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Research Article

Studying the impact of different amount of Phosphorus on lipids in Canola seeds in strain of Sarigol

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ABSTRACT

Phosphorus is an essential element for plants which has role in different processes. In the present study the impact of different consistency of phosphorus (0, 0.02, 0.2, 2 mmol/Lit) have been estimated on some physiological, biochemical parameters also, the amount of chlorophyll a,b & total chlorophyll and lipids in seed of Canola plant in the strain of Sarigol in stage of production. The results shown that high relatively consistency of phosphorus in stage of production caused to increase the length of shell in bush and the number of shell with seeds and the weight of thousand of seeds. Also, by studying the results of the study we can report that there was a meaningful difference about 1% in witness treatment as compared with 3 other treatments, the amount of chlorophyll a,b & total chlorophyll in studying the results of impact of phosphorus on fatty acids of Canola seeds we can found that the high amount of phosphorus in cultivation step caused increasing of fatty acids with a binary bond (oleic acid) but it does not have considerable impact on other saturated unsaturated fatty acids.

Keywords: Phosphorus production steps, Chlorophyll, Canola seed, Fatty acids

INTRODUCTION

Oily seeds are the second nutritional resources after cereals in the world. These production beside having fatty acids, rich resources owns proteins as well. The oil of Canola eatable seed has the best quality and after oilpressing, the meal remaining is full of protein and it is useful for nutrition of livestock (7). Canola seed includes 40% to 44% oil and 21% protein. Fatty acids forming oil are much more diverse than fatty acids of other vegetable oils. Canola oil containing 5 to 8% of saturation oil and 60 to 65% of unsaturation oil and 30 to 35% of multiple unsaturation oil such as linolenic acid and alpha linoleic acid.Also, Canola oilers contains more than 40% of Erucicacid which is used as lubricant, component manufacturer of tires, fiber, plastic and wax. Canola mainly used as Salad oil, margarine in food industry also in soap factory and lighting (23).

Phosphorus, is one of essential and high- Power element for growth and differentiation of the plant and play main role such as photosynthesis and nitrogen fixation, flowering, fruit production and maturity in plant. It used often in the process

of plant reproduction and only 9 to 16% of it used in vegetative (14). In comparison of other oil seeds Canola has a lot of phytic acid. Phosphorus in the soil impacts on the amount of phytic acid in seed.

Lots of phosphorus increases the production of seed and amount of oil. Shortage of phosphorus caused decreasing in the amount of pod in Canola. During investigation, the impact of phosphorus on oil will specified withIncreasing the amount of phosphorus to 40 Kg per hectare the amount of oil and oleic acid will increase with phosphorus and nitrogen.

Taylor &et alls (1986) had different opinions in their investigations regarding to the impact of phosphorus on the amount of oil so that, in some segment with increasing phosphorus caused increase the amount of oil and in other segment with increasing phosphorus decreased the amount of oil.

But before it, Singh &et alls in 1960 realized that the amount of oil has direct relation with phosphorus (13).

In recent years by more attention to Canolacaused considerable addition in cultivation. The aim of selection Canola is the important of cultivation of Canola based on the weather condition of north of Iran. By the way, using chemical fertilizers and responding plants to fertilizers(them) depends on the environmental conditions and type of it.

Furthermore, the aim of selecting type of Canola (Sarigol) because of clay average cultivation andits more crap.

This study has been done with the aim of reviewing the important and role of different consistency of phosphorus on quality and quantity of fatty acids which organizing the seed of Canola in type of Sarigol.

MATERIAL & METHODS

In this study, first of all the type of Sarigol, Canola selected in order to review different consistencies of phosphorus on lipids in seed after that it cultivated coincidental in planter for 5 times in the yard of a garden in chalus.

Its seeds packaged without antiseptic. About 400 kg soil on the basis of one (leave soil) to 4 (farming soil) used in this study.

1 Kg of farming soil sampled and transferred to pedology lab in order to determine the elements and texture of soil (based on Table2).

KOH 1.4 gr and Urea 1.4 gr (one- third before cultivation, one- third during start step when it is going to the stem and other of it added before blooming) added to the soil. 10 kg of soil after measuring the weight of it with digital scale added to the planters and finally inscribed on the planters the numbers of 0, 0.02, 0.2 and 2 mmol/Lit per lit with creation plate, the planters put on them in the soil. 20 seeds cultivated at depth of 1.5 cm in the soil.The irrigation done a little on the surface of soil till the plantler come out from the surface of soil every 2 days in between and in growth season it done depending on the need of plants considered in every other day. The mean temperature $20^{oC}$and relative humidity 75^{oC}.

For any treatment, 10 planters considered in this step. The space among planters was 35 cm in width and 10 cm in length.The budding took place at 4^{oC}.After 2 days, the primary leaves are visible. Four- leaf growth started from the surface of soil. With seedings treatment done for five plants.

1.3 gr of phosphorus in the form of NaH_2PO_4 melt in 4.5 Lit of water so, for treatment, the solution added just 1 time to the soil in 4 levels; witness= 0, 0.02, 0.2 and 2 mg/Lit based on the (table 1).

Table 1. Method of different treatment with phosphorus

The amount of added distilled water	The amount of phosphorus solution added	Treatment
100 cc	-	0

99 cc	1 cc	0.02
90 cc	10 cc	0.2
-	100 cc	2

Table 2. The physical and chemical characteristic of the useable soil for potted cultivation of Canola (Sarigol)

Zn ppm	Mg ppm	Ca (meq/lit)	Text	% clay	% silt	% sand	K (ppm)	P (ppm)	% o.c	% o.m	% N	PH oF paste	Ec ds/m	s.p	% s.m	Depth (Cm)
2.14	112	7.19	C	42.51	32.38	25.10	144	2.23	1.31	2.25	0.09	7.52	0.47	44.18	21.1	-300

(soil moisture) s.m
(soil p) s.p
DesiSimens/ meter (Electro conductivity) E.c
(Organic material) O.m
(Organic carbon) O.c

After 110 days of cultivation, mature and yellow pods removed and measured the length of stem andpod with a ruler andevaluated the number of pods in the secondary and main branch. The pods measured in oven at 60^{oC} for 24 h and calculated the weigh of pods (after putting in oven at 60^{oC} for 24 h) with a precise scale laboratory.

Lipids of seeds of Canola extracted by soxhlet extractor and isolation of lipids from additional solvent n- Hexane performed by using spinning band distillation system with method of (stauffer, C,E,1996) (fig 1, 2) (25).

To investigation fatty acids by GC method its necessary that the fatty acids be methylate. For this reason, 100 cc methanol 80% with 3 gr of sodium sulfate anhydrous added to the balloon for methanol dehydration, it was in vitro for 2 to 3 hours, at the end added 8 gr. of KOH to it. After all steps the solution should be reflux to the extent that the KOH completely melt and nothing seen at the bottom of the balloon.2 ml of n-hexane and 2 ml of methanol KOH added to 0.5 gr of extracted lipid.

The supernatant separated with spanterand pour to the stained glass tube. To pigment removal, the n-hexane and active carbon to glass tube should be put on shaker for half an hour. Then filtrated with filter paper and for injection to the GC device it should be hold in refrigerator (8).

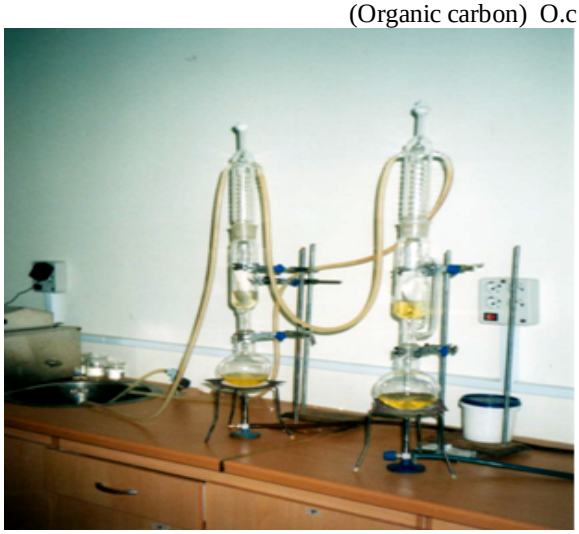

Fig 1. Extraction method of fatty acid of Canola seed (Sarigol) with soxhlet

Fig 2. Isolation method of fatty acid in Canola seed (Sarigol) from additional solvent, n-hexane with evaporator rotator.

In next step, to identify fatty acids derivative of injected to GC, the specification of GC device (N-6890 model) equipped with capillary column, Agilent model filled with Phenyl methyl cyclohexane 5%, with the final temperature 325^{oC}, internal diameter 250 micrometer, film thickness 0.5 micrometer with the initial rate of helium carrier gas one ml per minute and the amount of injection of sample was 1 microliter.

BACK INLET with mode: split, the initial temperature set on 280^{oC}, the pressure PSI 73.7 and ratio of speed 20 to 1 of helium carrier gas.

The initial temperature of the Oven considered 50^{oC}and the final tempreture 350^{oC} at 77 min. The measurement of chlorophyll in this study done based on Lichtenthaler method (1994) (20).

This study performed random[1]and repeated five times with 4 treatment and different consistency of phosphorus (0, 0.02, 0.2, 2mmol/Lit).

The model of randomized design following as:

$$Y_{ij} = \mu + \delta_j + \varepsilon_{ij}$$

In this formula, μ total average, δ_j the impact of phosphorus and ε_{ij} the effect of test error.

Analysis of data variance and comparison of the average done with (LSR or Dun can test) and the Orthogonal test done by using Mstatc software. The coefficient of variation examined for all specifications in order to be acceptable the accuracy of test. Pearson's correlation coefficient among specifications done by using SPSS software.The diagram drawn with Excel software.

CONCLUSION AND DISCUSSION

The results of the studied variance shown considerable difference among different consistency of phosphorus in level of possibility 1 or 5% as regards the length of stem, the number of seed in pod, weight of pod with seed, weight of one thousand of seeds, length of pod, the number of pod in the main branch and the number of pod in the secondary branch.

The difference of witness treatment with 3 treatments phosphorus for the mentioned specifications was considerable with level 1% to 5% and based on orthogonal test.

In this test the maximum length of stem, number of seed in pod, weight of pod with seed length of pod, weight of one thousand of seeds, number of pod in the main and secondary branch calculated 2 mmol/lit and only this treatment is different from the other treatments. (based on Table 4).

The role of phosphorus is in increasing the number of pod, the number of seed, the weight of seed and weight of one thousands seeds also, the importance presence of it is in metabolic step such as flowering, podand seed forming in reproductive stage. Since, phosphorus is an active element so, it used more in energy process and this factor in phosphorus shows its necessity to some actions after flowering in the Plant.

In this regard, we can refer to the reports by other researchers such as (Mckenzie, 2003, Black show, 2004, Berglund, 2000) (15, 17, 22).

The amount of phosphorus in this study (2 mmol/L) caused increasing in stem length and these results is conformity with increasing the amount of phosphorus in Alfalfa which caused increasing the ratio of shoot to Root and increasing growth in stem (18).

According to these results phosphorus caused precocious maturity in Canola. Some planters that consumed high concentration of phosphorus, its pod grow sooner in this treatment and shows that the time of ripen in these pods are shotter than other treatments.

This study also confirmed other researches which done by others concerning that phosphorus against nitrogen is a ripening early factor for agriculture products andsprouting, fertilizing and ripening of products depend on the presence of phosphorus (19, 21, 24).

[1] .Completely Randomized Design (CRD)

The maximum correlation seen between the weight of one thousand seeds and the weight of pod with seed (r α 0.98).

Except the correlation between the length of pod with the number of pod in the secondary branch there was meaningful correlation between morphological traits in two according to the Table 5.

So, there is connection between increasing of phosphorus (2 mmol/L) and meaningful correlation of other morphological traits (except chlorophyll a, b & total chlorophyll) with the function of seed andthe lack of meaningful correlation in length of pod with the number of pod in the secondary branches therefore, other results are consistent with other results which reported by other researchers (1).

There was meaningful difference among used treatments it means the amount of chlorophyll a, b & total chlorophyll and the level of possibility was 1% or 5% (according to the Table 4).

Also, the difference of witness treatment with 3 other treatments in (orthogonal test) was meaningful with level of Possibility 1%.

The maximum amount of chlorophyll a, b & total chlorophyll determined in witness treatment and was seen the meaningful difference about the amount of chlorophyll a only in this type of treatment as comparison with other treatments.

This difference about the amount of chlorophyll b in this treatment was 2 mmol/L and about total chlorophyll as comparison with two treatments the difference were in order 0.2 & 2 mmol/L (according to the Table 4).

The results obtained in the study reported the amount of chlorophyll a, b & total chlorophyll is more in witness treatment than 3 other treatments and there is meaningful difference among other treatments which are in agreement with the results of other researchers that in condition of shortage phosphorus, cell and leaf expansion are slower than the formation of chlorophyll also, the amount of chlorophyll is higher.on the other hand, increasing use of phosphorus in generative step can be related with the role of inorganic phosphate as regulator in Photosynthesis process, Sugar metabolism in leaf and transfer the Photosynthesis of material in order to fill the grain which are in agreement with reports (2, 3, 4, 5, 11).

The statistical meaningful difference observed among different treatments of phosphorus on the amount of oleic acid was about 5% (according to the Table 4).

The difference between witness treatment and other treatments for phosphorus was meaningful about 1% (orthogonal test). The maximum amount of oleic acid in treatment of phosphorus observed 2 mmol/L.

Although, the amount of it in other treatments (0.02 & 0.2 mmol/L) were not meaningful also, the amount of oleic acid in witness treatment as comparison with two treatment (0.02 & 0.2 mmol/L) were not meaningful (according to the Table 4).

The effect of concentrations of phosphorus which used on traits such as Palmitic acid, Stearic acid andLinoleic acid was not meaningful (according to the Table 3).On the other hand, the difference between witness treatment with other concentrations of phosphorus about the mentioned traits were not meaningful (orthogonal test).

The maximum amount of Palmitic acid, Stearic acid andLinoleic acid in terms of concentration observed 2 mmol/L (according to Table 4).

In research performed with increasing the amount of phosphorus caused increasing all amount of saturated and unsaturated fatty acids. Also, with increasing the amount of phosphorus (2 mmol/L) will be effective only in the amount of Oleic acid (an acid with 18 carbon atoms which contains a double transplant in carbon 9). but it does not have meaningful effect on other fatty acids in Canola which can be matched the results regarding increase in consumption of phosphorus are in agreement with increasing the quantity of

fatty acids that are not against the other reports by researchers (6, 12, 16).

Meanwhile, the correlation between Palmitic acid with Stearic acid was positiveand meaningful, but this connection was not meaningful with Oleic acid.Among 4 studied acids, only Oleic acid had meaningful studied morphology traits (according to Table 5).

Qualitatively, the meaningful correlation is only between Oleic acid and other morphologic traits (except chlorophyll a, b & total chlorophyll) which relates to high temperature when the oil accumulate in seed and caused the maturity in seed in this step the seed strongly happen influenced by environmental factors or in relation to the function of non- membrane enzyme stromaand the function of Elongation and desaturation enzymes which found in the smooth endoplasmic reticulum membrane (SER).

Fatty acid can be synthesized in chloroplasts, such as Palmitat, Stearat&Oleate. In addition to the mentioned acids, other fatty acids can be synthesized in the smooth endoplasmic reticulum membrane (6, 10, 12).

Table 3. The results of variance analysis of studied traits in Canola (Sarigol)

Weight of	**1000 seeds**	**weight of pod with seed**	**number of seed in pod**	**length of stem**	**linoleic acid**	**oleic acid**	**stearic acid**	**source changes**
0.715	0.013	21.6	81.25	2.89	1.244	0.236	------	consistency of phosphorus
0.013	0.00019	4	4.37	7.06	0.312	0,102	------	Errors of test
3.8	6	14.8	2.8	29.3	27.2	26.2	29.8	changes
**	**	**	*	ns	**	ns	ns	------

Ns,*,**: in order, non- meaningful ,meaningful with possibility level 5%, meaningful with possibility level 1%
+: the aim of orthogonal test is comparison witness treatment (phosphorus 0%) with other consistency of phosphorus (0.02, 0.2,2 mmol/ Lit)

Table 3. The results of variance analysis of studied traits in Canola (Sarigol)

Chlorophyll a	**Chlorophyll b**	**Chlorophyll a**	**the number of pod in secondary branch**	**the number of pod in main branch**	**the length of pod**
64.56	**3.12**	**37.6**	**167.6**	**135**	**128.3**
8.43	0.795	2.37	40.6	7.12	4.5
20.1	22	15.4	29.2	10.9	3.6
**	**	**	ns	ns	**

Ns,*,**: in order, non- meaningful ,meaningful with possibility level 5%, meaningful with possibility level 1%
+: the aim of orthogonal test is comparison witness treatment (phosphorus 0%) with other consistency of phosphorus (0.02, 0.2,2 mmol/Lit)

Table 4. The results of comparison among studied traits in Canola (Sarigol)

total Chlorophyll	Chlorophyll b	chlorophyll a	The number pod in secondary branch	The number of pod in main branch	The length of pod (mm)	Weight of 1000 seeds(gr)	Weight of pod with seed(gr)	Number of seed in pod	Length of stem	Oleic acid	treatment
18.94 a	5.23 a	13.71 a	16.6 b	23 b	52 b	2.65 c	0.195 b	11 b	72 b	1.41 b	0
14.19 ab	3.79 ab	10.4 b	16 b	20 b	61 a	2.74 bc	0.204 b	13 ab	71 b	2.13 ab	0.02
11.37 b	3.67 ab	7.7 b	14.2 b	23 b	62 a	2.89 b	0.214 b	14 ab	74 b	2.01 ab	0.2
11.71 b	3.53 b	8.18 b	27 a	32 a	63 a	3.49 a	0.303 a	16 a	80 a	2.63 a	2

Unlike alphabet means meaningful difference. Traits which has no meaningful variance analysis (table 3) comparison test did not performed on them

Table 5. The correlation coefficient between studied traits in Canola (Sarigol)

	Acid palmitic	2	3	4	5	6	7	8	9	10	11	12	13
Stearic acid	0.82												
Oleic acid	0.34	0.06											
Linoleic acid	0.92	0.70	0.12										
Length of stem	0.32	0.25	0.52	0.22									
Number of seed in pod	0.32	0.17	0.51	0.27	0.83								
Weight of pod with seed	0.34	0.29	0.53	0.24	0.91	0.76							
Weight of 1000 seeds	0.41	0.35	0.54	0.31	0.94	0.82	0.98						
Length of pod	0.2	0.04	0.6	0.16	0.56	0.84	0.6	0.67					
Number of pod in main branch	0.38	0.3	0.44	0.3	0.96	0.77	0.92	0.91	0.45				
Number of pod in second branch	0.37	0.18	0.46	0.3	0.79	0.78	0.76	0.73	0.44	0.86			
Cholorophyl a	-0.14	-0.11	-0.53	-0.02	-0.53	0.64	-1.5	0.6	-0.82	-0.38	-0.19		
Chlorophyll b	-0.14	-0.16	-0.46	-0.005	0.17	0.18-	-0.28	-0.3	0.48	-0.08	-0.05	0.45	
Total cholorophyll	-0.25	-0.26	-0.5	-0.08	-0.53	-0.58	-0.47	-0.57	-0.69	-0.4	-0.2	0.91	

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