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Verification and Validation of Dandelion (Taraxacum officinal) Seeds- Gamma Irradiated under Elicitation with Nano- and Micro- Zinc for Potential Optimization Biomass and enhancing Phenolics, Flavonoids and Antioxidant Activity

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Abstract:- Dandelion, Taraxacum officinal (TO)is medicinal wild plant, for its medicinal raw material high contents of, bioactive secondary metabolites which possess relief and treatment of several diseases.. Before flowering, plants were picked – up for quantification biomass fresh leaves and roots Kg and its quality for determination of total phenolics for leaves (TPCL) and roots (TPCR) total flavonoid for leaves (TPCL) and roots (TFCR) as well as antioxidant activity for leaves (AAL) and roots (AAR) extract. Then obtained data were subjected to statistically analysis of variance, that declared that G25, 35,50Gy; N20, 40ppb and M2 ppm actuated positive significant biomass production over that of control, On the side (G) excel (N) which surpassed (M) application. On the contrary, (N) 60ppb and M4, 6ppm achieved significant biomass reduction. TPC, TFC and AA were signicantly enhanced over that of control in response to N20, 40ppb transcend G25, 35 Gy that excel M2ppm.Meanwhile, N60ppb,and M4,6ppm reduced significantly these four traits. These results give evidence to sustain nanoparticle zinc(N) as oriental biotechnological potential tool substituted micro zinc (M) or gamma irradiation for optimization biomass production and enhancing bioactive secondary metabolites, phenolics and flavonoids and antioxidant activity for dandelion wild medicinal plant.

Keywords *:- Dandelion, Gamma irradiation, Nanoparticles, Elicitation, Medicinal plant, Phenolics, Flavonoids, Antioxidant, Secondary metabolites.*

I. INTRODUCTION

Dandelion, Taraxacum officinal L. (TO) is medicinal wild plant (Family Asteraceae) used as a food martial owing to its high nutritive value[1,2,3] and used for centuries as traditional medicine in relief and treatment of several diseases [4,5,6.3,7,8). The entire TO plant (roots, leaves, flowers) is medicinal raw material and has received increasing attention due its health beneficial properties and pharmaceutical function according to its high contents of bioactive secondary metabolites which possess; anticancer [9,6,7,10], antioxidant [11,2,12,13,14,11], antiobesty [16,17,18,8]. Ant-inflammatory [19,20,21,22], anti- diabetic [13]; anti-microbial [23,7,24,25,26] anti-HCV [27], hepato protective [28], divretic [29] Also, TO has shown therapeutic in Alzheimer's Parkinsonism disease [30], lowering the risk of heart diseases and enhance the immunity or immune system [3.31,32] and could be used in foods with the potential to delay the onset of diseases linked to metabolic derived reactive oxygen species, ROS, [33,2] These uses are attributed to the presence, mainly of phenolics and flavonoids and the others [34,35,36].

Gamma irradiation [37,38,39,40,41,42,43] and elicitation[44,45] have beneficial effects on morphological, physiological and biochemical characteristics of plant that led to stimulate growth, upraise yield production and enhanced formation and accumulation of secondary metabolites.

Nano biotechnology, through nanoparticles application have been increased in agriculture, industry, medicine and military [4, 46] Nanoparticles can be beneficial to for future application of plants as a noble metal nanoparticles are very attractive and Co-friendly alternative to chemical methods [47,48,49,50,51,52,53].

On the faith of that has been mentioned herein before, this comparative study was conducted to verify, validation of gamma irradiation Nano and micro zinc for potential optimization biomass and enhancing. bioactive secondary metabolites, phenolics, flavonoids, and antioxidant activity, for dandelion (TO) wild medicinal plant.

II. MATERIAL AND METHODS

A. *Execute field experiment*

At 1st April,(2017) Dandelion (TO), Seeds were subjected to gamma irradiation (G) for 0,25,35,50Gy doses, emitted from C60, source at 1.5 KGy/h, directly were sowing into trays contained soil, sand, beat mixed (1: 1: 1 ratio V/V)

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subsequently established in greenhouse. After 7 days, seedlings were fertilized with nutritive solution; 1.5gmZn, 0.49gm Cu, 1.2gm Fe, 1.29mgB, 0.29gm MO/20L water. Seedling 4 weeks – age were transplanting to the field, sand soil, in 2x4m/plot consisted 5 rows 4mlong 40Cm apart and 20Cm inter spacing (100plant/ plot i.e. 12.5 plant/m2 to give target plant population 52500 plant / F. i.e. 125000 plant/ha.). Brackish shallow well water 1100ppm was used for irrigation as well fertigation with 20: 20: 20Kg NP K/har through surface drip irrigation system.

B. Elicitation Treatments

Plants aged 3 months after sowing were foliarly sprayed with Nano- Zinc oxide particles (N), 20mm, for 0.20, 40, 60ppb and 0, 2, 4, 6ppm micro zinc sup hate (M) com centration's, both Nano- zinc and micro – zinc treatments as well as gamma irradiation treatments were a replicated for three replicates.

C. Biometric field traits

At 15 August plants were harvested, fresh leaves and roots, were recorded per plot then estimated biomass, leaves (LB) and roots (RB) per Fadden that converted to biomass. yild per hectare.

D. Bioactive secondary Metabolites

➢ *Extraction*

Ultrasonic extraction has been used for extraction of flavonoids [36] and Phenolics [54]. The output power is 100w and the frequency is 40 HZ, 60% ethanol alcohol extraction time 30min and solid – liquid ratio 1:20 dry leaf and root sample, 0.1mg- was sonicated, in an ultrasonic water path after the extraction has ended, the extract was filtered and the residue were then rinsed with rinsed with the extraction solvent in triplicate, and then the first extract and the later extract were pooled. Finally, the volume was made up to the mark with the extract solvent.

➢ *Total phenolics content (TPC):*

Total phenolic was determined by Folin–ciocalu method [55]. A1ml of aliquot of extract solution was mixed with 1.0ml of distilled water and 100µl Folin-ciocatu reagent followed by 300 µl of 200g/L N2Cl3 solution. The mixture was incubated in shaking incubator at 40°C for 3m and its absorbance at 700nm was measured. Gallic acid was used as standard for calibration cuave. Total phenolic content were represented as gallic mg equivalent /g (mg GAE/gDW.).

➢ *Total flavonoids (TFC)*

Total flavonoids was determined by the method of A 0.5mL aliquot of 20g./L Al Cl3 ethanolic solution was added to 0.5ml of extract solution. After/h. at room temperature, the absorbance at 240nm was measured. Total flavonoids content expressed rutin equivalent (mg RE/ g D.W.).

➢ *Determination of DPPH- Based Antioxidant Activity (AA)*

DPPH radical scavenging activity (DRSA) in different samples was determined according to the method of [56] Briefly, the ethanol extract (2ml; 10mg/ 25ml) of each leaf and root sample was mixed with 2ml of DPPH- free radical solution (0.25mg/ 25 ml X4). The mixture was incubated in the dark for approximately 30min. the absorbance of the resulted mixture was measured at 517nm at room temberative by using aUV- visible spectrophotometer.

The radical scavenging activity was calculated as percentage of DPPH discoloration using the following equation:

DRSA% = (1-Ap/AD) x100

Where Ap represents the absorbance of extract at 517nm and AD is the absorbance of the DPPH solution without leaf, root extract.

III. STATISTICAL ANALYSIS

The obtained data were subjected to computerize analysis of variance using M- state program The deference's between mean treatments were statistically tested by the calculated LSD at 1% level.

IV. RESULTS AND DISCUSSION

Statistical analysis for the analysis of variance revealed that G,N,M application actuated significantly for fresh leaves and roots biomass (LB, RB), total phenolics for leaves (TPCL) and roots (TPCR),Total flavonoids contents for leaves (TFCL) and rots (TFCR),antioxidant activity for leaves extract (AAEL) and roots extract (AAER). The deference's between mean treatments were tested according the calculated LSD at 1% level.

A. Biomass production

Table (1) show that Go, No, Mo control BL/BR were 2.050t/ 1.301t / h, G25, 35, 50Gy, N20, 40ppb, M2ppm achieved significant increment for BL/BR (as percent of control) up to (123/123, 120/119, 115/119), (117/117,113/114), (102/103), respectively, as shown table (1) and represented Figure (1) these findings revealed that(G) excel (N) which surpassed (M)application. On the contrary N60ppb and M4,6ppm resulted in significant reduction BL/ BR (as percent of control); (98/98), (95/95, 81/8), respectively. (Table 1, Figure 1) Data were in line with that has been previously reported for G application [39,40], N application [57,52,48,53].

B. Bioactive secondary Metabolites

Table (1), Figure (2,3)declared that GoGy, Noppb and Moppm control for TPCL,(mg GAE/ g.D.W) and TFCR, (mg. RE/g.D.W) were 4.71 and 5.50, respectively, G 25, 35, 50Gy and N20, 40 ppb; M2 ppm enhanced significantly TPCL/ TFL (as% control) up to; (126/133,117/124,104/107), (304/322,200/215), (138/136), respectively. Whereas, TPCR/TFCR at G,N, M control, were 5.25/6.14, and G25, 35,

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50 Gy, N20,40ppm, M2ppm achieved significant enhanced TPCR/ TFCR (as percent of control) up to (127/131,120/125,110/115), (321/335,266/282), (140/145). Even more, N surpassed M in both (L) and (R). On the contrary, N60ppb and M4.6ppm. Attained significant reduction in TPCL/ TFCL (as % of control); (94/96), (92/75,79/69) and TPCR/TFCR; (98/97), (90/85,75/75) as represented in Figure (2). Data were in agreement with that has been stated previously [58,52,59,4].

C. DPPH free radical scavenging activity, Antioxidant activity (AA)

Since phenolics and flavonoids acts as antioxidants [60] the AA for leaf extract (AAEL) and root extract, (AAER) at GoGY, Noppb and Moppm were AAEL / AAER, 65.15/72.12. whereas, G25, 35, 50 Gy, N20, 40ppb and M2ppm upraised significantly up to % of control (120/118,115/122,112/111), (135/132, 131/125), (110/114). On the contrary, N60ppb and M4,6ppm acted significant reduction for AAEL/AAER were, (87/86), (85/94,85/90), respectively. (Table 1 and Figure 4).

In all N20, 40ppb, G25, 35, 50Gy, M2ppm resulted in significant optimization BL and BL production and significantly enhancing; TPCL, TPCR, TFCL, TFCR, AAEL and AAER, alongside N surpassed G and M application these findings were attributed beneficial effects for applicated treatment on physiological, biochemical chacteristics, promote photosynthesis and nitrogen metabolism, regulation of genes [61,62,63,46,58]Précis results highlight that (TO) plants show good physiological performance which means that this species is particularly constrained conditions (Sandy poor soil irrigated with saline water, 1100ppm). This seems attributed to the high phenolics and flavonoids centents due G, N, M, application, in return determines a high antioxidant activity, that compatting the oxidative stress induced by saline water. Based on the above results, metal nanoparticles zinc can potentially be used as reliable biotechnological tool for (TO) biomass improvement and enhancing bioactive secondary metabolites, phenolics, flavonoids and antioxidant activity, exceedingly N could be substituted M and G, these results were in accordance with that had been declared previously [46,51,52,12].

V. CONCLUSION

The precise results assert elicitation – field application with gamma irradiation (25,35,50 GY) as physical elicitor; abiotic elicitors, Nano – zinc oxide (20,40, ppb) and micro zinc (2ppm) could be considered, as potential reliable biotechnological tool to enhance, significantly biomass, bioactive secondary metabolites (phenols, flavonoids) and antioxidant activity . At that physical elicitor transient abiotic Nano–zinc which excel micro–zincto highly valuable wild medicinal Taraxacum officinal.

Treatments	B,t/ha		TPC,mgGAE/gDw.		TFC,MgRE/g.D.W		AAE	
	Leaves (L)							
O Control	2.050	(100)	4.71	(100)	5.50	(100)	65.15	(100)
G 25Gy	2.528	(123)	5.94	(126)	7.32	(133)	78.318	(120)
G 35 Gy	2.452	(120)	5.51	(117)	6.82	(124)	74.92	(115)
G 50GY	2.356	(115)	4.90	(104)	5.89	(107)	72.97	(112)
N20ppb	2.396	(117)	14.22	(304)	17.71	(322)	87.95	(135)
N40ppb	2.321	(113)	9.42	(200)	11.83	(215)	85.35	(131)
N60ppb	2.009	(98)	4.43	(94)	5.28	(96)	56.68	(87)
M2ppm	2.095	(102)	6.50	(138)	7.48	(136)	71.67	(110)
M4ppm	1.947	(95)	4.33	(92)	4.13	(75)	55.38	(85)
M6ppm	1.660	(81)	3.72	(79)	3.80	(69)	50.82	(78)
LSD1%	0.011	-	0.15	-	0.18	-	0.74	-
	Roots (R)							
O Control	1.301	(100)	5.25	(100)	6.14	(100)	72.12	(100)
G 25Gy	1.598	(123)	6.67	(127)	8.04	(131)	85.10	(118)
G 35 Gy	1.544	(119)	6.30	(120)	7.68	(125)	87.99	(122)
G 50GY	1.509	(116)	5.76	(110)	7.06	(115)	80.05	(111)
N20ppb	1.522	(117)	16.85	(321)	20.57	(335)	95.20	(132)
N40ppb	1.479	(114)	13.96	(266)	17.42	(282)	90.15	(125)
N60ppb	1.275	(98)	5.15	(98)	5.96	(97)	62.02	(86)
M2ppm	1.343	(103)	7.35	(140)	8.90	(145)	82.22	(114)
M4ppm	1.236	(95)	4.73	(90)	5.22	(85)	67.79	(94)
M6ppm	1.054	(81)	3.94	(75)	4.60	(75)	64.91	(90)
LSD1%	0.007	-	0.17	-	0.21	-	0.87	-

Table 1:- L& R; t/h, TPC and AAE in response to G, N and M application treatments.

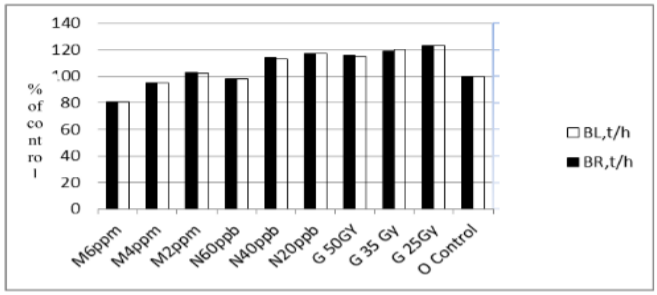

Fig 1:- BL, BR, t /h, as % of control, in response to G,N,M.

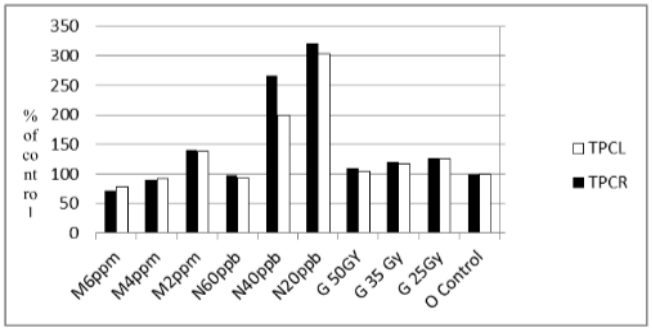

Fig 2:- TPCL, TPCR, as % of control in response to G, N, M

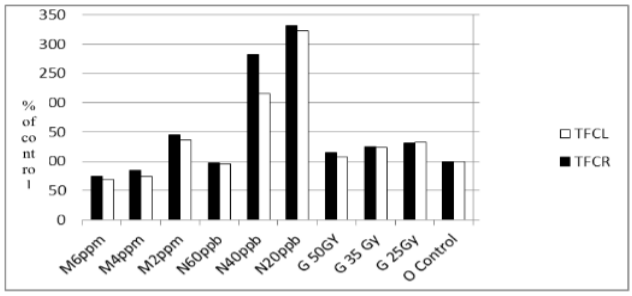

Fig 3:- TFC,TFCR, as % of control in response to G,N,M.

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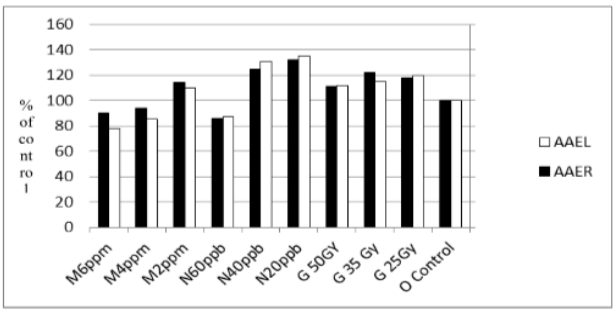

Fig 4:- AAEL, AAER, as % of control in response to G, N, M.

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