(100)	1463.5	(120)	1438.9	(118)	24.4
G2	1390.3	(114)	1609.7	(132)	1670.1	(137)	
G3	1414.7	(116)	1646.2	(135)	1585.9	(130)	

Sy

S/y	y_1		y_2		y_3		LSD 1%
S1	1209.2	(100)	1529.5	(126)	1487.6	(123)	26.6
S2	1450.6	(120)	1632.3	(135)	1668.6	(138)	
S3	1511.8	(125)	1693.7	(140)	1717.8	(142)	

GSY

GSY	S1			S2			S3			LSD 1%
	y_1	y_2	y_3	y_1	y_2	y_3	y_1	y_2	y_3	
G1	1217.1	1545.7	1570.1	1223.2	1577.9	1602.4	1219.6	1646.5	1683.0	9.7
	(100)	(127)	(129)	(100)	(129)	(131)	(100)	(135)	(138)	
G2	1375.3	1606.6	1646.1	1382.2	1675.8	1712.5	1378.2	1695.2	1768.4	
	(113)	(132)	(135)	(113)	(137)	(140)	(113)	(139)	(145)	
G3	1363.3	1655.3	1667.4	1369.9	1675.8	1700.2	1365.9	1707.4	1719.6	
	(112)	(136)	(137)	(112)	(137)	(139)	(112)	(140)	(141)	

Table 1:- BGY,Kg/h in response to G,S,Y elicitors and their interactions

Gamma ray doses, $G_{1\text{-}3}$(0, 20,25GY)-Selenium concentration, $S_{1\text{-}3}$(0, 20,40ppb)-Yeast concentration,$Y_{1\text{-}3}$ (0,0.1,1.0 g/L.) Values between parentheses were percent of control.

G, S,Y.

G,S,Y	1		2		3		LSD 1%
G	1.23	(100)	1.66	(135)	1.48	(120)	0.09
S	1.31	(100)	2.25	(172)	2.45	(186)	0.10
Y	1.35	(100)	2.86	(210)	3.11	(230)	0.12

GS

G/S	S_1		S_2		S_3		LSD 1%
G1	1.42	(100)	2.64	(186)	2.70	(190)	0.07
G2	1.96	(138)	3.12	(220)	3.20	(225)	
G3	1.78	(125)	3.05	(215)	3.00	(211)	

GY

Gyy	y_1		y_2		y_3		LSD1%
G1	1.56	(100)	2.89	(185)	3.06	(196)	0.08
G2	2.25	(144)	3.85	(247)	4.21	(270)	
G3	2.11	(135)	4.14	(265)	3.98	(255)	

Sy

S/y	y_1		y_2		y_3		LSD 1%
S1	1.51	(100)	3.55	(235)	3.32	(220)	0.11
S2	9.73	(181)	4.15	(275)	4.12	(273)	
S3	2.94	(195)	4.30	(285)	4.38	(290)	

GSY

GSY	S1			S2			S3			LSD 1%
	y_1	y_2	y_3	y_1	y_2	y_3	y_1	y_2	y_3	
G1	1.55	2.38	2.45	1.65	2.66	2.78	1.53	2.59	26.8	0.09
	(100)	(127)	(129)	(100)	(129)	(131)	(100)	(135)	(138)	
G2	2.19	3.02	3.10	2.32	3.98	5.20	2.17	5.43	5.66	
	(141)	(195)	(200)	(145)	(249)	(325)	(142)	(355)	(370)	
G3	1.97	2.82	3.08	2.10	4.08	4.56	2.00	4.06	4.21	
	(127)	(182)	(199)	(131)	(255)	(285)	(131)	(265)	(275)	

Table 2:- TFC,g.QE/100g.d.w.TBWgrains in response to G,S,Y elicitors and their interaction

Gamma ray doses, G_{1-3}(0,20,25$_{GY}$)-Selenium concentration, S_{1-3}(0,20,40$_{ppb}$)-Yeast concentration,Y_{1-3} (0,0.1,1.0 g/L.)
Values between parenthesis were percent of control.

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G, S,Y.							
G,S,Y	1		2		3		LSD 1%
G	3.15	(100)	3.88	(123)	3.69	(117)	0.03
S	3.16	(100)	4.27	(135)	4.65	(147)	0.04
Y	3.16	(100)	4.49	(142)	5.06	(160)	0.05
GS							
G/S	S_1		S_2		S_3		LSD 1%
G1	3.17	(100)	4.91	(155)	5.14	(162)	0.04
G2	4.31	(136)	5.17	(163)	5.48	(173)	
G3	3.74	(118)	4.79	(151)	4.69	(148)	
GY							
Gyy	y_1		y_2		y_3		LSD1%
G1	3.18	(100)	5.12	(161)	5.41	(170)	0.05
G2	4.93	(155)	5.44	(171)	5.79	(182)	
G3	4.52	(142)	4.18	(151)	5.25	(165)	
Sy							
S/y	y_1		y_2		y_3		LSD 1%
S1	320	(100)	5.12	(160)	5.38	(168)	0.06
S2	5.09	(159)	5.33	(165)	5.92	(172)	
S3	5.28	(165)	5.44	(170)	5.95	(186)	

GSY	S1			S2			S3			LSD 1%
	y_1	y_2	y_3	y_1	y_2	y_3	y_1	y_2	y_3	
G1	3.21	3.30	4.53	3.20	4.67	5.15	3.22	5.09	5.67	0.03
	(100)	(134)	(141)	(100)	(146)	(161)	(100)	(158)	(176)	
G2	4.01	4.66	5.04	4.06	5.38	5.60	4.06	6.12	6.44	
	(125)	(145)	(157)	(127)	(168)	(175)	(126)	(190)	(200)	
G3	3.82	4.24	4.53	3.84	4.86	5.12	3.90	5.51	5.96	
	(119)	(132)	(141)	(120)	(152)	(160)	(121)	(171)	(185)	

Table 3:- RL,g./100 g.&.**w** in response to G,S,Y elicitors and their interaction**.**

Gamma ray doses, $G_{1\text{-}3}$(0,20,25$_{GY}$)-Selenium concentration, $S_{1\text{-}3}$(0,20,40$_{ppb}$)-Yeast concentration,$Y_{1\text{-}3}$ (0,0.1,1.0 g/L.)
Values between parenthesis were percent of control.

G, S,Y.										
G,S,Y	1			2			3			LSD 1%
G	1.052		(100)	1.894		(180)	1.704		(162)	0.08
S	1.052		(100)	2.157		(205)	2.420		(230)	0.09
Y	1.052		(100)	2.314		(220)	2.683		(255)	0.11
GS										
G/S	S_1			S_2			S_3			LSD 1%
G1	1.065		(100)	2.556		(240)	2.396		(225)	0.05
G2	2.450		(230)	2.790		(262)	2.929		(275)	
G3	2.364		(222)	2.716		(255)	2.780		(261)	
GY										
Gyy	y_1			y_2			y_3			LSD1%
G1	1.071		(100)	2.699		(252)	2.870		(268)	0.06
G2	2.581		(241)	2.945		(275)	3.106		(290)	
G3	2.517		(235)	2.870		(268)	2.945		(275)	
Sy										
S/y	y_1			y_2			y_3			LSD 1%
S1	1.062		(100)	2.942		(277)	3.069		(289)	0.08
S2	2.708		(255)	3.133		(295)	3.239		(305)	
S3	2.570		(242)	2.995		(282)	3.876		(365)	
GSY										
GSY	S1			S2			S3			LSD 1%
	y_1	y_2	y_3	y_1	y_2	y_3	y_1	y_2	y_3	
G1	1.058	1.852	2.857	1.068	2.777	3.290	1.069	3.742	4.030	
	(100)	(175)	(270)	(100)	(260)	(308)	(100)	(350)	(377)	
G2	1.957	2.325	3.703	1.965	3.898	4.196	1.956	4.272	4.543	
	(185)	(220)	(350)	(184)	(365)	(390)	(183)	(400)	(425)	
G3	1.746	2.190	3.269	1.837	3.759	4.005	1.869	4.009	4.073	
	(165)	(207)	(309)	(172)	(352)	(375)	(175)	(375)	(381)	

Table 4:- GRC % for grains in response to G,S,Y elicitors and their interaction.

Gamma ray doses, $G_{1\text{-}3}$(0,20,25$_{GY}$)-Selenium concentration, $S_{1\text{-}3}$(0,20,40$_{ppb}$)-Yeast concentration,$Y_{1\text{-}3}$ (0,0.1,1.0 g/L.)
Values between parenthesis were percent of control.

Application:	TAC%	
Control	60	100
G_2	80	133
3	70	117
S_2	85	142
3	95	158
y_2	95	158
3	115	192
G_2S_2	120	200
3	130	217
G_3S_2	105	175
3	110	183
G_2y_2	130	217
3	145	242
G_3y_2	125	208
3	135	225
S_2y_2	125	208
3	135	225
S_3y_2	140	233
3	150	243
$G_2S_2y_2$	140	233
3	150	250
$G_2S_3y_2$	145	242
3	170	283
3	120	200
$G_3S_2y_2$	120	200
3	130	217
$G_3S_3y_2$	130	217

Table 5:- TAOC for grains extract in response to G,S,Y elicitors and their interaction

Gamma ray doses, G_{1-3}(0,20,25$_{GY}$)-Selenium concentration, S_{1-3}(0,20,40$_{ppb}$)-Yeast concentration,Y_{1-3} (0,0.1,1.0 g/L.)
Values between parenthesis were percent of control.

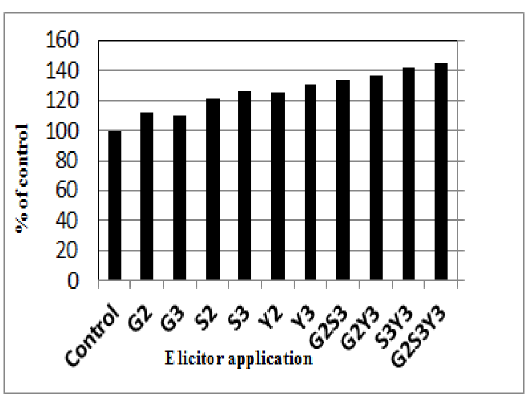

Fig 1:- BGY/h as% of control

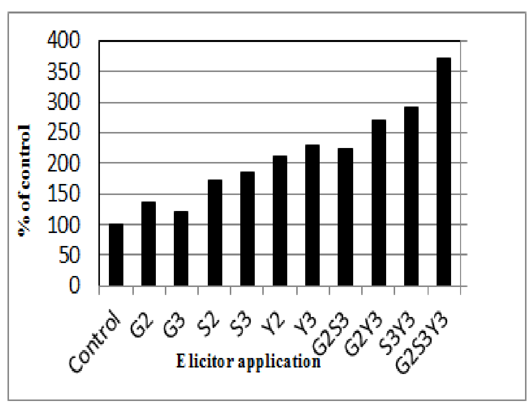

Fig 2:- GTFC as% of control

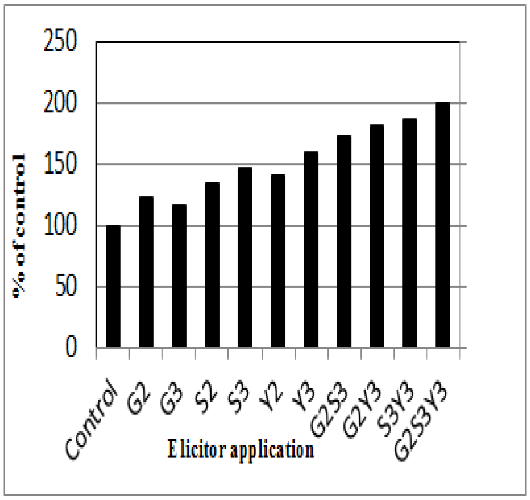

Fig 3:- RLC as % of control

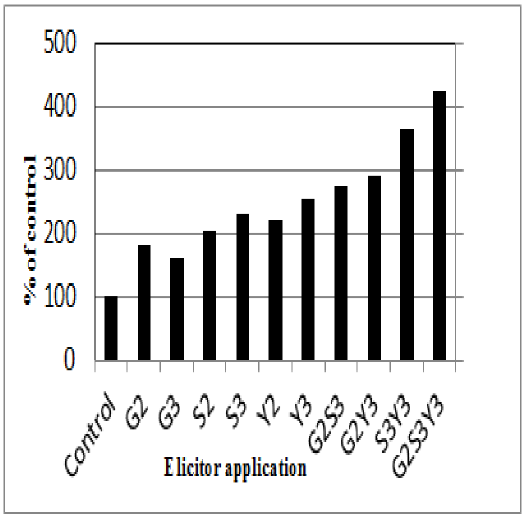

Fig 4:- GRC as % of control

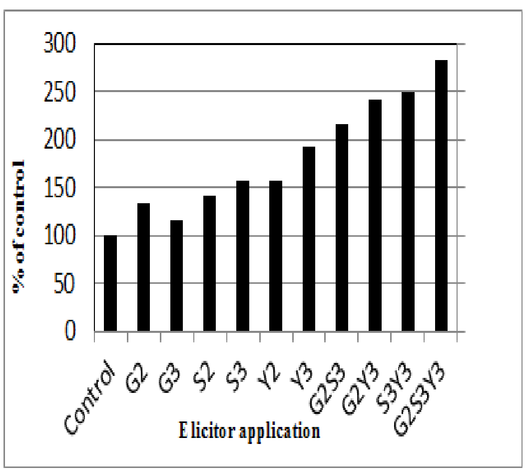

Fig 5:- TAOC as % of control.

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