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

Ezzat A. Kotb[1] and Ahmed A. Moursy[1*]

[1]Nuclear Research Center, Department of Soil and Water Research, Atomic Energy Authority, Abou-Zaabal, 13759, Egypt.

Original Research Article

ABSTRACT

A greenhouse experiment was carried out to evaluate the role of Humic acid (HA) in improving canola (*Brassica napus L.*) growth when irrigated with treated sewage. We applied different concentrations of HA (0, 5, 10, 15, and 20 mg kg^{-1}) during this study. Canola seeds were exposed to different doses of gamma rays (0, 100, 200, 300, 400 and 500 Gy). The response of canola plant to HA and irradiation was evaluated and found to rank as following; 15 > 20 > 10 > 5 > 0 mg.kg^{-1} and 300 > 200 > 100 > 400 > 0 > 500 Gy respectively. We also found that treatment with irradiation 200 Gy and 300 Gy enhanced the growth, micro-nutrients (Fe, Zn, Mn and Cu) uptake and the yield of oil.

Keywords: Canola; humic acid; gamma ray; treated sewage effluent.

**Corresponding author: E-mail: ahmad1a2m3@yahoo.com;*

1. INTRODUCTION

Rapeseeds are cultivated in area of 32 million hectare worldwide (FAO, 2010). They provide the raw materials for oil production and account for 16% of world vegetable oil occupying the third position as the most important oilseed crop worldwide (Fediol, 2014). Between the years of 2000 and 2009 only rapeseeds were used to produce 10 to 15 % of the world oil. Rapeseed production in Europe increased significantly from 12 to 20 million tons during the last decade (FAOSTAT, 2014), mainly due to the higher demands for biofuel. The small round rapeseed seed contains 38 to 45% oil. In addition to high oil content, rapeseed seeds contain approximately 17 - 26% protein (Uppstrom, 1995). Thus, rapeseeds provide an important resource of oil in food and food-related industry (Hidalgo and Zamora, 2006).

Water is becoming an increasingly scarce with increasing demand in arid and semi-arid countries. Thus, it is essential to consider all sources of water that can be effectively used (Ahmadifard and Kalbasi 2014). These resources include low quality water such as wastewater that can be used to minimize dependence on agricultural fresh water (Galavi *et al*., 2010). However, precautions should be taken into consideration when reusing contaminated or wastewater resources. In this regards, many researchers identified contaminants in such low quality water resources (Zeng et al., 2011, 2013a and, Deng et al., 2013; Chen et al., 2013, Lesmana et al., 2009, Tang et al., 2012; Rahman and Islam, 2009).

Up to 80% of soil organic matters are composed of humic substances (Brady & Weil, 2008). Carbon, oxygen, hydrogen, nitrogen, and sulfur are the most common elements in humic substances. Humic acids are water soluble in alkaline conditions and encompass high moiety of carbon rings and carbon chains (Pettit, 2004). They 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 canola growth and oil production.

2. MATERIALS AND METHODS

Pot experiment was carried out during 2017 and 2018 in a randomized complete block design with 5 replicates under greenhouse conditions. The latitude and longitude of the experimental site are 30o 24` N, 31o 35` E respectively, while the altitude is 20 m above the sea level. One part of the experiment was irrigated with fresh water and the other part was irrigated with treated sewage. Experimental seeds were Canola (*Brassica napus L.)* irradiated with different doses of gamma rays at 0, 100, 200, 300, 400 and 500 Gy and referred to as R0, R1, R2, R3, R4, and R5 respectively. Gamma irradiation was conducted using 60Co gamma source (Cyclotron Department, Nuclear Research Center, Atomic Energy Authority, Egypt). Humic acid (HA) was added at different concentrations 0, 5, 10, 15, and 20 mg kg-1 and referred to as H0, H1, H2, H3 and H4 respectively. HA contains 90% Humic and10% potassium. Poly-vinyl chloride (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 from seeds was carried out by using Soxhlet extractor as described by Akbar et al. (2009). Chemical and physical properties of the experimental soil were determined according to the standard methods outlined by Hamdy (2005) and presented in (Table 1). Chemical properties of treated sewage effluent used for irrigation are presented in (Table 2).

Table 1. Chemical and physical properties of experimental soil

EC dS m^{-1}	pH	OC (mg kg^{-1})	(mg kg^{-1}) N	P	K	Coarse sand%	Clay %	Silt %	Sand %	Soil texture
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
COD; Chemical oxygen demand

3. RESULTS AND DISCUSSION

3.1 Effect of HA and Sewage Water Irrigation on Yield Seeds

Seeds yield was affected by irrigation water, HA, and different irradiation doses as indicated in Fig. 1. Plants irrigated with treated sewage effluent produced higher seed yield than those with fresh water irrigation. This phenomenon was observed under all radiation and HA treatments. Slight increase was observed in the fresh weight of the seeds in the presence of HA compared to untreated plants. While no significant changes were observed between different HA concentration, radiation doses of R_2 (200 Gy) and R_3 (300 Gy) showed superiority over other 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 on seed fresh weight could be arranged as following: 300 Gry > 200 Gry > 100 Gry > 400 Gry > 0 Gry > 500 Gry giving rise to 136,133,130,129,124 and 116 g $plant^{-1}$ respectively. In the case of fresh water values could be rank as following: 300 Gry > 200 Gry ≥ 100 Gry > 400 Gry > 0 Gry > 500 Gry giving rise to weights of 128,123,116,116,112 and 108 g $plant^{-1}$ respectively.

Seeds fresh weight yield was also affected by HA and was ranked as following: 15 mg kg^{-1} > 10 mg kg^{-1} > 20 mg kg^{-1} > 5 mg kg-1 > 0 mg kg^{-1} HA with weights of 132, 129, 124, 126, and 123 g $plant^{-1}$ respectively when plants irrigated with treated sewage effluent. HA concentrations affected the plants irrigated with fresh water and it was ranked as follows 15 mg kg^{-1} > 10 mg kg^{-1} ≥ 20 mg kg^{-1} > 5 mg kg^{-1} > 0 mg kg^{-1} giving rise to weights of 121,120,120,115, and 111 g $plant^{-1}$ respectively.

Several studies indicated the positive effect of reusing sewage effluent on canola biomass and seed yield is attributed to the nutrients in such water resources (Chen and Cutright, 2001 and Peralta-Videa et al, 2002). Nasiri et al. (2017) reported that HA increase seed yield and seed oil. The efficiency of humic substances depends on their origin and the processing methods (Senesi, 2007). The number of seeds per plant and seed weight per plant were reported by Kafeel et al. (2011) to increase with irrigation using sewage water.

Canola dry weight (g plant-1) was affected by HA when seeds exposed to different doses of gamma rays under two water tubes; Fresh water irrigation and treated irrigation with sewage effluent as shown in Fig. 2. Generally, under irrigation with sewage effluent, it was clearly observed that doses of gamma rays treatment either solely or in combination with HA enhanced canola dry weight, in most cases, compared to the irrigation lacking HA. The dry weight yield of canola under doses of gamma rays treatment with two water tube (Fresh water irrigation and treated irrigation with sewage effluent) was ranked as following: 300 Gry > 200 Gry > 400 Gry > 100 Gry > 0 Gry > 500 Gry giving rise to the weights of 31, 27, 25.4, 24.8, 22.2 and 20.6 g plant-1 respectively. Fresh water irrigation weight yields were ranked as followes; 300 Gry > 200 Gry > 400 Gry > 100 Gry > 0 Gry > 500 Gry giving rise to weights of 20.4,18, 16,14.4,12.8, and 11.2 g plant-1 respectively. Dry weight yield of canola under HA 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, 24.5and 22.8 g plant-1 respectively. Fresh water irrigation was ranked as 15 mg kg-1 > 20 mg kg-1 >10 mg kg-1 > 5 mg kg-1 > 0 mg kg-1 HA and gave rise to weights of 17.3, 16.8, 16.2, 14.5, and 12.6 g plant-1 respectively. Furthermore, similar trends were observed in seeds dry weight (Table 4).

Although, Schiavon et al., (2010) and Berbara and Garcia (2014) reported different mechanisms responsible for enhanced growth by HA, Shakeel Ahmad et al., (2016) reported that the growth and the yield of canola were increased by HA.

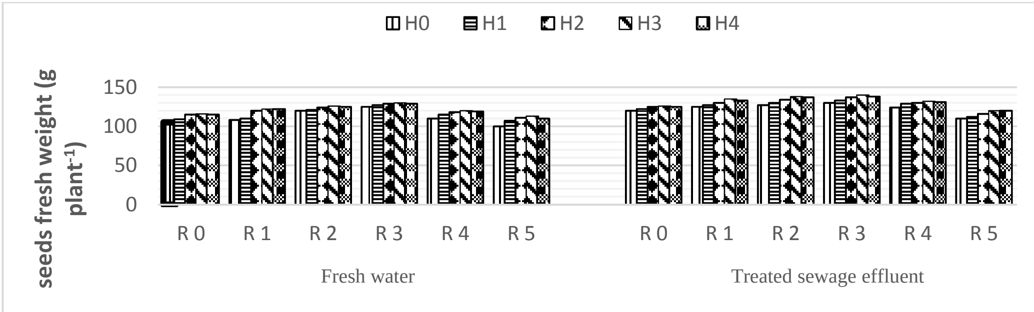

Fig. 1. Effect of HA and gamma ray irradiation on seed fresh weight (g plant^{-1}) irrigated with sewage effluent and fresh water

Notes: R0, R 1, R 2, 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 HA kg^{-1} respectively

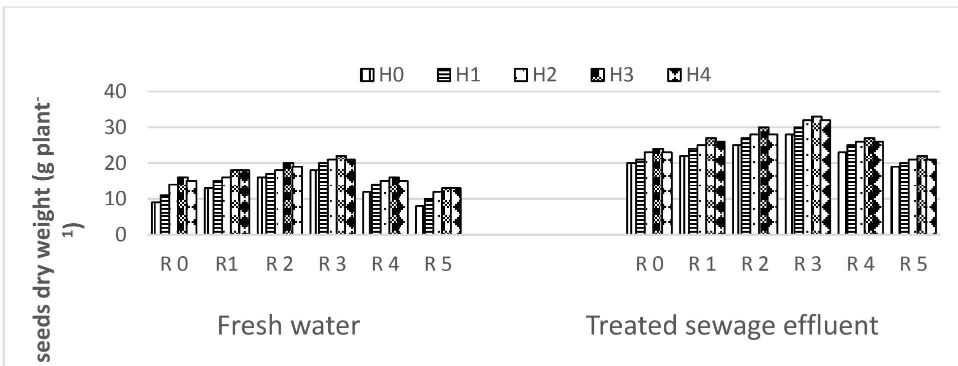

Fig. 2. Effect of HA and gamma rays on canola seeds dry weight (g plant^{-1}) irrigated with sewage effluent and fresh water

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 HA kg-1 respectively

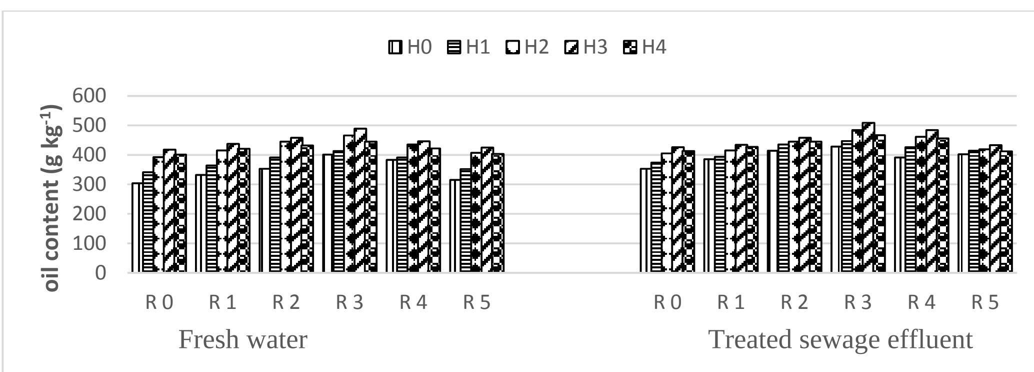

Fig. 3. Effect of humic acid and gamma rays on oil content (g kg^{-1}) in canola seeds under irrigation with treated sewage effluent and fresh water irrigation

See footnotes of Fig. 1 for treatment designations

3.2 Effect of HA and Sewage Water Irrigation on Oil Yield

As show in Fig. 3 above, HA rates and irradiation have positive effects on oil production. Oil contents varied among HA treatments. The most significant amounts were observed with H_3 (15 mg kg^{-1}), H_2 (10), and H_4 (20) respectively compared to H_0 and H_1 (5) treatments. The oil yield of canola under doses of gamma rays treatment with two water tube (Fresh water irrigation and treated irrigation with sewage effluent) was ranked as following: R3 (300 Gy), R 2(200), and R4 (400) respectively. The same was observed under fresh water and treated sewage effluent with but slightly higher in treated sewage effluent irrigation. These findings agree with previous studies by (Oregani et al. 2014) indicating that municipal wastewater irrigation significantly affected the biomass and the yield of canola.

3.3 The Effects of HA and Sewage Water Irrigation on Metal Content

Micronutrients contents in canola plants was affected by irrigation sources, HA, and gamma irradiation as indicated in (Tables 3, 4, 5 and 6), for Fe, Zn, Mn, and Cu, respectively. Iron content (Table 3) was enhanced by irradiation doses R1, R2, and R4 (mean values). However, the content of Fe was higher in R3 under fresh water irrigation. Slightly higher contents were observed in treated sewage effluent irrigation. HA significantly increased iron content compared to untreated plants. Particularly, 15 mg kg^{-1} resulted in the highest iron contents either under fresh water or treated sewage effluent. Thus, both irradiation and HA addition showed positive impact on plant growth and iron content of.

Several studies explained the mechanisms leading to enhanced metal contents. Aiken et al., (1985) reported that HA contains functional acidic moiety such as phenolic, hydroxyl, and carboxyl groups, which can bind several metal ions existing in soil and aquatic environments. In presence of metal ions HA forms complexes with these ions and hence provide plants with many microelements (Lobartini et al., 1998). In addition, HA were also found to serve as carriers of Fe (II) forming Fe (II)-HA complex (Rose and Wait, 2003and Willy et al., 2008).

Zn contents (Table 4) followed the similar trend as Fe but in very low amounts. However, it was slightly high in the case of sewage effluent irrigation. While both irradiation and HA enhanced Zn uptake, HA enhanced the uptake Cu and Cd since they are organic and inorganic chelates (Lesage et al. 2005, Meers et al. 2005, Finžgar et al. 2006).

Table 3. Effect of HA and gamma rays on Fe content in canola shoot (mg kg^{-1}) under treated sewage effluent and fresh water irrigation. See footnotes of Fig. 1 for treatment designations

	Humic acid "mg kg^{-1}" (H)					
Irradiation (R)	**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**		

Table 4. Effect of HA and gamma rays on Zn content of canola (mg kg-1) under sewage effluent and fresh water irrigation. See footnotes of Fig. 1 for treatment designations

	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**			

Table 5. Effect of HA and gamma rays on Mn content of canola (mg kg^{-1}) under treated sewage effluent and fresh water irrigation. See footnotes of Fig. 1 for treatment designations

	Humic acid "g kg^{-1}" (H)					
Irradiation (R)	**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**		

Table 6. Effect of HA and gamma rays on Cu content of canola (mg kg^{-1}) under treated sewage effluent and fresh water irrigation. See footnotes of Fig. 1 for treatment designations

	Humic acid "g kg^{-1}" (H)					
Irradiation (R)	**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**		

4. CONCLUSIONS

Irradiation and HA effectively enhanced canola growth, seed yield, oil content, and micronutrients including Fe, Zn, Mn, and Cu. Moderate HA concentrations and irradiation doses resulted in significant increase in plant growth, seed, and seed oil yields. Recent results indicated that HA is beneficial for plant and oil production when used alone or in combination with gamma irradiation. Therefore, we recommend further future studies for better understanding of the mechanisms that mediate the beneficial impacts of HA and radiation on plant.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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