Future of Food: Journal on Food, Agriculture and Society
11 (1) December 2022

Physiological response of some canola genotypes to proline concentration under saltwater irrigation conditions

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Data of the article

First received : 04 October 2021 | Last revision received : 30 May 2022
Accepted : 15 November 2022| Published online :29 December 2022
DOI : 10.17170/kobra-202210056935

Keywords

salinity; cultivars; proline; yield; pigments; chemical composition

A pot experiment was conducted in split-split plot design with four replications to study proline foliar application with 0, 50, and 100 ppm and four canola genotypes cultivated under irrigation of tap water and salinity irrigation water at 4500 ppm and their interactive effects on growth characters, yield, and yield components and some chemical composition of the canola plants. Results indicated that a higher salinity level at 4500 ppm reduced growth, photosynthetic pigments, yield, and yield attributes as well as the chemical composition of seeds as compared with tap water. Results also indicated that Serw 6 cultivar had the greatest values of most characters under study. Trapper cultivar came in the second rank. Meanwhile, proline treatment at 100 ppm was the optimum treatment. Results indicated that there was an interaction between salinity x cultivars x proline concentration. Pots irrigated tap water secured the highest values of most characters with Serw 6 or Trapper cultivar x 100 ppm proline treatment. It could be concluded that proline especially at 100 ppm partially alleviated the harmful effects of salinity stress on the growth, yield, and yield components as well as the chemical composition of seeds of Serw 6 or Trapper cultivar of canola plants and nutritive value of the yielded seeds.

1. Introduction

Canola (*Brassica napus L.*) is the third most important oilseed crop worldwide and accounts for 12% of the total annual global oil production (FAOSTAT 2014). Seeds of canola have an oil content of more than 40% and produce post-crushing meals with 35% to 40% protein which is used mainly for animal feed (Snowdon *et al*., 2007). Canola is important due to the low Erucic acid in its oil which makes it good quality edible oil, but it also has high Erucic acid containing varieties that are used for manufacturing purposes. Seed oils are an important source of fatty acids for human nutrition and hydrocarbon chains for industrial products such as oleochemistry or as a replacement for petroleum products for combustion engines (Friedt and Lühs, 1998). It is the preferred oil seed crop under Egyptian conditions, especially where salinity and drought are commonplace and in newly reclaimed soil (Weiss, 1983). Canola oil contains a suitable profile of saturated fatty acids (7%), high levels of unsaturated fatty acids such as oleic acids (61%), and medium levels of linoleic acid (21%) and linolenic acid (11%). (El-Sabagh *et al*., 2018). As a result, it is a healthy edible oil.

Abiotic stress that limits plant growth and development is largely confined to salinity and drought in the realm of agriculture (Chandrasekaran *et al*., 2014; Augé *et al*., 2015). Osmotic factors owing to salinity and drought create rampage adversities upon plant production and productivity due to water constraints. Various biotic and abiotic stresses limit the successful cultivation of canola, with salinity being one of the major abiotic factors limiting production (Ashraf, 2001 and Qasim *et al*., 2003). Over 800 million ha of land is under salinity stress, accounting for 6% of the total cultivated land on earth (Arzani, 2008 and Munns and Tester, 2008). It has been reported that 20% of cultivated and 50% of croplands all over the world are affected by salinity (Kaya *et al*., 2002). Salinity can cause an assortment of changes in the metabolisms of plants including suppression of photosynthesis and respiratory, osmotic stress, ion toxicity, oxidative stress, and nutrient paucity (Tuteja, 2007 and Bandehagh *et al*., 2011). A surfeit of NaCl in soil solution, obstructs mineral nutrition and water uptake thus causing to accumulation of toxic ions in plants. Several authors, who have studied the effects of salinity on Brassicas report reductions in plant height, shoot and root dry weight, leaf number, leaf area, pod number/plant, seed number/pod, 100-seed weight, seed yield/plant, oil and protein content in the seeds (Ashrafijou *et al*., 2010; Saadia Sakr *et al*., 2012 and El Habbasha and Mekki, 2014) on canola. Currently, there is intensive work by many researchers to study the responses of plants to salt stress in order to try to overcome salt injury.

One approach is the exogenous application of substances that have been identified at a cellular level to be involved in resistance to stresses such as glycine betaines, proline, and antioxidants (Lopez and Satti, 1996). Proline is an amino acid and is one of the most commonly occurring compatible solutes, it plays a crucial major role in osmoregulation and osmotolerance (Hasegawa *et al*., 2000). It protects membranes and proteins against the destabilizing effects of dehydration during abiotic stress. In addition, it has some ability to scavenge free radicals generated under stress conditions (Ashraf and Foolad, 2007). Exogenous application of proline counteracted the adverse effects of salt stress by stimulating the growth of cells and plants (Ali *et al.* 2008) improving metabolism (Rai and Rana, 1996) and reducing oxidation of membrane lipids (Okuma *et al*., 2004; Yazici *et al*., 2007) under stress conditions. Athar and Ashraf (2009) also showed that exogenously applied proline at the germination and seedling stages alleviated the adverse effects of salt stress on canola cultivars and Okuma *et al.* (2004) illustrated that proline induced alleviation of the adverse effects of salt stress on growth. The work reported in this paper is the result of trying to determine whether proline can applied exogenously in the field to alleviate field-induced salt stress under Egyptian conditions. This may then provide an agronomic option for the alleviation of stress which could be used whilst plant breeders and biotechnologists search for genetic and physiological solutions to this problem. Therefore, the objective of this research studying the effect of different proline concentrations on growth parameters, yield and yield attributes, and some chemical analysis of canola varieties under saline conditions.

2. Material and Methods

2.1. Experimental site layout

A pot experiment was conducted at the wire-house of the National Research Centre, Dokky, Cairo, Egypt, where the cultivation process for canola seeds were done in the middle of November (15 November) during the growing season (2019/2020). The temperature ranged from 10–27 ºC and relative humidity ranged from 21–87 %. The chemical analysis of the experimental soil was determined according to Chapman and Pratt (1978) and included the following characteristics: pH 7.8, organic matter 0.21%, CaCo3 1.0%, E.C. 0.5 mhos cm-3 and available total N, P, K were 0.10, 3.20, 20.0 ppm, respectively. To reduce compaction and improve drainage, the soil was mixed with sand granules in a proportion of 2:1 (v:v). Nitrogen fertilizer was applied at the rate of 72 units of (N) ha^{-1}

ISSN-Internet 2197-411x OLCL 862804632

and Phosphorus fertilizer was applied at a rate of 24 units of (P2O5) ha^{-1} to each pot. The fertilizer was divided into three doses, the first dose mixed with the soil before sowing, the second dose after three weeks of sowing, and the third dose after six weeks of sowing. The experimental design was a split-split plot design with six replicates. The main plots included two salinity levels (tap water (600 ppm) and 4500 ppm) were prepared by dissolving sea salt with tap water. Sub-plots were assigned to four canola varieties (two genotypes imported from Germany (Agamax and Trapper) and two Egyptian genotypes (Serw 4 and Serw 6). Sub-subplot was divided into the foliar application of proline (0 as (control), 50 and 100 ppm) at 30 and 45 days from sowing.

2.2. Data recorded

2.2.1. Growth characters

Plants were sampled during vegetative stages (60 and 90 days after sowing) for measurement of some growth parameters (plant height, number of leaves/ plants, number of branches/ plant, fresh and dry weights of plant), fresh leaves were used for determination of photosynthetic pigments, where chlorophyll (A and B) and carotenoids contents in canola leaves were determined according to Witham *et al.* (1971).

2.2.2. Yield and yield attributes

At harvest, five plants were sampled randomly to estimate, plant height, number of siliqua $plant^{-1}$, 1000-seed weight (g), and seed, straw, and biological yields $plant^{-1}$ (g).

2.2.3. Chemical analysis

Macronutrients (N, P, and K) and micronutrients (Fe, Mn, and Zn) of canola seeds were determined according to Cottenie *et al.* (1982). The percentages of oil and total protein in canola seeds were determined according to (Helrich K., 1990). Seed protein content will calculate by multiplying N (%) by 5.75. Seed oil content will estimate by using the Soxhlet apparatus and petroleum ether at 60-80°C as a solvent.

2.3. Statistical analyses

The combined analysis of variance for the data of the two seasons was performed after testing the error homogeneity and Fisher's Least Significant Difference (LSD) test at 0.05 level obtained data from each season were subjected to the proper statistical analysis of variance of significance was used for the comparison between means according to Gomez and Gomez (1984).

3. Results and Discussion

3.1. Morphological characters and photosynthetic pigments after 60 days from sowing

3.1.1 Effect of salinity levels on some morphological characters and photosynthetic pigments after 60 days from sowing

The mean values for growth parameters (plant height, number of leaves/ plants, number of branches/plant, fresh and dry weights of plant) and photosynthetic pigments (chlorophyll a, chlorophyll b, carotenoids, and total pigments) after 60 days from sowing are presented in Table (1). The salinity level (4500 ppm) significantly affected the studied growth parameters and photosynthetic pigments compared with the tap water treatment, where it reduced the aforementioned characteristics. Farouk, (2011) stated that such reduction may be due to the inhibiting effect of salinity on cell division and cell enlargement; increase energy required for mineral and water absorption; accumulation of some poisonous compounds in the plants; high respiration rate; inhibition of protein turnover and nucleic acid synthesis. These results are in accordance with those recorded by Ashrafijou *et al*., 2010 and Saadia *et al*., 2012.

3.1.2. Effect of varietal differences on some morphological characters and photosynthetic pigments after 60 days from sowing

Average of plant height, number of leaves/ plants, number of branches/ plant, fresh and dry weights of plant, chlorophyll a, chlorophyll b, carotenoids, and total pigments affected by canola cultivars are shown in Table (1). The four tested cultivars were significantly different in most of the aforementioned characters. The results indicated that Serw 6 cultivar had the greatest plant height, number of leaves /plant, fresh and dry weights of plant, and chlorophyll b compared with other cultivars after 60 days from sowing. In this connection, the Trapper cultivar had the maximum values of the number of branches/plants, chlorophyll a, carotenoids, and total pigments compared with the other tested cultivars after 60 days from sowing. Serw 4 cultivar had the greatest number of branches/plant and chlorophyll after 60 days from sowing. While it gave the lowest values of plant height, the number of branches/plants, and chlorophyll b. In this regard, the Agamax cultivar gave the minimum values of the number of leaves /plants, chlorophyll a, carotenoids, and total pigments. Moreover, the Trapper cultivar gave the lowest values of fresh and dry weights of plants. These results may be due to the superiority of Serw 6 cultivar in traits under study to increase vegetative growth and plant height compared to the rest of the varieties. The results of the present investigation are in trend with those obtained by Bybordi and Tabatabaei (2009) and El-Habbasha and Mekki, (2014).

3.1.3. Effect of proline concentration on some morphological characters and photosynthetic pigments after 60 days from sowing

Proline treatments caused significant increases in most of the growth parameters and photosynthetic pigments relative to the corresponding control (0 of proline concentration) (Table 1). Increasing the proline concentration from 0 to 100 ppm increased most of the studied characters, this increment reached a significant level with the characters, plant height, and fresh weight/ plant. Application of proline increased growth parameters under saline conditions as compared with the control plant (0 proline) with both varieties. The protein organic amino acid-proline functions as an osmolyte, radical scavenger, electron sink, stabilizer of macromolecules, and a cell wall component (Matysik *et al*., 2002). Increased accumulation of proline leads to the increase of enzyme activity of glutamate kinase and therefore increases proline biosynthesis (Vašáková and Štefl, 1982). Plants utilize increased content of proline to protein biosynthesis that has specific properties. Proline and hydroxyproline are found in specific compounds. Many of these compounds have specific characteristics and these proteins help to overcome plant stress. This reason for such stress may be soil salinity. For some stress proteins rich proline content is typical (Jofre and Becker, 2009; Roshandel and Flowers, 2009). This is consistent with the general argument that proline is one of the major organic osmolytes. Therefore, the rapid accumulation of free proline in plants is a typical response to a wide range of environmental stresses (Pavlíková *et al*., 2008). The positive effect of proline on growth parameters of photosynthetic pigments may be attributed to an important variable amino acid in determining protein and membrane structures and scavenging reactive oxygen species (ROS) under drought stress (Ashraf and Foolad, 2007). Proline not only acts as an osmotolerant but also acts as a nutritional source.

3.1.4. Effect of Interactions

3.1.4.1. Effect of interaction between salinity levels and proline concentration on some morphological characters and photosynthetic pigments after 60 days from sowing

Results indicate that irrigation of tap water treatment with application of 100 ppm proline gave the highest values of plant height, number of leaves/ plants, number of branches/ plants, and fresh and dry weights of the plant) and photosynthetic pigments (chlorophyll a, chlorophyll b, carotenoids, and total pigments as compared to other treatments (Table 2). In the contrast, salinity 4500 ppm with 0 proline concentration treatment produced the lowest values of previous characters.

3.1.4.2. Effect of interaction between varietal differences and proline concentration on some morphological characters and photosynthetic pigments after 60 days from sowing

The obtained results verified that 100 ppm proline treatments with Agamax cultivar were highly efficient in increasing plant height as compared with other treatments (Table 3). Also, 50 ppm proline treatments with the Trapper cultivar were highly efficient in increasing chlorophyll a, chlorophyll b, carotenoids, and total pigments as compared to other treatments. On the other side, 0 proline concentration with the Trapper cultivar gave the lowest values of plant height, chlorophyll a, chlorophyll b, carotenoids, and total pigments.

ISSN-Internet 2197-411x OLCL 862804632

Table 1. Effect of salinity levels, cultivars, and proline concentration on some growth characters of canola genotypes after 60 days from sowing

Treatments	Plant height	No. of leaves/ plant	No. of branches/ plant	Fresh weight/ plant	Dry weight/ plant	Chlorophyll A	Chlorophyll B	Carotenoids	Total pigments
Salinity	-	-	-	-	-	-	-	-	-
4500 ppm	36.99	6.51	4.63	40.36	7.65	0.69	0.81	0.46	2.14
Tap water	41.78	7.34	5.18	45.70	8.65	0.78	0.92	0.52	2.41
F.test	*	*	*	*	*	*	*	*	*
Cultivars	-	-	-	-	-	-	-	-	-
Agamax	41.14	6.65	4.21	42.92	8.44	0.35	0.93	0.23	1.63
Serw 4	36.35	6.67	4.95	40.81	7.70	0.60	0.81	0.40	1.95
Serw 6	42.47	7.03	4.63	43.96	8.51	0.41	0.97	0.27	1.79
Trapper	36.11	6.64	5.06	40.70	7.48	1.27	0.74	0.86	3.14
LSD 0.05	0.17	NS	0.21	0.29	0.10	0.11	NS	0.17	NS
Proline con.	-	-	-	-	-	-	-	-	-
0	37.63	6.89	4.88	42.88	8.17	0.74	0.87	0.49	2.29
50	38.43	6.94	4.91	42.97	8.11	0.74	0.87	0.49	2.27
100	42.10	6.94	4.94	43.24	8.17	0.72	0.87	0.48	2.26
LSD 0.05	0.30	NS	NS	0.15	NS	NS	NS	NS	NS

Table 2. Effect of interaction between salinity levels and spraying by proline concentrations on some growth characters of canola genotypes after 60 days from sowing

Treatments		Plant height	No. of leaves/ plant	No. of branches/ plant	Fresh weight/ plant	Dry weight/ plant	Chlorophyll A	Chlorophyll B	Carotenoids	Total pigments
4500ppm	0	34.21	6.52	4.64	40.35	7.65	0.69	0.81	0.46	2.14
	50	36.19	6.47	4.61	40.14	7.61	0.69	0.81	0.46	2.13
	100	40.56	6.54	4.66	40.58	7.68	0.69	0.82	0.46	2.15
Tap water	0	41.04	7.37	5.24	45.59	8.69	0.79	0.92	0.53	2.44
	50	40.67	7.31	5.22	45.61	8.61	0.78	0.92	0.52	2.41
	100	43.64	7.35	5.10	45.89	8.66	0.76	0.92	0.50	2.37
LSD 0.05		0.43	NS	NS	0.11	0.15	NS	NS	NS	NS

Table 3. Effect of interaction between varietal differences and spraying by proline concentrations on some growth characters of canola genotypes after 60 days from sowing

Treatments		Plant height	No. of leaves/ plant	No. of branches/ plant	Fresh weight/ plant	Dry weight/ plant	Chlorophyll A	Chlorophyll B	Carotenoids	Total pigments
Agamax	0	37.53	7.16	4.41	44.97	8.76	0.38	0.98	0.25	1.75
	50	40.46	6.51	4.21	42.96	8.54	0.36	0.97	0.24	1.70
	100	45.43	6.28	4.01	40.83	8.01	0.31	0.84	0.20	1.45
Serw 4	0	34.06	6.57	5.30	39.15	7.09	0.93	0.74	0.62	2.49
	50	36.35	6.41	4.47	40.71	7.90	0.41	0.81	0.27	1.61
	100	38.65	7.03	5.08	42.58	8.12	0.46	0.87	0.30	1.76
Serw 6	0	40.53	7.50	5.32	44.45	8.31	0.49	0.98	0.33	1.95
	50	42.88	7.11	4.38	44.70	8.73	0.38	0.97	0.25	1.74
	100	44.00	6.47	4.20	42.72	8.49	0.36	0.97	0.24	1.70
Trapper	0	31.09	6.82	4.38	39.68	8.29	0.97	0.72	0.65	2.55
	50	34.69	6.65	5.19	41.21	7.59	1.53	0.78	1.03	3.66
	100	42.53	6.46	5.63	41.19	6.56	1.33	0.73	0.89	3.22
LSD0.05		0.95	NS	0.45	0.26	NS	0.14	NS	0.21	0.30

3.1.4.3. Effect of interaction between salinity levels and varietal differences on some morphological characters and photosynthetic pigments after 60 days from sowing

Data presented in Table (4) illustrated the effect of interaction between salinity levels and varietal differences on some morphological characters and photosynthetic pigments after 60 days from sowing, where the interaction between salinity levels and varietal differences significantly affected most of the studied characters except, number of leaves/ plants, Chb, and carotenoids. Where the treatments tap water + Serw 6 and tap water + Agamax records the highest values of plant height, number of leaves/ plants, number of branches/ plant, fresh weight/ plant, and Chb, while the treatments saline water (4500 ppm) + Serw 6 and saline water + Trapper recorded the lowest values of plant height, number of leaves/ plant and fresh weight/ plant. Also, the treatment saline water (4500 ppm) + Serw 6 recorded the lowest values of dry weight/ plant, Cha, Chb, Car, and total pigments.

3.1.4.4. Effect of interaction among salinity levels, varietal differences, and proline concentration on some morphological characters and photosynthetic pigments after 60 days from sowing

The second-order interaction among the three tested factors clearly showed the beneficial effects of salinity and proline growth parameters and photosynthetic pigments under canola cultivars (Figs 1, 2, 3, and 4). Pots irrigated with tap water secured the highest values of plant height with Agamax genotype and 100 ppm proline, while the lowest value of the plant height was recorded by the treatment salinity level of 4500 ppm with Trapper and 0 proline concentration (Fig 1). Pots irrigated with tap water recorded the highest values of fresh weight/ plant with Agamax genotype and 0 ppm proline as well as tap water with Serw 6 and 50 ppm proline concentration with no significant differences between both treatments (Fig 2), while the treatment of 4500 ppm salinity level with Serw 4 genotype and 0 proline concentration recorded the lowest value for fresh weight/ plant character. Dry weight/ plant showed the highest value with the treatment of tap water with the Agamax genotype and 50 ppm proline concentration while the treatment 4500 ppm salinity water with Trapper genotype and 50 ppm proline concentration showed the lowest value of dry weight/ plant (Fig 3). The pots sowing by Trapper genotype and irrigated with tap water and sprayed by 50 or 100 ppm proline showed the highest total pigments as illustrated in (Fig 4).

ISSN-Internet 2197-411x OLCL 862804632

Table 4. Effect of interaction between salinity levels and varietal differences on some morphological characters and photosynthetic pigments after 60 days from sowing

		Plant height	No. of leaves/ plant	No. of branches/ plant	Fresh weight/ plant	Dry weight/ plant	Chlorophyll A	Chlorophyll B	Carotenoids	Total pigments
4500	Agamax	38.88	6.56	3.91	40.37	7.06	0.30	0.84	0.30	1.57
	Serw 4	34.58	6.52	4.76	39.79	7.65	0.45	0.82	0.29	1.69
Ppm	Serw 6	41.62	6.75	4.32	43.61	6.45	0.31	0.77	0.27	1.35
	Trapper	34.20	6.53	5.29	39.56	7.07	0.91	0.72	0.75	2.38
Tap water	Agamax	43.39	7.04	4.50	44.47	8.83	0.39	1.01	0.25	1.79
	Serw 4	39.12	6.81	5.13	41.82	8.73	0.74	0.79	0.49	2.21
	Serw 6	43.37	7.29	5.94	44.29	8.56	0.45	0.97	0.30	1.86
	Trapper	38.01	6.73	5.84	43.83	8.91	1.12	0.77	0.75	2.90
L S D 0.05		1.12	NS	0.45	0.84	0.64	0.21	NS	NS	0.67

Fig (1): Effect of interaction between salinity levels, cultivar and spraying by proline concentrations on plant height of canola after 60 DAS

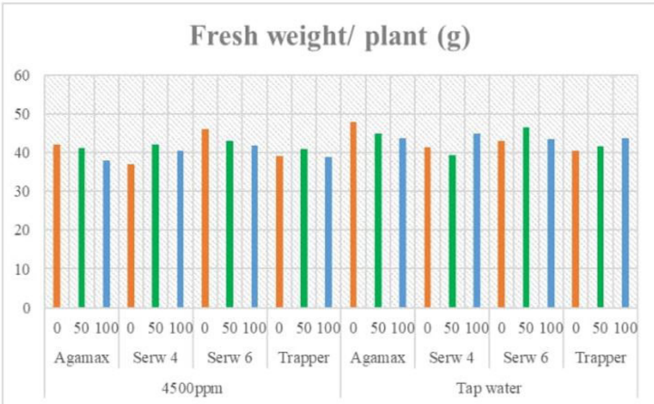

Fig (2): Effect of interaction between salinity levels, cultivar and spraying by proline concentrations on fresh weight/plant of canola after 60 DAS

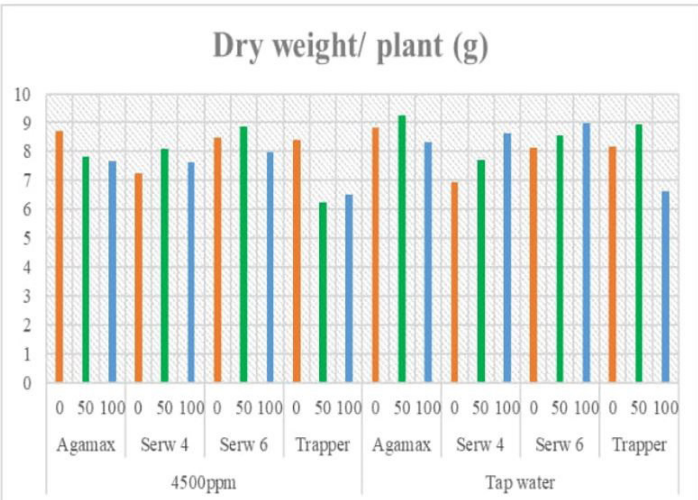

Fig (3): Effect of interaction between salinity levels, cultivar and spraying by proline concentrations on dry weight/plant of canola after 60 DAS

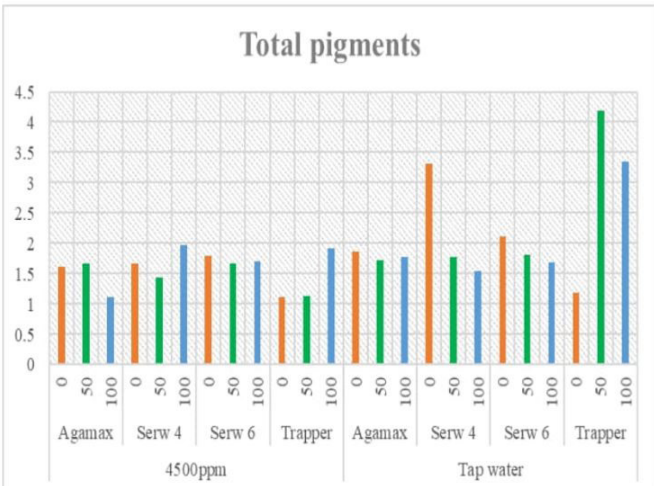

Fig (4): Effect of interaction between salinity levels, cultivar and spraying by proline concentrations on total pigments of canola after 60 DAS

3.2. Yield and yield attributes of some canola genotypes

3.2.1. Effect of salinity levels on some yield and yield attributes of some canola genotypes

The results illustrated in (Table 5) shows the effect of saline water (4500 ppm) and tap water on some yield and yield attributes characters, where, salinity stress caused decreases in 1000 seed weight, seed yield/plant, pod yield/plant, straw yield/plant, and biological yield/plant. Farouk *et al.* (2011) stated that such reduction in yield and yield attributes result from the reduction in the supply of carbon assimilation due to decreasing the net photosynthetic rate and biomass accumulation. Van Hoorn *et al.*, (2001) mentioned that the reduction in yield of soybean plants under salinity stress was attributed to the decrease in photosynthetic rate, carbohydrate accumulation, nitrogenase activity, and consequently seed yield. The results of the present investigation are in trend with those obtained by Eyvazlou *et al.*, 2019.

3.2.2. Effect of varietal differences on some yield and yield attributes of some canola genotypes

The results in (Table 5) indicated that the effect of four canola cultivars on yield and yield attributes were significantly pod yield/plant, straw yield/plant, and biological yield/plant. Agamax cultivars gave better values to the previous characters as compared to other cultivars. This increase in pod yield/plant, straw yield/plant, and biological yield/plant amounted to 11.52, 10.19, and 10.48 % more than Serw 4 cultivar. In this regard, the increase in Agamax yield may be due to increasing vegetative growth, which led to an increase in yield component resulting in increased plant seed yield compared to the rest of the varieties. These results are in coinciding with those detected by El-Habbasha and Mekki (2014).

3.2.3. Effect of proline concentration on some yield and yield attributes of some canola genotypes

Data presented in Table (5) showed that increasing proline concentration from 0 to 100 ppm tended to significantly increment 1000 seed weight, seed yield/plant, pod yield/plant, straw yield/plant, and biological yield/plant. While, increasing proline concentration from 50 to 100 ppm tended to no significant increment in most of the studied characters except, pod yield/plant and biological yield/plant.

Table 5. Effect of salinity levels, cultivars, and proline concentration on yield and yield attributes of canola plants

Treatments	1000 seed weight	Seed yield/plant	Pod yield/plant	Straw yield/plant	Biological yield/plant
Salinity	-	-	-	-	-
4500 ppm	3.08	4.31	8.66	14.11	27.08
Tap water	3.56	4.93	9.89	16.17	30.99
F. test	*	*	*	*	*
Cultivars	-	-	-	-	-
Agamax	3.40	4.65	9.49	15.25	29.39
Serw 4	3.08	4.25	8.51	13.84	26.60
Serw 6	3.34	4.61	9.24	15.03	28.88
Trapper	3.30	4.56	9.03	15.02	28.61
LSD 0.05	NS	NS	0.54	0.84	0.64
Proline con.	-	-	-	-	-
0	2.90	4.40	8.23	14.11	26.74
50	3.32	4.51	9.24	15.07	28.82
100	3.34	4.64	9.86	15.94	30.44
LSD 0.05	0.21	0.15	0.42	1.05	1.14

ISSN-Internet 2197-411x OLCL 862804632

3.2.4. Effect of interactions

3.2.4.1. Effect of interaction between salinity levels and proline concentration on some yield and yield attributes of some canola genotypes

The interactive effects between salinity and proline concentration significantly affected significantly of pod yield/plant, straw yield/plant, and biological yield/plant (Table 6). Pots that received an application of salinity at the rate of 4500 ppm and untreated proline treatments produced the lowest pod yield/plant, straw yield/plant, and biological yield/plant. Meanwhile, the maximum values of the previous characters were reported with tap water treatment and foliar application of 100 ppm proline treatment. El-Moukhtari *et al.* (2020) reported that under high-salt conditions, proline application enhances plant growth with increases in seed germination, biomass, photosynthesis, gas exchange, and grain yield. These positive effects are mainly driven by better nutrient acquisition, water uptake, and biological nitrogen fixation. Exogenous proline also alleviates salt stress by improving antioxidant activities and reducing Na+ and Cl− uptake and translocation while enhancing K+ assimilation by plants. These results are in general agreement with those recorded by Gyawali *et al.*, 2019 and Sadak *et al.*, 2020.

3.2.4.2. Effect of interaction between varietal differences and proline concentration on some yield and yield attributes of some canola genotypes

Concerning the interaction effect between varietal differences and proline concentration, where the studied characters were significantly affected by the interaction between treatments, except, for 1000-seed weight and seed yield/ plant. The maximum values of pod yield/plant, straw yield/plant, and biological yield/plant were recorded with Serw 6 and spraying of 100 ppm proline treatment (Table 7). In contrast, the lowest values of the aforementioned characters were obtained by Serw 4 cultivars and untreated proline combination.

Table 6. Effect of interaction between salinity levels and spraying by proline concentrations on yield and yield attributes of canola plants.

		1000 seed weight	Seed yield/ plant	Pod yield/ plant	Straw yield/ plant	Biological yield/ plant
4500 Ppm	0	3.10	4.29	8.60	14.02	26.91
	50	3.09	4.34	8.70	14.15	27.19
	100	3.07	4.29	8.68	14.17	27.14
Tap water	0	3.51	4.91	9.82	16.10	30.83
	50	3.56	4.88	9.79	15.99	30.66
	100	3.61	4.99	10.07	16.40	31.46
LSD 0.05		NS	NS	0.64	1.05	1.25

Table 7. Effect of interaction between varietal differences and spraying by proline concentrations on some yield and yield components of canola genotypes

		1000 seed weight	Seed yield/ plant	Pod yield/ plant	Straw yield/plant	Biological yield/plant
Agamax	0	3.39	4.57	9.64	15.03	29.24
	50	3.48	4.89	9.58	15.20	29.67
	100	3.34	4.48	9.25	14.51	28.24
Serw 4	0	3.06	4.21	8.36	13.43	26.00
	50	3.28	4.45	8.45	14.58	27.48
	100	2.91	4.09	8.74	13.52	26.35
Serw 6	0	3.13	4.35	8.49	14.14	26.98
	50	3.40	4.61	9.52	15.13	29.26
	100	3.48	4.85	9.71	16.83	31.39

3.2.4.3. Effect of interaction between salinity levels and varietal differences on some yield and yield attributes of some canola genotypes

The results in (Table 8) showed that there were significant interactions between salinity levels and varietal differences in straw yield/plant and biological yield/plant. The highest values were obtained from irrigation of tap water integrated with Agamax cultivars. On the other hand, the lowest values were recorded from the irrigated with 4500 ppm salt treatment integrated with Serw 4 cultivar. These results are in accordance with those recorded by Bybordi and Tabatabaei (2009) and El-Habbasha and Mekki (2014).

3.2.4.4. Effect of interaction among salinity levels, varietal differences, and proline concentration on some yield attributes of some canola genotypes

Data presented in Figs 5, 6, 7, and 8 clear that, the third interaction among salinity levels, varietal differences, and proline concentration on seed yield/ plant, pod yield/plant, straw yield/plant, and biological yield/plant, where these characters significantly affected by the interactions among salinity levels, varietal differences and proline concentration where the treatment tap water with the cultivar Agamax and spraying with 50 ppm proline concentration recorded the highest values of the studied characters. While, the lowest values of the studied characters were recorded by the treatment 4500 ppm saline water with the cultivar Serw 4 and without sparing proline for the characters seed yield/ plant, straw yield/plant, and biological yield/plant, also the treatment of 4500 ppm saline water with the cultivar Serw 6 and without sparing proline for the character pod yield/plant.

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Table 8. Effect of interaction between salinity levels and varietal differences on some yield and yield components of canola genotypes

IRRI	TRT	1000 seed weight	Seed yield/plant	Pod yield/plant	Straw yield/ plant	Biological yield/ plant
4500 ppm	Agamax	3.29	4.48	9.23	14.76	28.47
	Serw 4	2.94	4.12	8.24	13.31	25.67
	Serw 6	3.23	4.53	9.07	14.80	28.4
	Trapper	3.13	4.41	8.74	14.55	27.7
Tap water	Agamax	3.51	4.81	9.75	15.73	30.29
	Serw 4	3.22	4.37	8.77	14.38	27.52
	Serw 6	3.44	4.68	9.41	15.26	29.35
	Trapper	3.46	4.70	9.31	15.50	29.51
LSD0.05		NS	NS	NS	0.94	1.14

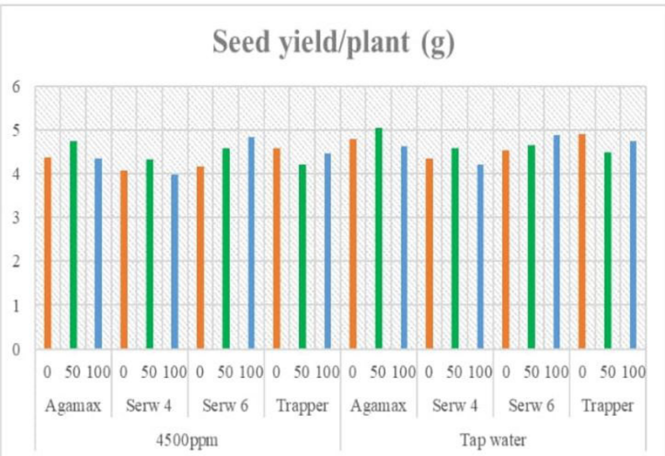

Fig (5): Effect of interaction among salinity levels, varietal differences and spraying by proline concentrations on seed yield/plant of canola genotypes

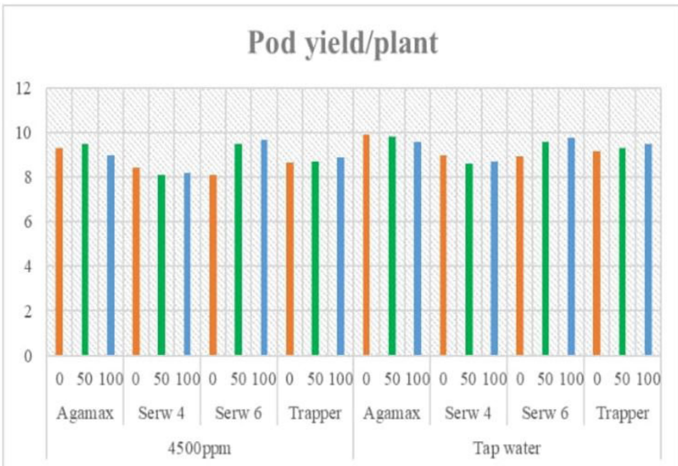

Fig (6): Effect of interaction among salinity levels, varietal differences and spraying by proline concentrations on pod yield/plant of canola genotypes

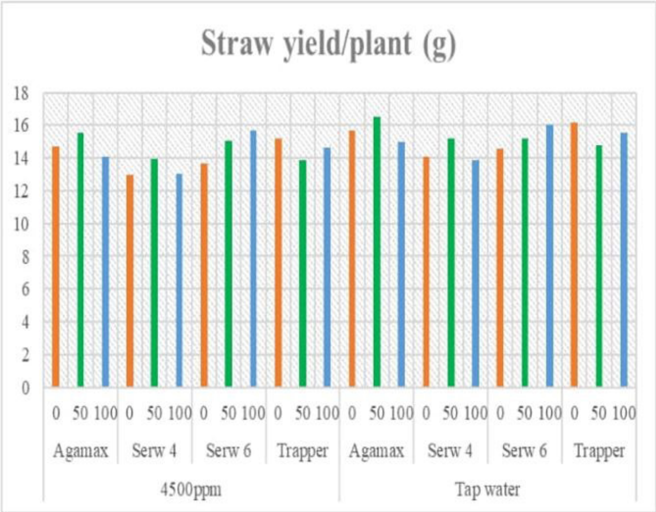

Fig (7): Effect of interaction among salinity levels, varietal differences and spraying by proline concentrations on straw yield/plant of canola genotypes

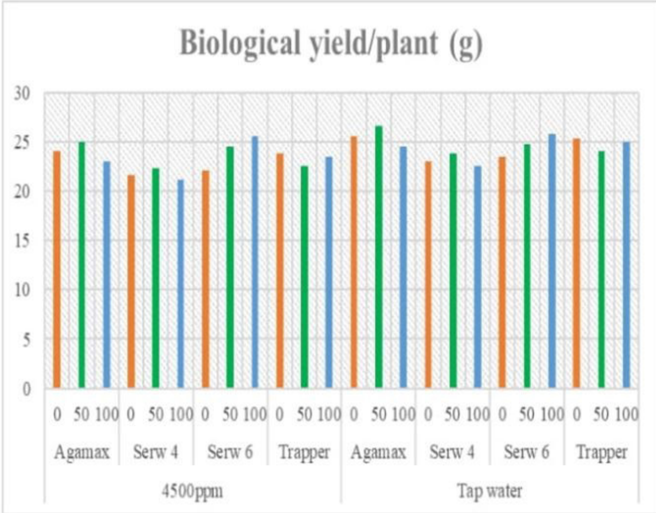

Fig (8): Effect of interaction among salinity levels, varietal differences and spraying by proline concentrations on biological yield/plant of canola genotypes

3.3. Seed chemical composition of some canola genotypes

3.3.1. Effect of salinity levels on seed chemical composition of some canola genotypes

Results in (Table 8) show that protein %, N%, P%, K%, Fe ppm, Mn ppm, and Zn ppm concentrations were significantly decreased by increasing salinity level relative to a corresponding control, while oil% was significantly decreased by increasing salinity. These results may be due to the reduction in the growth of canola plants under salinity stress was attributed to the decrease in photosynthetic rate, protein and carbohydrate accumulation as well as macro and micronutrients. Hussain *et al.* (2018) concluded that soil salinity affects various plant physiological activities through increased oxidative damage, decreased turgor, as well as changes in leaf gas exchange, ultimately leading to reduced plant growth, development, and yield (Hussain *et al.*, 2016 and Roy *et al.*, 2014).

3.3.2. Effect of varietal differences on seed chemical composition of some canola genotypes

Results indicated that four canola genotypes significantly differ in percentages of protein %, N%, P%, K%, Fe ppm, Mn ppm, and Zn ppm as shown in (Table 8). Trapper cultivar gave better values of the oil %, P%, Fe ppm, Mn ppm, and Zn ppm as compared to other cultivars. While Agamax gave the maximum values of the protein % and N%, compared to other cultivars. In this connection, Serw 6 cultivar gave the highest values of K% as compared to other cultivars. In contrast, Serw 4 cultivar produced the lowest values of protein %, N%, P%, and K% when compared with the other cultivars. A similar trend was reported by Bybordi (2010) and El-Habbasha and Mekki (2014).

3.3.3. Effect of proline concentration on seed chemical composition of some canola genotypes

Data in Table (9) showed the effect of increasing proline concentration from 0 to 100 µM on protein content, seed oil content, some macro (Ca, Mg, K, and Na), micro (Fe, Mn, and Zn) nutrients, of some canola varieties (Agamax, Trapper, Serw 4 and Serw 6). Where the results indicated that there was no significant effect of proline concentration on the chemical composition of canola seeds.

3.3.4. Effect of interactions

3.3.4.1. Effect of interaction between salinity levels and proline concentrations on seed chemical composition of some canola genotypes

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Table 9. Effect of salinity levels, cultivars, and proline concentration on some chemical characters of canola.

Treatments	Protein %	Oil %	N %	P %	K %	Fe ppm	Mn ppm	Zn ppm
Salinity:	-	-	-	-	-	-	-	-
4500 ppm	22.88	47.78	3.97	0.46	0.90	71.53	13.13	25.17
Tap water	24.89	46.83	4.53	0.52	1.04	81.67	15.00	28.74
F.test	*	*	*	*	*	*	*	*
Cultivars:	-	-	-	-	-	-	-	-
Agamax	25.13	46.42	4.36	0.48	0.95	69.51	13.00	26.35
Serw 4	22.54	47.88	3.91	0.44	0.90	76.25	13.57	25.17
Serw 6	24.33	46.71	4.22	0.47	0.97	63.47	13.51	26.23
Trapper	23.76	47.98	4.12	0.50	0.95	84.89	14.18	27.16
LSD0.05	0.10	0.07	0.03	0.00	0.01	0.93	0.10	0.17
Proline con.	-	-	-	-	-	-	-	-
0	23.92	47.23	4.24	0.49	0.97	76.73	14.04	26.93
50	23.90	47.32	4.24	0.49	0.96	76.12	14.00	26.84
100	23.85	47.36	4.28	0.49	0.98	76.95	14.16	27.09
LSD0.05	NS	NS	NS	NS	NS	NS	NS	NS

In pots irrigated with tape water with proline concentration produced the maximum values of protein %, N%, Fe ppm, Mn ppm, and Zn ppm (Table 10). On the other side, irrigating the plants with salinity at 4500 ppm gave the minimum values of previous characters. These results are in coinciding with those detected by Eyvazlou *et al.* (2019).

3.3.4.2. Effect of interaction between varietal differences and proline concentrations on seed chemical composition of some canola genotypes

Data in (Table 11) showed that there was a significant effect due to the interaction between canola cultivars and proline concentration on protein %, N%, Fe ppm, Mn ppm, and Zn ppm. Cultivation of Serw 6 and application of proline at 100 ppm produced the highest values of protein % and N%. In this connection, Serw 4 gave the maximum values of oil % when addition to proline at the rate of 100 ppm. In this regard, the Trapper cultivar with 100 ppm proline produced the largest values of Fe ppm, Mn ppm, and Zn ppm. While the lowest values of previous characters were recorded with Serw 4 treatment with the addition of 50 or 100 ppm proline concentrations. These results are in accordance with those recorded by El-Habbasha and Mekki (2014).

3.3.4.3. Effect of interaction between salinity levels and varietal differences on seed chemical composition of some canola genotypes

Results presented in (Table 12) illustrated those significant differences in the most of studied characters, except P% and K% as affected by the interaction of salinity levels and varietal differences. Tape water x Agamax was the more efficient interaction for enhancing seeds protein % and N%. While Trapper x tape water gave the maximum seeds of Fe ppm, Mn ppm, and Zn ppm contents (Table 11). Moreover, Serw 4 treatment with salinity produced the largest values of oil %. In contrast, the lowest values of protein %, N%, Fe ppm, Mn ppm, and Zn ppm were recorded with Serw 4 treatment with pots irrigated with saline water at the rate of 4500 ppm. Similar results have been reported by Bybordi (2010) and El-Habbasha and Mekki (2014).

Table 10. Effect of interaction between salinity levels and spraying by proline concentrations on some chemical characters of canola.

		Protein %	Oil %	N %	P %	K %	Fe ppm	Mn ppm	Zn ppm
4500	0	22.89	47.71	3.97	0.46	0.90	71.40	13.13	25.16
Ppm	50	22.94	47.92	3.98	0.46	0.90	71.74	13.17	25.23
	100	22.82	47.71	3.96	0.46	0.90	71.44	13.10	25.12
Tap water	0	24.88	46.74	4.50	0.52	1.03	82.07	14.96	28.71
	50	24.85	46.73	4.49	0.52	1.02	80.50	14.82	28.44
	100	24.94	47.01	4.60	0.53	1.05	82.45	15.22	29.07
LSD0.05		0.05	0.03	0.01	NS	NS	0.53	0.04	0.09

Table 11. Effect of interaction between varietal differences and spraying by proline concentrations on some chemical characters of canola.

		Protein %	Oil %	N %	P %	K %	Fe ppm	Mn ppm	Zn ppm
Agamax	0	24.45	47.04	4.24	0.42	0.95	65.95	13.32	27.15
	50	25.92	45.56	4.50	0.50	0.98	75.83	13.46	27.03
	100	25.02	46.65	4.34	0.51	0.91	66.75	12.22	24.87
Serw 4	0	23.53	46.46	4.08	0.41	0.94	83.12	14.17	23.73
	50	22.51	48.57	3.91	0.44	0.92	92.62	14.75	28.98
	100	21.58	48.61	3.74	0.47	0.83	43.00	11.78	22.80
Serw 6	0	22.88	47.80	3.96	0.48	0.98	49.36	13.88	24.82
	50	24.32	46.59	4.22	0.42	0.95	65.61	13.25	27.01
	100	25.78	45.72	4.47	0.50	0.98	75.44	13.39	26.88
Trapper	0	23.95	47.82	4.15	0.50	0.93	82.14	12.59	24.53
	50	22.98	48.60	3.98	0.53	0.94	79.08	14.59	28.33
	100	24.36	47.52	4.23	0.48	0.98	93.44	15.37	28.62
LSD0.05		0.11	0.07	0.02	NS	NS	1.19	0.08	0.20

ISSN-Internet 2197-411x OLCL 862804632

Table 12. Effect of interaction between salinity levels and varietal differences on some chemical characters of canola.

		Protein %	Oil %	N %	P %	K %	Fe ppm	Mn ppm	Zn ppm
4500 Ppm	Agamax	24.34	47.16	4.22	0.46	0.92	67.31	12.59	25.52
	Serw 4	21.83	47.91	3.79	0.42	0.87	73.84	13.13	24.38
	Serw 6	24.00	46.79	4.16	0.46	0.96	62.70	13.31	25.88
	Trapper	23.01	48.17	3.99	0.49	0.92	82.21	13.73	26.30
Tap water	Agamax	25.93	45.68	4.50	0.49	0.98	71.71	13.41	27.18
	Serw 4	23.25	47.85	4.04	0.45	0.93	78.65	14.00	25.97
	Serw 6	24.66	46.62	4.28	0.47	0.98	64.24	13.70	26.59
	Trapper	24.52	47.79	4.25	0.52	0.98	87.56	14.63	28.01
LSD0.05		0.27	0.35	0.22	NS	NS	0.72	0.37	0.13

3.3.4.4. Effect of interaction among salinity levels, varietal differences, and proline concentration on seed chemical composition of some canola genotypes

Data presented in (Figs 9, 10, 11, 12, 13, and 14) cleared that the third interaction among salinity levels, varietal differences, and proline concentration on some seed chemical composition of canola genotypes, where these characters significantly affected by the interactions among salinity levels, varietal differences, and proline concentration. The treatment of tap water with the cultivar Agamax and spraying with 50 ppm proline concentration recorded the highest values of protein % (Fig 9). The treatment of tap water in addition to Serw 4 genotype and application of 50 ppm proline recorded the highest values of Fe and Zn (Fig 12 and 14). While, the treatments tap water+ Serw 6 genotype + 100 ppm proline concentration and 4500 ppm + Trapper genotype + 100 ppm concentration recorded the highest value of Mn (Fig 11 and Fig 13).

While the lowest values of the studied characters were recorded by the treatment of 4500 ppm saline water with the cultivar Serw 4 and 100 ppm proline for the characters protein %, N %, Fe, Mn, and Zn (Figs 9, 11, 12, 13 and 14), the same treatment recorded the highest oil % (Fig 10).

4. Conclusions

According to the previous results, it could be concluded that the use of exogenous application of plant growth regulating compounds like proline alleviation the adverse effects of salt stress on canola varieties by improving metabolism and stimulating the growth of plants this tended to significantly increase the most of growth, yield and yield attributes as well as some chemical constituents, proline especially at 100 ppm partially alleviated the harmful effects of salinity stress as well as the chemical composition of seeds of Serw 6 or Trapper cultivar of canola plants and nutritive value of the yielded seeds.

Acknowledgment

The authors gratefully acknowledge Science, Technology, and Innovation Funding Authority (STIFA), Egypt for funding the bilateral GERF project number (23149) which gave us the opportunity for carrying out this research. Also, special appreciation extends to National Research Center (NRC), Egypt for offering the facilities through the project and this research.

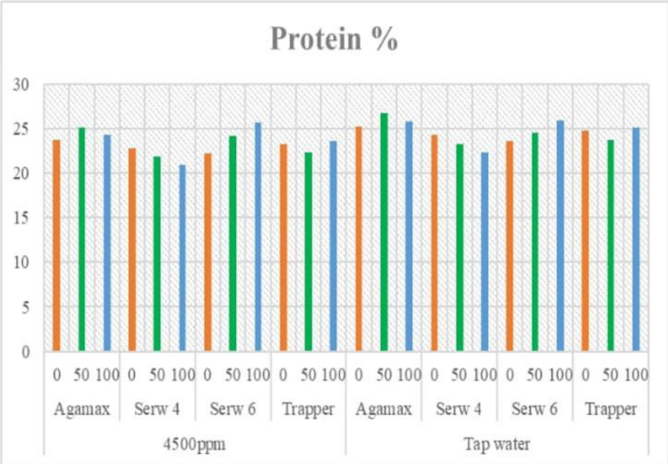

Fig (9): Effect of interaction among salinity levels, varietal differences and spraying by proline concentrations on protein % of canola seed

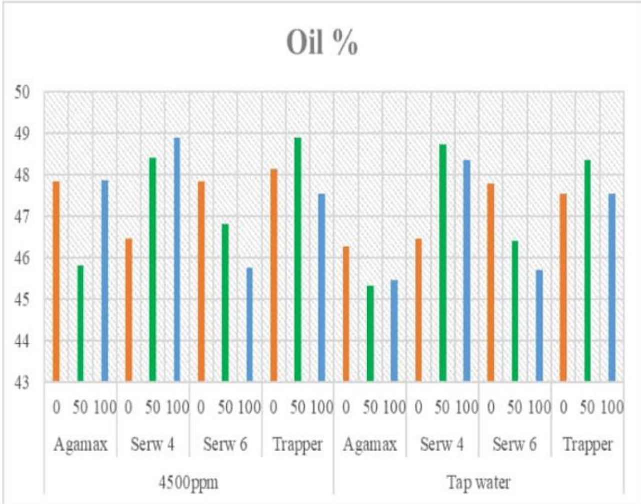

Fig (10): Effect of interaction among salinity levels, varietal differences and spraying by proline concentrations on oil % of canola seed

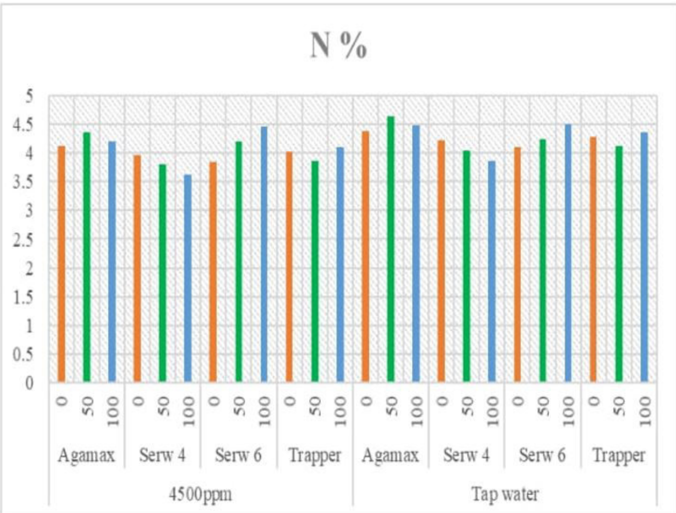

Fig (11): Effect of interaction among salinity levels, varietal differences and spraying by proline concentrations on N % of canola seed

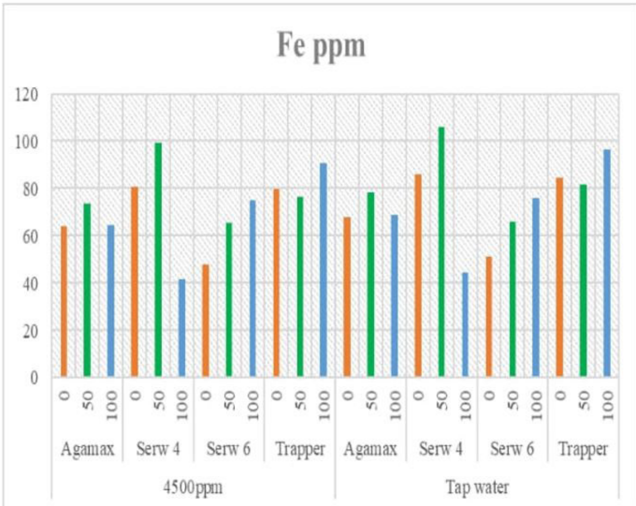

Fig (12): Effect of interaction among salinity levels, varietal differences and spraying by proline concentrations on Fe content of canola

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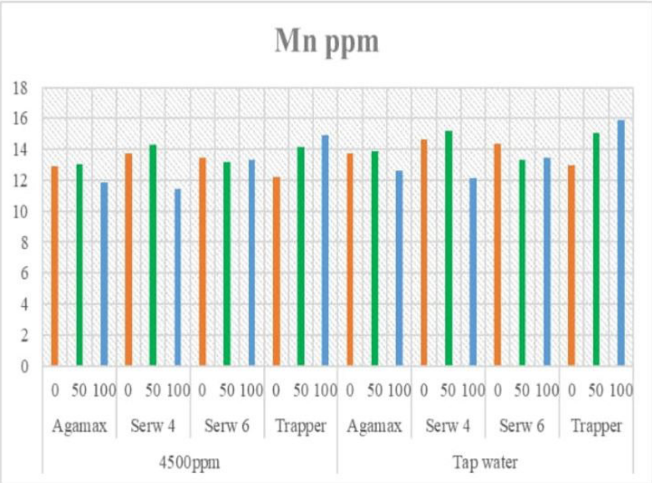

Fig (13): Effect of interaction among salinity levels, varietal differences and spraying by proline concentrations on Mn content of canola seed

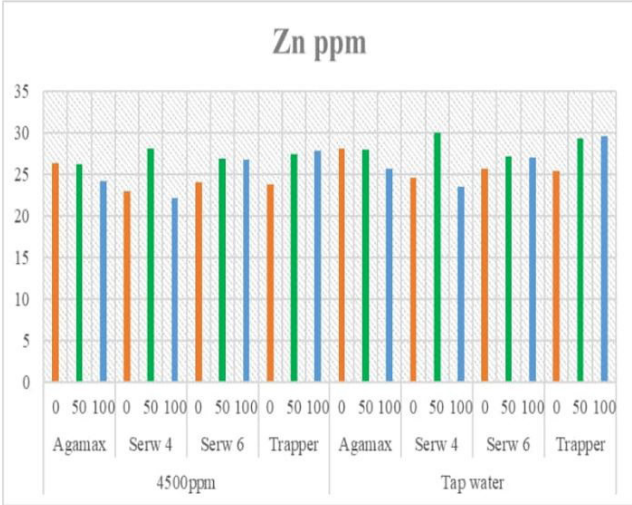

Fig (14): Effect of interaction among salinity levels, varietal differences and spraying by proline concentrations on Zn content of canola seed

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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ISSN-Internet 2197-411x OLCL 862804632

UniKassel & VDW, Germany- December 2022