




Effect of Humic Acid, Treated Sewage Effluent and Radiation on Canola Growth and Oil Production


Abstract

A greenhouse experiment was carried out to evaluate the role of Humic acid (H) applied at different rates i.e. 0, 5, 10, 15, and 20 mg kg⁻¹ in improving canola (*Brassica napus* L.) growth when irrigated with treated sewage effluent. Before planting, Canola seeds were exposed to different doses of gamma rays (0, 100, 200, 300, 400 and 500 Gy). Response of canola plant to humic acid rates and irradiation doses have the following rank, 15 > 20 > 10 > 5 > 0 mg.kg⁻¹ and 300 > 200 > 100 > 400 > 0 > 500 Gy, respectively. The positive effect of irradiation was indicated by ancement of both canola wth and ro-nutrients uptake.

Key words: Canola, Humic acid, Gamma ray, Treated sewage effluent.

Introduction

Rapeseed as a raw material for oil production is cultivated in area of 32 million hectare (FAO, 2010). It provides about 16% of world vegetable oil and occupies the third position as the most important oilseed crop worldwide (Fediol, 2014). Rapeseed production represented 10 - 15 percent of the world oil crop production between years 2000 and 2009. During the last decade, European rapeseed production increased sharply from 12 million tons in 2000 to 20 million tons in 2010 (FAOSTAT, 2014), mainly due to higher demands for biofuel. The small, round rapeseed seed contains 38 to 45 percent oil. In addition to high oil content, rapeseed seeds contain approximately 17 - 26% protein (Uppstrom, 1995). Mainly, the importance of rapeseed is considered from the food sector viewpoint (Hidalgo and Zamora, 2006).

Water is becoming an increasingly scarce resource in arid and semi-arid countries and planners are forced to consider any source of water that might be used economically and effectively to meet increasing demands for water (Ahmadifard and Kalbasi 2014). Therefore, low quality water resources including wastewater are considered a solution to minimize dependence on agricultural fresh water requirement (Galavi *et al.*, 2010). Precautions should be taken into consideration when planning to reuse the contaminated or wastewater resources. In this regard many researches  dealt with identification of contamination and low quality of such water resources (Chong *et al.*, 2010; Zeng *et al.*,

2011, 2013a and b, Deng et al., 2013; Chen et al., 2013, Lesmana et al., 2009, Tang et al., 2012; Rahman and Islam, 2009).

Humic substances compose up to 80% of soil organic matter (Brady & Weil, 2008). Carbon, oxygen, hydrogen, nitrogen, and sulfur are the most common elements in humic substances (Pettit, 2004). Humic acids are water soluble in alkaline conditions (Pettit, 2004). High portions of the humic acids structure are carbon rings and carbon chains (Pettit, 2004). Humic acids are typically composed of 54 to 58% carbon, 33 to 38 % oxygen, 36 % hydrogen, 0.8 to 4.3% nitrogen and 0.1 to 1.5% sulfur (Steelink, 2002).

The aim of the present study is to evaluate the effect of seed irradiation and humic acid on stimulation of canola growth and oil production.

Materials and Methods

Pot experiment was carried out during 2017 / 2018, in randomized complete block design with 5 replicates under greenhouse conditions. One part of the experiment was irrigated with fresh water and another was irrigated with treated sewage effluent. Experimental factors were: Factor 1: Irradiation with gamma rays at 0, 100, 200, 300, 400 and 500 Gy; symbol as R_0 , R_1 , R_2 , R_3 , R_4 , and R_5 , respectively. Factor 2: Humic acid addition (H); 0, 5, 10, 15, and 20 mg kg⁻¹ symbol as H_0 , H_1 , H_2 , H_3 and H_4 , respectively. PVC pots with dimensions, 30 cm width and 30 cm depth, filled with 10 kg soil per pot were used. Seeds were sown 10 per pot thinned to 5 after 10 days from seeding. Extraction of oil by Soxhlet extractor was carried out as described by Akbar et al. (2009). Some of chemical and physical properties of the experimental soil were determined according to the standard methods outlined by Hamdy (2005), and presented in Table (1). Some of chemical properties of treated sewage effluent used for irrigation are presented in Table (2).

Table 1. Some chemical and physical properties of experimental soil

EC dS m ⁻¹	pH	OC (mg kg ⁻¹)	(mg kg ⁻¹)			Coarse sand%	Clay %	Silt %	Sand %	Soil texture
			N	P	K					
1.21	7.85	4	3.21	1.58	1.65	4.0	7.0	2.5	86.5	Sand

EC= Electrical Conductivity of saturation extract, OC= Organic Carbon %, FC=Field Capacity

Table 2. Main properties of the sewage water

EC ds/m	pH	BOD	COD	OC gkg ⁻¹	(mg L ⁻¹)							
					N	P	K	Fe	Zn	Mn	Cu	Pb
1.62	7.31	190	375	36	22.5	4.5	1.87	1.55	0.11	0.12	0.07	0.08

BOD= Biochemical oxygen demand, and COD= Chemical oxygen demand

Results and discussion

Seeds yield:

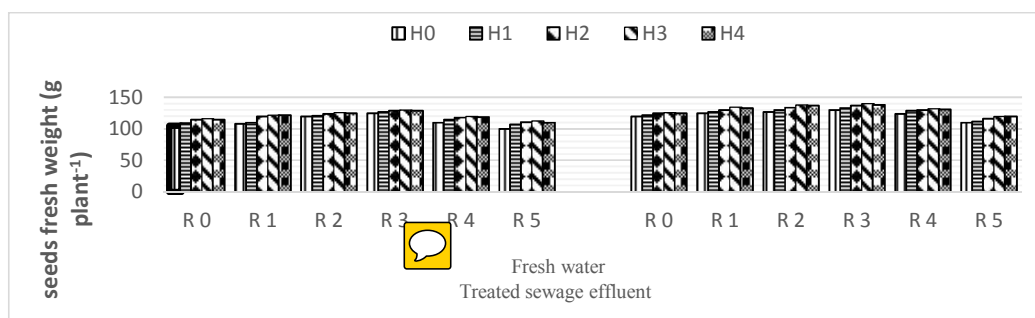
Seeds yield as affected by irrigation water, humic acid rate and different irradiation doses is presented in Fig (1). Plants irrigated with treated sewage effluent produced higher seed yield than those recorded with fresh water irrigation. This phenomenon was true under all radiation and humic acid treatments. Slight increase in fresh weight of seeds was detected with application of humic acid comparing to the untreated plants. In this regard, there was no remarkable variation between humic acid rates. Concerning the effect of radiation, data reflected the superiority of R_2 (200 Gy) and R_3 (300 Gy) over other radiation doses. On the other hand, the lowest values were recorded with 400 Gry, 0 Gry and 500 Gry, respectively. Under irrigation with treated sewage effluent, the effect of gamma irradiation of seed fresh weight could be arranged as following: 300 Gry > 200 Gry > 100 Gry > 400 Gry > 0 Gry > 500 Gry recording 136,133,130,129,124 and 116 g plant⁻¹, respectively. In case of fresh water, values could be rank as following: 300 Gry > 200 Gry ≥ 100 Gry > 400 Gry > 0 Gry > 500 Gry representing 128,123,116,116,112 and 108 g plant⁻¹, respectively.

Seeds fresh weight yield as affected by humic acid rates indicated that the best treatments could be ranked as following: 15 mg kg⁻¹ > 10 mg kg⁻¹ > 20 mg kg⁻¹ > 5 mg kg⁻¹ > 0 mg kg⁻¹ humic acid, recording 132,129,124,126 and 123 g plant⁻¹, respectively when plants irrigated with treated sewage effluent.

In case of fresh water irrigation, effect of humic rates have the following order: 15 mg kg⁻¹ > 10 mg kg⁻¹ ≥ 20 mg kg⁻¹ > 5 mg kg⁻¹ > 0 mg kg⁻¹, recording 121,120,120,115 and 111 g plant⁻¹, respectively.



In this respect, many workers have indicated the positive effect of reuse of sewage effluent on canola biomass and seed yield which attributed to the nutrients contained in such water resources (Chen and Cutright, 2001; Peralta-Videa et al, 2002; Zhao et al., 2003; Gardea-Torresdey et al., 2004; Turan and Angin 2004; Turgut et al. 2004; Hajiboland 2005; Tlustoš et al. 2006).



Notes: R_0, R_1, R_2, R_3, R_4 and R_5 are 0, 100, 200, 300, 400 and 500 Gy respectively. H_0, H_1, H_2, H_3 and H_4 are 0, 5, 10, 15 and 20 mg humic acid kg^{-1} respectively

Fig1. Effect of humic acid and gamma ray irradiation on seed fresh weight (g plant^{-1}) irrigated with sewage effluent and fresh water.

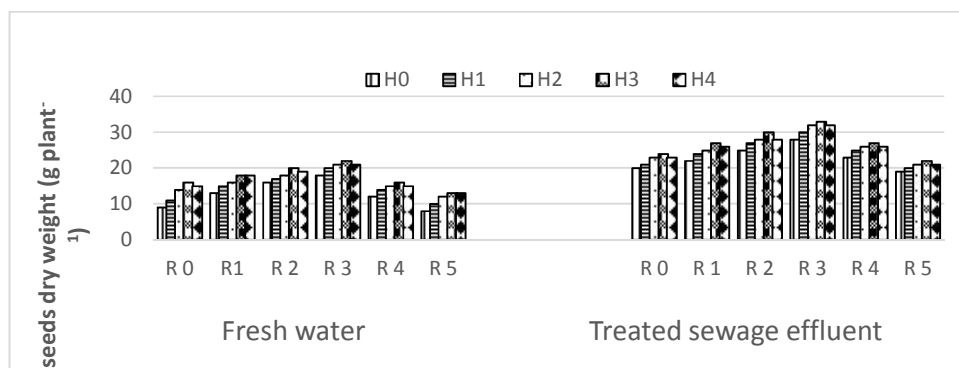
Canola dry weight (g plant^{-1}) as affected by application of humic acid rate and canola seeds were exposed to different doses of gamma rays under two water tube (Fresh water irrigation and treated Irrigation with sewage effluent) was presented in Fig (2). Generally, under treated Irrigation with sewage effluent, it was clear that application of doses of gamma rays treatment either solely or in combination with humic acid enhanced Canola dry weight, in most treatments, comparing to the non-addition humic acid. dry weight yield of canola under doses of gamma rays treatment with two water tube (Fresh water irrigation and treated Irrigation with sewage effluent), the best treatments could be ranked as following: Treated Irrigation with sewage effluent: - 300 Gry > 200 Gry > 400 Gry > 100 Gry > 0 Gry > 500 Gry recorded, 31,27,25.4,24.8,22.2 and 20.6 g plant^{-1} respectively.

Fresh water irrigation: - 300 Gry > 200 Gry > 400 Gry > 100 Gry > 0 Gry > 500 Gry recorded, 20.4,18, 16,14.4,12.8 and 11.2 g plant^{-1} respectively.

Dry weight yield of canola under humic acid rate with two water tube (Fresh water irrigation and treated Irrigation with sewage effluent), the best treatments could be ranked as following: Treated Irrigation with sewage effluent: - 15 mg kg^{-1} > 20 mg kg^{-1} > 10 mg kg^{-1} > 5 mg kg^{-1} > 0 mg kg^{-1} humic acid, recorded 27.1, 26.0, 25.8, 24.5 and 22.8 g plant^{-1} respectively.

Fresh water irrigation: - 15 mg kg^{-1} > 20 mg kg^{-1} > 10 mg kg^{-1} > 5 mg kg^{-1} > 0 mg kg^{-1} humic acid, recorded 17.3, 16.8, 16.2, 14.5 and 12.6 g plant^{-1} respectively.

Approximately, similar trends were noticed when seeds dry weight as affected by experimental treatments was considered (Table 4). In accordance, **Schiavon et al., (2010)**; **Berbara and Garcia, (2014)** reviewed some different mechanisms responsible for enhancement of plant growth as affected by humic acid addition. In harmony, **Shakeel Ahmad et al., (2016)** reported that growth and yield of canola were increased by application of humic acid.

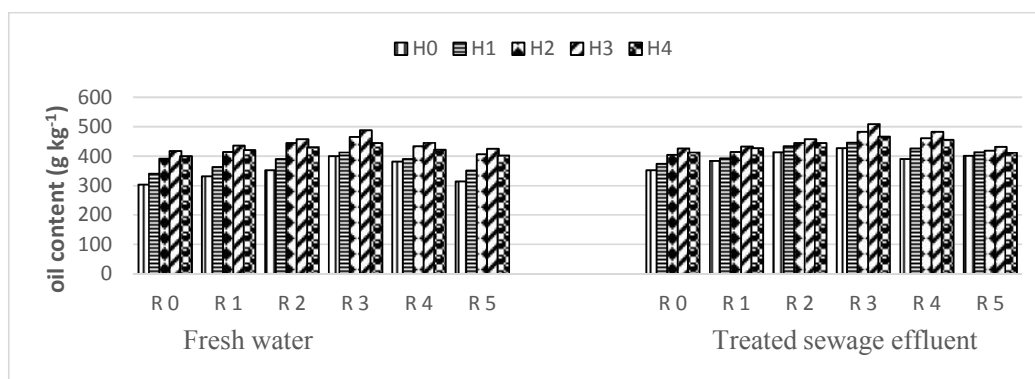


Notes: R₀, R₁, R₂, R₃, R₄ and R₅ are 0, 100, 200, 300, 400 and 500 Gy respectively. H₀, H₁, H₂, H₃ and H₄ are 0, 5, 10, 15 and 20 mg humic acid kg⁻¹ respectively

Fig2. Effect of humic acid and gamma rays on canola seeds dry weight (g plant⁻¹) irrigated with sewage effluent and fresh water.

Oil yield

As show in Fig (3), humic acid rates as well as irradiation doses have a positive effect on production of oil. Values of oil content were varied among humic acid rates. The best values were recorded with H₃ (15 mg kg⁻¹), H₂ (10) and H₄ (20), respectively comparing to H₀ and H₁ (5) treatments. Concerning the irradiation treatments, the best values of oil content were recorded with R₃ (300 Gy), R₂(200) and R₄ (400), respectively. This holds true under fresh water and treated sewage effluent but the values were to some extent, higher in case of treated sewage effluent than those recorded with fresh water irrigation. These findings are consistent with those reported by (**Oregani et al.2014**) who indicated municipal wastewater irrigation affected significantly on the biomass and yield of canola.



See footnotes of Fig 1 for treatment designations.

Fig 3. Effect of humic acid and gamma rays on oil content (g kg^{-1}) in canola seeds under irrigation with treated sewage effluent and fresh water irrigation

Metal content

Micronutrients content in canola plants as affected by irrigation sources, humic acid and gamma irradiation are presented in Tables (3, 4, 5 and 6), for Fe, Zn, Mn and Cu, respectively. Iron content (Table 3), was enhanced by irradiation doses and values recorded with I₁, I₂ and I₄ (mean value) were nearly closed to each other while the best one was recorded with I₃, under fresh water irrigation. Similar trend, but to somewhat higher extent, was with treated sewage effluent irrigation. Application of humic acid induced significant increases in Fe content as compared to the untreated plants. Application at rate of 15 mg kg^{-1} resulted in the highest Fe value either under fresh water or treated sewage effluent treatments. In general, both irradiation and humic addition reflected positive effects on plant growth and consequently it's content of iron. Many researchers had explained the mechanisms related to enhancement of metal content. **Aiken et al., (1985)** reported that humic acids contain acidic functional groups such as phenolic, hydroxyl and carboxyl groups which can bind to many metal ions, and they exist in soil and aquatic environments. In presence of metal ions, the resulting humic acids could complex with them and provide plants with many microelements (**Lobartini et al., 1998**). Humic acids serve as carriers of Fe (II) and Fe (II)-HA (**Rose and Wait, 2003** and **Willy et al., 2008**).



Table 3. Effect of humic acid and gamma rays on Fe content in canola shoot (mg kg^{-1}) under treated sewage effluent and fresh water irrigation.

Humic acid “mg kg ⁻¹ ” (H)						
Irradiation (I)	H ₀	H ₁	H ₂	H ₃	H ₄	Mean
Fresh water						
R ₀	556	594	618	650	640	611
R ₁	600	627	649	675	670	644
R ₂	619	645	661	689	680	658
R ₃	645	672	688	699	685	677
R ₄	620	650	670	680	675	659
R ₅	570	622	643	654	650	627
Mean	601	635	654	674	666	
LSD	R= 4.530	H = 4.135	R x H = 10.13			
Treated sewage effluent						
R ₀	726	750	775	790	785	765
R ₁	755	775	790	841	810	794
R ₂	796	824	842	885	825	834
R ₃	850	877	895	950	844	883
R ₄	820	855	860	880	835	850
R ₅	770	836	850	862	828	829
Mean	786	819	835	868	821	
LSD	R= 9.78	H = 8.92	R x H = 21.86			

See footnotes of Fig 1 for treatment designations.

Zn content (Table 4), followed the same trend recorded with Fe but in very low values. Also, it was, to somewhat extent, high in case of sewage effluent irrigation. Both irradiation and humic addition had enhanced Zn uptake by treated plants. On line, humic acids found to be as enhancer for Cu, Cd and Zn uptake since they are organic and inorganic chelates (Fi scherov et al. 2005, Lesage et al. 2005, Meers et al. 2005, Finžgar et al. 2006).

Table 4. Effect of humic acid and gamma rays on Zn content of canola (mg kg^{-1}) under sewage effluent and fresh water irrigation.

Humic acid “mg kg ⁻¹ ” (H)						
Irradiation (I)	H ₀	H ₁	H ₂	H ₃	H ₄	Mean
Fresh water						
R ₀	3.5	4.2	5	5.6	5.3	4.7
R ₁	3.7	4.8	5.3	6.1	5.9	5.1
R ₂	4.1	5.3	6.2	7	6.5	5.8
R ₃	4.4	5.9	6.5	7.4	7.1	6.2
R ₄	4.2	4.6	5.8	6.9	6.5	5.6
R ₅	3.2	4.1	5.2	5.8	5.4	4.7
Mean	3.8	4.8	5.6	6.4	6.1	
LSD	R= 0.86	H = 0.79	R x H = 0.194			
Treated sewage effluent						

R ₀	5.5	6.8	7.5	8.2	8	7.2
R ₁	6.7	7.5	8.3	8.7	8.5	7.9
R ₂	8.4	8.8	9.4	9.9	9.4	9.1
R ₃	9.9	10.1	12.1	13.4	9.7	11.0
R ₄	8.2	8.4	9.3	11.5	8.8	9.2
R ₅	6.1	7.1	7.4	9.7	8.5	7.7
Mean	7.4	8.1	9	10.2	8.8	
LSD	R= 0.12 H = 0.11 R x H = 0.27					

See footnotes of Fig 1 for treatment designations.

Table 5. Effect of humic acid and gamma rays on **Mn** content of canola (mg kg⁻¹) under treated sewage effluent and fresh water irrigation.

Humic acid “g kg ⁻¹ ” (H)						
Irradiation (I)	H ₀	H ₁	H ₂	H ₃	H ₄	Mean
Fresh water irrigation						
R ₀	12	16	19	22	21	18
R ₁	15	20	22	25	23	21
R ₂	18	23	27	30	28	25
R ₃	21	25	29	35	33	28
R ₄	17	22	24	26	25	22
R ₅	13	15	18	20	20	17
Mean	16	20	23	26	25	
LSD	R= 0.632	HA = 0,577	R x HA = 1.415			
Treated sewage effluent						
R ₀	80	85	89	97	93	88
R ₁	87	98	125	129	120	111
R ₂	110	124	136	140	127	127
R ₃	117	140	152	158	138	141
R ₄	96	122	144	145	141	129
R ₅	88	114	131	134	130	119
Mean	96	113	129	1133	124	
LSD	R= 1.47	H = 1.34	R x H = 3.28			

See footnotes of Fig 1 for treatment designations.

Table 6. Effect of humic acid and gamma rays on **Cu** content of canola (mg kg⁻¹) under treated sewage effluent and fresh water irrigation.

Humic acid “g kg ⁻¹ ” (H)						
Irradiation (I)	H ₀	H ₁	H ₂	H ₃	H ₄	Mean
Fresh water						
R ₀	3	6	9	10	8	7.2
R ₁	5	7	8	11	9	8
R ₂	8	9	12	13	10	10.4
R ₃	9	10	13	15	12	11.8
R ₄	6	8	9	10	8	8.2
R ₅	4	7	8	9	8	7.2
Mean	5.8	7.8	9.8	11	9	
LSD	R= 0.233	H = 0.213	R x H = 0.522			
Treated sewage effluent						

R ₀	8	10	13	15	14	12
R ₁	9	13	16	17	15	14
R ₂	12	15	18	20	17	16
R ₃	15	18	21	23	20	19
R ₄	12	13	15	17	16	14
R ₅	9	11	12	13	11	11
Mean	10.8	13.3	15.8	17.5	15.5	
LSD	R = 0.18	H = 0.16	R x H = 0.40			

See footnotes of Fig 1 for treatment designations.

Conclusion

Irradiation and humic acid were effective in enhancing canola growth, seed yield, oil content and micronutrients i.e Fe, Zn, Mn and Cu. Applications of moderate humic acid rate and irradiation doses resulted in remarkable values of plant growth, seed and seed oil yields. Recent results proved that humic acid could be considered beneficial either applied individually or in combination with gamma irradiation for plant and oil production. Therefore, we recommend further field research for good explanation and more discussion of the responsible mechanisms of humic acid and radiation impacts on plant.

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