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Phytoalexin Elicitation- Potency As A Novel Technology for Biological Control and Protection *Digitalis purpurea* L. plants from Pre-Sowing Seed Treatment with Gamma Ray and Electric Current

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ABSTRACT

***Digitalis purpurea* L seeds treated with gamma ray, (G) 0, 2.5, 5, 7.5 KR, and electric current (E), 0, 100, 150, 200 mA, then planted in splite-splite plot design for 3 replicates (R) and 2 successive seasons, in sandy soil irrigated with brackish water (900ppm) through surface drip irrigation system (SDI).The formed plants were foliar sprayed with manganese (M),0,3ppm-The formed Phytoalexin (PA) was bioassayed and chemically quantified.M3ppm could induce sistemic resistance (ISR) which initiate to farm 0.064, 0.070 mg PA 100mg fresh leaves. The formed PA exhibited PA-glycosidal structure wherefore, achieve therapeutic potency. (G) depress significantlly PA 73-91% of control while (E) activated (PA) significantly 134-154% of control. At (GE) interaction, such (G) dose PA increased significantlly by increasing (E) dose up to E200mA. Hence (E) efface a serious depression of (G). At (GEM) interaction, (G) 2.5, 5, 7.5 KR EO mA, M3ppm the formed (PA) were ; 91, 75, 63 were increased significantlly by increasing (E) dose up to E200mA M3ppm 128, 119, 109 – 129, 117, 107% of control for first and second season, respectively. Therefore, M elicitor application, in GEM combination, could be considered as a novel strategy for biological control and plant protection, from economic and environmental benefit point of view. It would occur by decreasing the cost of fungicides, bactericides and pesticides in *Digitalis purpurea* L biomass production, grown in extended agriculture area.**

Key Words: Phytoalexin/ Elicitors/ Secondary – Metabolites/ Gamma Ray/ ElectricCurrent/ Induced Resistance / Stress Response.

INTRODUCTION

Utilization of gamma rays for crop improvement achieve serious depressing innate defense mechanism [1-3] that aggravate the predisposing factors to infectious agents; especially at semi-tropical agriculture, as EAR, where the extending agriculture are against the sever conditions [4] of developing saline, sandy area and use low quality water. These deviation from environmental condition could be conside as stresses that leading as predisposing factors. Phytoalexin (PA), stressed secondary metabolite and natural antibiotic synthesis de novo in response diverse forms of stress; fungi, [5,6] bacteria, [7] reckettcia, [8] viruses [9], nematode [10] and insect as [11] biochemical defense mechanism [12,13] which inhibit the growth of parasite [14]. More than 350 PAs have been chemically characterized from 30 plant families, including medicinal plants, contributing to many of major group of secondary metabolites including; glycosides, alkaloids, terpenes, flavonoids, isoflavonoids, stilbenes, simple phenolic compounds [15]. Wild species formed about abundant PA more than cultivated varieties [16]. At that, electric current could enhance biosynthesis and accumulation of these secondary metabolites [17] and PA as well [18]. On the contrary, gamma rays inhibit PA formation [3] as well as long UV radiation [19].

PAs also produced by biotic elicitor; non-pathogenic agent [20], osmotic stress [21] and abiotic elicitor; heavy metals and their salt [22] growth regulators and tranquilizers [23], sulfur compounds[24], oligosaccharides [25], physiological agents such as short UV [26] and wounding[27]. The use of elicitors could induce systemic resistance (ISR) in plants against parasite agents through induced activation of formation and accumulation of PAs [28,29]. PAs, resveratrol, glycoside structure [30,31] have a wide array of biological activities such as anti-inflamemmatory, reduced heart diseases (arterioclerosis, anti-angionic) and are also recently reported to be potentially important on human health as natural chemopreventive and chemotherapeutic, supestitutive to synthetic anti-cancer that used in clinical trails but sever effects are serious problem for them. [32,33] Owing to hereinbefore, the present investigation targeted to validity and reliability of practical application of PA elicitor, from technological point of view, to protect and biological control for Digitalis purpurea L plants from pre-sowing seeds treated with gamma rays and electric current.

MATERIALS AND METHODS

Seed treatment and field experiment execution.

Wild Digitalis purpurea L seed, imported from Thompson and Morgan (UK) Ltd., Were pre-sowing subjected to 0, 2.5, 5, 7.5 KR gamma rays, emitted from Cobalt-60 source followed by 0, 100, 150, 200 mA for 60 seconds in an electric cell filled with 0.25% potassium nitrate and 0.25% calcium nitrate (1:1). Hereafter, sown in greenhouse for two subsequent seasons, the layout of such season was applied by transplanting seedlings in sandy soil, at Sharkia province, in splite-splite plot designee with 3 replications. Seedling were planted under surface drip irrigation system, PE 18mm rows 60cm apart on drips 4L/h.35 cm apart, irrigated and fertigated with brackish water; shallow well depth below 15m., 900ppm. The unite area was consisted a plat 1/200 Feddan. Plants 4 month old as well as every month were foliarly sprayed with 3ppm manganese sulfate (Mn SO_4 H_2O) with 0.5V/V Tween-80 as surface active agent and pH was adjusted to 6 by H_2SO_4 .

PA, extraction, bioassay detection and quantitation.

After 48 hours from Manganese application for 6 month old plants, fresh leaves samples were extracted for glycosides with chloroform-methanol (1:1), evaporated on water bath at 60-80^oC then stored in refrigerator, 2ml chloroform-methanol dissolved the residue of the extract, then were used for spotting with known digitoxin and gitoxin on TLC plated coated with silica gel-254, 250 µm thickness activated for 1 hour at 110^oC. TLC plates were developed by Benzene-Ethanol (4:1) saturated with 75% water, let for dryed to remove traces of developing solvent. For bioassay detection TLC sprayed with a dense spore suspension of Cladosporium Czapec dox solution (a glucose mineral medium at pH5). The plates were then incubated in dark at 30^oC for 5 days. [34] PA apparent indicating by white areas where fungus growth has been inhibited, then R_f was determined for PA area. TLC plat, spotted with the extract and known bioactive glycosides, digitoxin and gitoxin and developed with the previous solvent, after development sprayed with trichloroacetic acid 25% in chloroform aged by few drops of hydrogen peroxide, [35] for determination R_f for the spots. For quantitation PA, silica gel layer from each zone (R_f) were quantitatively scraped then extracted with 10ml. chloroform and was dried completely by heating on hot air (110^oC) oven. For each residue 5ml Baljet reagent [53] was added for colorimetric measurement mg PA as mg gitoxin /100gm. fresh leaves.

Statistical analysis

The obtained data were subjected to statistical analysis by computer using SPPS-15 Windows Evaluation Version, as splite-splite design. Whereas, the differences between means were statististically tested by the calculated LSD at 1% level [36].

RESULTS AND DISCUSSION

Phytoalexin (PA) did not detected at zero manganese (Mo) as well its interaction with gamma ray (G), and electric current (E) under investigation. Whereas, M3ppm could induce PA, which achieve glycoside structure, formation of 0.071 mg/0.076 mg /100g fresh leaves, for the first and second season, respectively as presented at Table (1). Wherefore, the formed PA it may consider as natural cancer preventing agent, novel – plant derived natural drug. [30, 32] Owing to the reason that it inhibit of DNA topoisomerases enzymes, cellular enzymes that changes the topological state of DNA through breakage and rejoining of DNA strand. Moreover, that PA substitute synthetic anticancer drugs as inhibitors of topoisomerases that have been developed and use in clinical trails but severe effects are serious for them.[31,33] M3ppm achieved its elicitation to induced systemic resistance (ISR) in Digitalis purpurea L. plants through induced activation of formation and accumulation PA, [23,27] was attributed to its effect on gene expression [66] since, PA metabolism are under unusual genetic control [3]. Statistical analysis indicated that G, E, M, and their interactions, GE, GM, EM, GEM highly significant affect on the formed PA. the differences between mean treatments were tested for its significancy by the corresponding calculated LSD at 1% level. (Tale :1).

G within EM suppressed significantlly PA for 90-73, 91-75% of control at first and second season, respectively. Also, GM decreased PA for 91-63% of control for the two seasons. This finding sustain by-effect for utilization G, even low doses, in weakening innate systemic resistance. On the contrary, an activation (ISR) by inhaced formation and accumulation for significant increase 138-154, 134-148% of control due to E and 138-151, 139-153% of control due to EM at first and second season, respectively. More or less similar trends would be declared regarding E in increasing formation of secondary metabolites include PAs [28]. The inhibition of G and stimulation of E attributed to difference in the wave length of radiation [19]. GE resulted G 2.5, 5, 7.5 KR form 91, 75, 63% and 91, 74, 63% of control at Eo, were found to increase by increasing E up to 128, 119, 109% and 129, 117, 107% of control at E200 mA for the first and second season, respectively. The same trend has been achieved at GEM, G 2.5, 5, 7.5 KR formed PA 91, 75, 64% and 90, 74, 63% of control at Eo mA increased significantly by increasing E dose up to 128,119, 109% and 129, 117, 107% of control at E200mA for the first and second season, respectively as expressed in table (1) and represented at Fig.(1).

Overall, the results assured the reliably for practability of elicitor (M), as novel strategy for biological control and plant protection in Digitalis purpurea L. This conclusion was in harmony with those has been reported. [28,29]

CONCLUSION

The results strongly attained significantly the validation of abiotic elicitor to induce systemic resistance that initiate (PA) formation and accumulation for biological control and plant protection of *Digitalis purpurea* L plants from seeds treated with gamma ray and electric current grown in sandy soil irrigated with brackish water through surface drip irrigation system.

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Table(1): phytoalexin, as mg.digitoxin / 100g.fresh leaves for *Digitalis purpurea L.* in response to pre- sowing seed tretment with gamm(G1-4:0,25,50,75,GY.) , electric current (E1-4 : 0,100,150,200mA.), foliar plant application with manganese (M1-2 :0,3PPm) for the formed plants and their commbinations (GE ,GM ,EM ,GEM for two susequent seasons .

S.V.	Means				LSD 1%	S.V.	Means				LSD 1%
	First season 2008-2010 .						Second season 2009-2011 .				
R1-3	0.035	0.036		0.036	-	R1-3	0.037	0.037		0.039	-
G1-4	0.041(100)	0.037(90)	0.033(80)	0.030(73)	0.003	G1-4	0.044(100)	0.040(91)	0.036(82)	0.033(75)	0.002
E1-4	0.026(100)	0.036(138)	0.038(146)	0.040(154)	0.005	E1-4	0.029(100)	0.039(134)	0.041(141)	0.043(148)	0.004
GE 1	0.032(100)	0.041(128)	0.044(138)	0.046(144)	0.002	GE 1	0.035(100)	0.044(126)	0.047(134)	0.050(143)	0.003
GE 2	0.029 (91)	0.037(116)	0.039(122)	0.041(128)		GE 2	0.032 (92)	0.040(114)	0.042(120)	0.045(129)	
GE 3	0.024 (75)	0.035(109)	0.036(113)	0.038(119)		GE 3	0.026 (74)	0.038(109)	0.040(114)	0.041(117)	
GE 4	0.020 (63)	0.033(103)	0.034(106)	0.035(109)		GE 4	0.022 (63)	0.037(106)	0.037(106)	0.037(109)	
M1-2	0.000		0.071		0.001	M1-2	0.000		0.076		0.002
GM 1	0.000		0.081(100)		0.002	GM 1	0.000		0.088(100)		0.004
GM 2	0.000		0.073(90)			GM 2	0.000		0.080(91)		
GM 3	0.000		0.067(83)			GM 3	0.000		0.072(82)		
GM 4	0.000		0.060 (75)			GM 4	0.000		0.066(75)		
EM 1	0.000		0.053(100)		0.002	EM 1	0.000		0.057(100)		0.004
EM 2	0.000		0.073(138)			EM 2	0.000		0.079(139)		
EM 3	0.000		0.076(143)			EM 3	0.000		0.083(146)		
EM 4	0.000		0.080(151)			EM 4	0.000		0.087(153)		

GEM	E1		E2		E3		E4			E1		E2		E3		E4		
	M1	M2	M1	M2	M1	M2	M1	M2		M1	M2	M1	M2	M1	M2	M1	M2	
1	0.000	0.064 (100)	0.000	0.081 (127)	0.000	0.087 (136)	0.000	0.092 (144)	0.003	0.000	0.070 (100)	0.000	0.088 (126)	0.000	0.094 (134)	0.000	0.100 (143)	0.004
2	0.000	0.058 (91)	0.000	0.074 (116)	0.000	0.087 (122)	0.000	0.082 (128)		0.000	0.063 (90)	0.000	0.081 (116)	0.000	0.084 (120)	0.000	0.090 (129)	
3	0.000	0.048 (75)	0.000	0.070 (109)	0.000	0.073 (114)	0.000	0.076 (119)		0.000	0.052 (74)	0.000	0.076 (109)	0.000	0.08 (114)	0.000	0.082 (117)	
4	0.000	0.041 (64)	0.000	0.066 (103)	0.000	0.067 (105)	0.000	0.07 (109)		0.000	0.044 (63)	0.000	0.071 (101)	0.000	0.073 (104)	0.000	0.075 (107)	

N.B: Number of observations for calcution the tabulted means were: 32(R1-3 replications) ,24(G1-4), 24(E1-4),8(GE),48(M1-2),12(GM),12(ME),3(GEM). Values between parenthesis were percent of control .

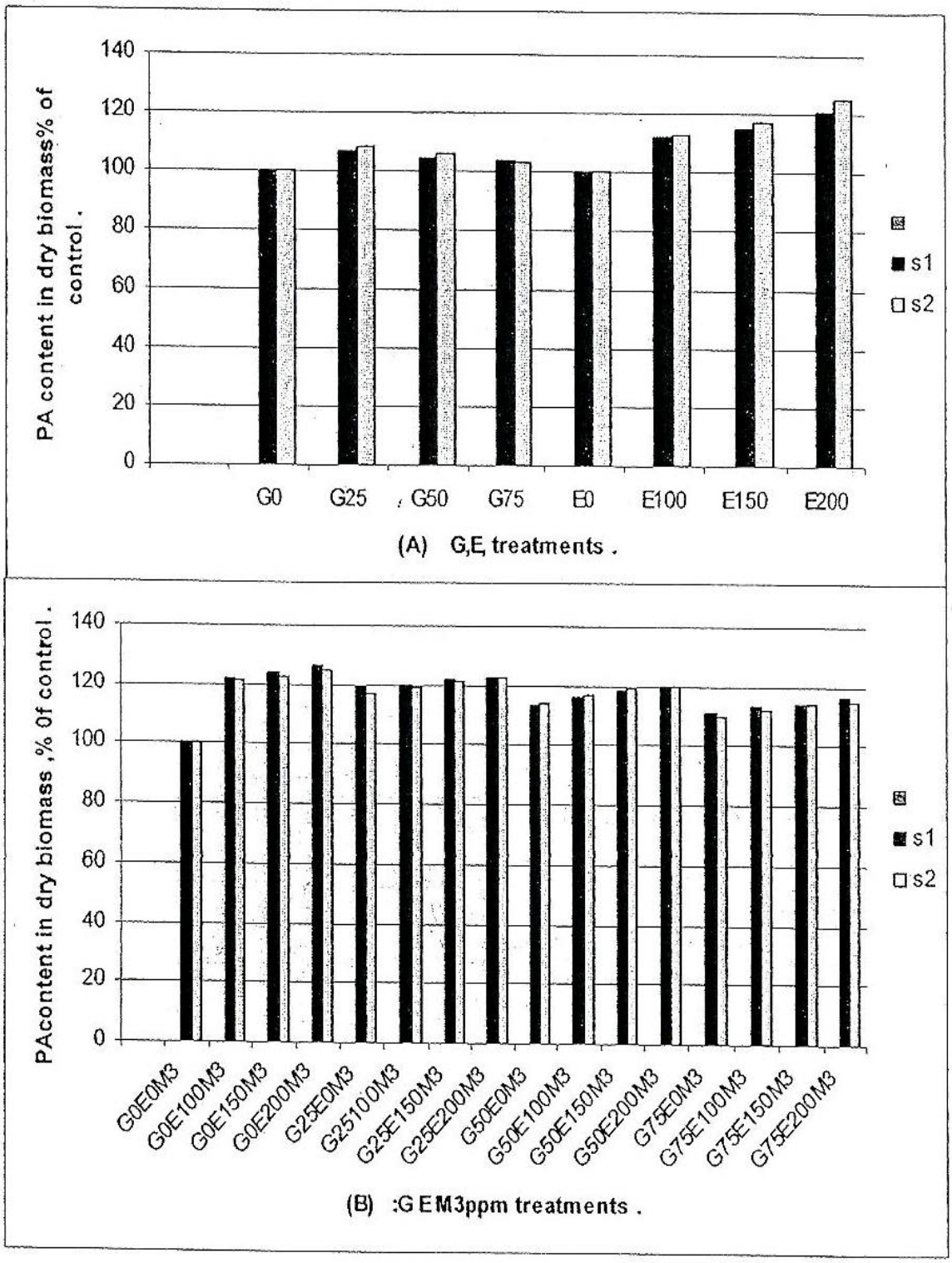

Figure (1): Elicited PA by M3ppm , as percent of control , in response to G,E and GEM treatments .

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