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Response of rapeseed fatty acid composition to foliar application of humic acid under different plant densities

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Abstract: In order to the assessment of humic acid application on the qualitative characteristics of rapeseed in various plant densities, a factorial split-plot test was conducted for two cultivation years in Karaj, Iran. In this experiment, plant density considered in three levels (40, 60, and 80 plants/m^2), humic acid at two concentrations (non-application and application at the concentration of 0.3%) in the main plots, and six cultivars of rapeseed embracing RGS003, Zafar, Julius, Jerry, Zabol10, and Hyola4815 in the sub-plots. The interaction effect of plant density × humic acid × cultivar on seed yield, oil yield, oleic acid, linolenic acid, linoleic acid, palmitic acid, erucic acid, canopy temperature, and seed glucosinolate content as well as the interaction effect of plant density × cultivar on the oil content and total chlorophyll content were significant at 1% level. Cv. Jerry had the maximum seed and oil yields under humic acid application condition with the density of 40 plants/m^2, and the maximum contents of oleic, linoleic, and palmitic fatty acids as well. Moreover, this cultivar, through containing the lower and standard content of erucic acid and glucosinolate, is recommendable under the circumstance of the present research.

Keywords: *Brasica napus* L.; nutrition; oil quality; organic fertiliser; plant competition; semi-arid region

Oil seeds are the second important source of the energy supply after cereals in societies. Rapeseed is one of the most important oil seeds in the world, which has allocated the third position after soybean and oil palm in supplying the plant oil, and the fifth position in providing protein (Jaberi et al. 2015). Rapeseed importance is due to its oil and meal, which also has the potential of a renewable biofuels source. Rapeseed oil has greater nutritional value due to containing the lower amount of saturated fatty acids, a reasonable quantity of the unsaturated fatty acids, and without cholesterol (Starner et al. 2002).

One of the main objectives in agriculture is the necessity of the best plant density determination to achieve optimal productivity. The optimal plant density is the density, that because of it, the whole environmental factors to be consumed by a plant and at the same time the intra-plant and interplant competition to be the minimum, until the maximum possible yield with the desirable quality to be acquired (Khajehpour 2006). The different rapeseed cultivars contain 37 to 47 percent oil (Kadivar et al. 2010), and the yield, as well as seed oil percentage, are very influential in the rapeseed production profitability (Robertson and Holland 2004).

Humic acid is a natural organic polymeric compound, which is generated due to the degradation of soil organic materials, peat, lignin, and so forth, and probably utilised to increase crop quantity and quality. One of the significant advantages of humic acid is the chelating of various elements such as sodium, potassium, magnesium, zinc, calcium, iron, copper and so forth in order to overcome the nutrients deficiency, which occasions the increase of root

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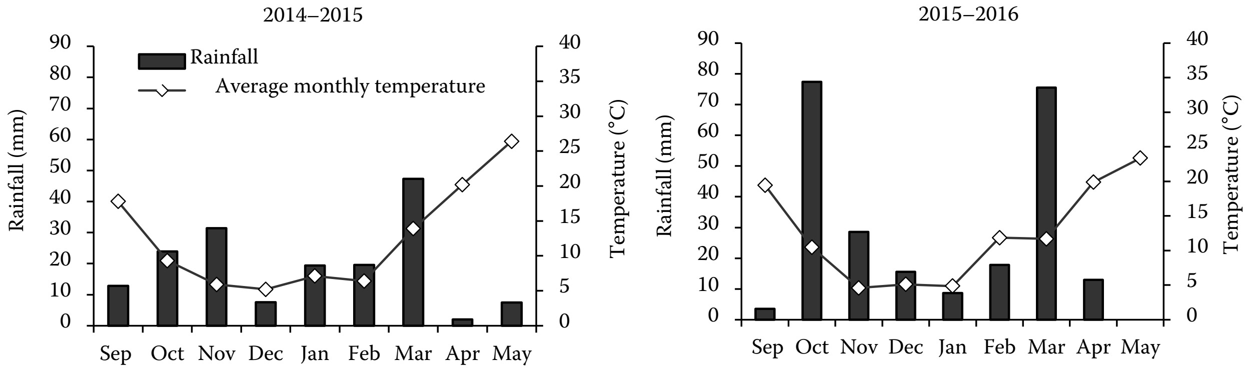

Figure 1. Variation of temperature and rainfall in Karaj meteorology station during 2014–2016 growing seasons

length and weight as well as the creation of lateral roots (Abedi and Pakniat 2010). In the experiment conducted by Nasiri et al. (2017), foliar humic acid application in the rapeseed budding stage resulted in the significant increase of chlorophyll content, seed, and oil yield. In research, the application of humic acid by 500, 1 000, and 2 000 mg/kg soil, brought about the increase of hypocotyl length, stem diameter, stem length, dry weight, and the nutrients content in pepper plant (*Capsicum annum* L.) (Tan 2003). This experiment conducted in order to the evaluation of humic acid application efficacy on the qualitative traits of rapeseed genotypes in the various plant densities, and the selection of the compatible genotypes with the environmental conditions, to develop rapeseed cultivation in the cold temperate and semi-arid regions.

MATERIAL AND METHODS

To evaluate the effect of the humic acid application on the qualitative traits and yield of rapeseed cultivars in different plant densities, a factorial split-plot experiment was performed in a randomised complete block design with three replications in the crop years of 2014–2015 and 2015–2016 in Karaj of Iran. In this research, the main factors were, humic acid at two concentration including; the spray with pure water (non-application of humic acid) and humic acid application (spraying by 0.3% in 4–6 leaf and budding stages), and plant density at three levels of 40, 60, and 80 plants/m^2. Rapeseed fall cultivars, comprising RGS003, Zafar, Julius Jerry, Zabol10, and Hyola4815, were the subfactor. Figure 1 shows the meteorological data of the experiment site during the two crop years. The soil texture of the experimental farm was clay loam, and the soil specification of the experiment site has been illustrated in Table 1.

Every experimental plot was included 6 planting rows, six meters in length, and the distance between the rows was 30 cm, that the two sidelines considered as the margins. The plant's distance on the lines was 5 cm. Fertilisers' applications based on the soil test were: (1) 150 kg/ha ammonium phosphate and 150 kg/ha potassium sulfate as a basis with preparing seedbed simultaneously; (2) 300 kg/ha urea (100 kg in the four-leaf stage, 100 kg in the stem emerging stage and 100 kg in the bud formation stage). The first foliar application of humic acid, with the concentration of 0.3% (as Humax 95-WSG containing about 80% humic acid, and about 15% fulvic acid), carried out in 4–6 leaf stage and the second one was at budding, as well as the pure water spray was done as the humic acid non-application treatment. To

Table 1. Physicochemical properties of soil collected from study site

Year	Depth (cm)	EC (dS/m)	pH	Organic carbon	N	P	K	Sand	Clay	Silt	Texture
				(%)		(ppm)		(%)			
2014	0–30	2.2	7.4	0.62	0.8	13.7	264	21	29	50	Clay loam
	30–60	1.9	7.1	0.65	0.11	14.5	274	24	33	43	
2015	0–30	1.57	7.9	0.90	0.9	14.9	195	27	31	42	Clay loam
	30–60	1.34	7.3	0.94	0.98	15.5	206	32	28	40	

EC – electrical conductivity

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determine the seed yield, the plants in the 4.8 square meter area of each plot were separately submerged and precisely weighed and calculated. To determine the seed oil percentage, a sample of 5 g was opted from every plot, and then its percentage was designated by nuclear magnetic resonance (NMR) (International Standard ISO 5511, 1992). After the determination of seed oil percentage, the seed oil yield was calculated by multiplying the seed oil percentage by the seed yield. To measure and determine the present fatty acids in the seed oil, the gas chromatography procedure was employed Azadmard-Damirchi et al. (2005). The seed glucosinolate content was measured by a spectrophotometer (Makkar et al. 2007).

Ultimately, after carrying out the Bartlett test and proving the homogeneity of the variances in every year, the analysis of the combined variances was accomplished by the SAS v.9.1 software (Cary, USA). The comparison of means was carried out by the least significant difference test at $P < 0.05$. The mean comparison of the interaction effect was made using the cutting procedure. The graphs were drawn by Excel software (Redmond, USA).

RESULTS AND DISCUSSION

Seed yield. The applied cultivars in this experiment had a significant difference in terms of seed yield in the humic acid application and non-application treatments in different plant densities. In the condition of humic acid application and 40 plants/m^2, cv. Jerry with the average of 5 412 kg/ha, and cv. RGS003 with the average of 3 832 and 2 586 kg/ha in the densities of 60 and 80 plants/m^2 had the maximum seed yield, respectively (Table 2). In the circumstance of non-application of humic acid and density of 40 plants/m^2, cv. Jerry had the maximum seed yield with the average of 5 313 kg/ha, and cv. RGS003 with the average of 3 780 and 2 493 kg/ha in the densities of 60 and 80 plants/m^2, respectively. Nardi et al. (2002) declared that humic acid increases plants yield through the positive physiological effects such as the effect on the metabolism of plant cells and the increase of leaf chlorophyll concentration. Humic acid enhances the permeability of the cell membrane, hence facilitates the entry of the potassium and increase the internal cell pressure, which results in cell division. On the other hand, the increase of the energy inside of cell occasions to further production of chlorophyll and eventually to the enhancement of photosynthesis, followed by the nitrogen assimilation into the cell intensifies, and nitrate production reduces, which is an important factor in growth, and ultimately these impacts bring about yield increase (Giasuddin et al. 2007).

Oil yield. The reaction of cultivars was different in terms of seed oil yield to the application and non-application of humic acid in the various plant densities. In the condition of humic acid application and 40 plants/m^2, cv. Jerry had the maximum oil yield by 2 196 kg/ha on average, and cv. RGS003, by 1 511 and 991 kg/ha on average in the densities of 60 and 80 plants/m^2, respectively (Table 2). In the condition of non-application of humic acid and density of 40 plants/m^2, cv. Jerry had the maximum oil yield by 2 149 kg/ha on average, and cv. RGS003 by 1 488 and 956 kg/ha on average in the densities of 60 and 80 plants/m^2, respectively. Totally, rapeseed oil yield decreased through the increase of plant density. In the optimal cultivation density, plants exploitation from the environmental factors rise and therefore the maximum assimilation and yield will be acquired, and since the oil yield achieves from the multiplication of seed yield by seed oil percentage (Läänsite et al. 2008), and on the other hand there is a straight correlation between seed and oil yields; hence the maximum oil yield was obtained under the condition of 40 plants/m^2 as well. The results of this research are in accord with the report of Rajpar et al. (2011) and Chris et al. (2005) that demonstrated, the humic acid application has a significant effect on rapeseed oil percentage and yield.

Oleic acid. The experimented cultivars had a significant difference in terms of oleic acid in the humic acid application and non-application treatments in the different plant densities. Cv. Jerry with an average of 63.07%, in the condition of humic acid application and the density of 40 plants/m^2, and cv. RGS003 with an average of 61.23% and 59.40% in the plant densities of 60 and 80 plants/m^2, respectively, had the maximum oleic acid content (Table 2). Under the condition of humic acid non-application and the density of 40 plants/m^2, cv. Jerry by 62.96% on average, and cv. RGS003, with an average of 61.14% and 59.33%, respectively, in the plant densities of 60 and 80 plants/m^2, had the maximum oleic acid content. The consequences of this assessment suggested that through the increase of plant density, oleic fatty acid content significantly decreased in the various cultivars. Rapeseed seed oil quality intensely affected by the environmental condition (Enjalbert et al. 2013).

Linolenic acid. The cultivars' reactions were different in terms of linolenic acid content to humic

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Table 2. Interaction effect of plant density × cultivars × humic acid on rapeseed characteristics

Humic acid application	Crop densities (plants/m^2)	Cultivar	Seed yield	Oil yield	Oleic acid	Linolenic acid	Linoleic acid	Erucic acid	Glucosinolate (μmol/g)
			(kg/ha)		(%)				
Non-application	40	RGS003	5 004^{c}	2 007^{c}	62.55^{c}	4.41^{d}	22.65^{c}	0.21^{c}	8.24^{d}
		Zafar	4 303^{d}	1 702^{d}	61.62^{d}	4.94^{c}	21.67^{d}	0.26^{b}	9.89^{c}
		Julius	4 074^{e}	1 607^{e}	61.38^{e}	5.06^{b}	21.45^{e}	0.28^{a}	10.27^{b}
		Jerry	5 313^{a}	2 149^{a}	62.96^{a}	4.14^{f}	23.24^{a}	0.189^{d}	7.58^{f}
		Zabol10	3 940^{f}	1 551^{f}	61.33^{e}	5.16^{a}	21.35^{f}	0.28^{a}	10.46^{a}
		Hyola4815	5 163^{b}	2 093^{b}	62.76^{b}	4.25^{e}	22.86^{b}	0.20^{cd}	7.92^{e}
	LSD		442.3	192.35	1.97	0.31	1.33	0.01	1.07
	60	RGS003	3 780^{a}	1 488^{a}	61.14^{a}	5.30^{f}	21.12^{a}	0.29^{c}	10.84^{f}
		Zafar	2 695^{d}	1 041^{d}	59.60^{e}	6.40^{b}	19.44^{e}	0.37^{a}	13.60^{b}
		Julius	2 728^{d}	1 052^{d}	59.68^{d}	6.36^{c}	19.52^{d}	0.36^{a}	13.44^{c}
		Jerry	3 639^{b}	1 427^{b}	60.93^{c}	5.42^{e}	20.93^{b}	0.30^{c}	11.25^{e}
		Zabol10	2 618^{e}	1 005^{e}	59.54^{e}	6.45^{a}	19.33^{f}	0.37^{a}	13.86^{a}
		Hyola4815	3 068^{c}	1 194^{c}	60.27^{b}	5.94^{d}	20.24^{c}	0.33^{b}	12.47^{d}
	LSD		294.8	140.6	2.31	0.28	1.65	0.02	1.25
	80	RGS003	2 493^{a}	956^{a}	59.33^{a}	6.61^{f}	19.11^{a}	0.38^{d}	14.25^{f}
		Zafar	2 126^{c}	809^{c}	58.70^{b}	7.03^{d}	18.52^{c}	0.42^{c}	15.47^{d}
		Julius	1 752^{e}	663^{e}	58.35^{d}	7.29^{b}	18.12^{e}	0.44^{b}	16.13^{b}
		Jerry	1 457^{f}	547^{f}	57.91^{e}	7.61^{a}	17.63^{f}	0.46^{a}	16.49^{a}
		Zabol10	1 929^{d}	731^{d}	58.51^{c}	7.18^{c}	18.29^{d}	0.43^{bc}	15.84^{c}
		Hyola4815	2 247^{b}	858^{b}	58.92^{a}	6.87^{e}	18.72^{b}	0.41^{c}	15.00^{e}
	LSD		184.64	98.65	1.88	0.40	1.41	0.02	1.25
Application	40	RGS003	5 040^{c}	2 083^{b}	62.64^{c}	4.34^{d}	22.78^{c}	0.21^{b}	8.07^{d}
		Zafar	4 723^{d}	1 894^{c}	62.11^{d}	4.65^{c}	22.26^{d}	0.24^{a}	8.96^{c}
		Julius	4 570^{e}	1 815^{d}	61.92^{e}	4.76^{b}	22.01^{e}	0.25^{a}	9.31^{b}
		Jerry	5 412^{a}	2 196^{a}	63.07^{a}	4.07^{f}	23.40^{a}	0.184^{c}	7.51^{f}
		Zabol10	4 469^{f}	1 771^{e}	61.85^{f}	4.84^{a}	21.91^{f}	0.25^{a}	9.51^{a}
		Hyola4815	5 238^{b}	2 113^{b}	62.82^{b}	4.21^{e}	23.04^{b}	0.19^{c}	7.77^{e}
	LSD		484.77	207.16	2.19	0.33	1.62	0.01	1
	60	RGS003	3 832^{a}	1 511^{a}	61.23^{a}	5.24^{f}	21.23^{a}	0.29^{c}	10.64^{f}
		Zafar	2 909^{f}	1 128^{d}	60.01^{e}	6.15^{b}	19.87^{e}	0.35^{a}	12.91^{b}
		Julius	2 937^{f}	1 136^{c}	60.10^{d}	6.07^{c}	19.98^{d}	0.35^{a}	12.76^{c}
		Jerry	3 711^{b}	1 461^{a}	61.04^{b}	6.34^{e}	21.04^{b}	0.30^{c}	11.04^{e}
		Zabol10	2 868^{f}	1 113^{c}	59.89^{f}	6.22^{a}	19.77^{f}	0.35^{a}	13.10^{a}
		Hyola4815	3 216^{d}	1 249^{b}	60.43^{c}	5.75^{d}	20.37^{c}	0.33^{b}	12.27^{d}
	LSD		236.19	135.06	2.18	0.44	1.60	0.02	1.19
	80	RGS003	2 586^{a}	991^{a}	59.40^{a}	6.53^{f}	19.20^{a}	0.38^{d}	14.07^{f}
		Zafar	2 208^{c}	840^{c}	58.84^{c}	6.97^{d}	18.61^{c}	0.41^{bc}	15.20^{d}
		Julius	1 851^{e}	700^{e}	58.42^{e}	7.24^{b}	18.22^{e}	0.43^{b}	15.99^{b}
		Jerry	1 567^{f}	589^{f}	58.05^{f}	7.51^{a}	17.76^{f}	0.45^{a}	16.40^{a}
		Zabol10	2 053^{d}	779^{d}	58.62^{d}	7.11^{c}	18.41^{d}	0.42^{b}	15.61^{c}
		Hyola4815	2 341^{b}	893^{b}	59.04^{b}	6.81^{e}	18.78^{b}	0.40^{c}	14.82^{e}
	LSD		220.37	85.80	2	0.53	1.17	0.02	1.31

Any two means sharing a common letter do not differ significantly from each other at 5% probability; *LSD* – least significant difference

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acid application and non-application in the various plant densities. Cv. Jerry by 4.07% on average, in the condition of humic acid application and the density of 40 plants/m^2, and cv. RGS003 with an average of 5.24% and 6.53% in the plant densities of 60 and 80 plants/m^2, respectively, had the minimum linolenic acid content (Table 2). Under the condition of humic acid non-application and the density of 40 plants/m^2, cv. Jerry by 4.14% on average, and cv. RGS003, with an average of 5.30% and 6.61%, respectively, in the plant densities of 60 and 80 plants/m^2, had the minimum linolenic acid content. Linolenic acid content increased through the increase of plant density. Then why is refers to the negative correlation of oleic and linolenic acids. In other words, through the increase of plant density, the oleic acid content in seed oil descends, but linolenic acid content ascends (Möllers and Schierholt 2002).

Linoleic acid. The evaluated cultivars had different reactions to the application and non-application of humic acid in terms of linoleic acid in different plant densities. Cv. Jerry by 23.40% on average, in the case of humic acid application and the density of 40 plants/m^2, and cv. RGS003 with an average of 21.23% and 19.20%, in the plant densities of 60 and 80 plants/m^2, respectively, had the maximum linoleic acid content (Table 2). Under the condition of humic acid non-application and the density of 40 plants/m^2, cv. Jerry by 23.24% on average, and cv. RGS003, with an average of 21.12% and 19.11%, respectively, in the plant densities of 60 and 80 plants/m^2, had the maximum linoleic acid content. Through the increase of the plant density, linoleic fatty acid content decreases, that the reason can be attributed to the genotypes differences (Fernandez-Martinez 2002) and their competition for light absorbance in higher densities (Badri et al. 2011).

Erucic acid. The cultivars' reaction was different from the application and non-application of humic acid in various plant density in terms of erucic acid content. Cv. Jerry had the minimum erucic acid content under the conditions of humic acid application and non-application by 0.189% and 0.184%, respectively, in the density of 40 plants/m^2 (Table 2). Likewise, cvs. Jerry and RGS003 by 0.30% and 0.29% on average in the density of 60 plants/m^2, as well as cv. RGS003 by 0.38% on average under the conditions of humic acid application and non-application in the density of 80 plants/m^2, had the minimum erucic acid content. With respect to the fact that erucic acid content is an important measure for rapeseed and its edible consumptions (Gecgel et al. 2007), but in this research, the content of this harmful fatty acid had been within the standard range (less than 2%).

Glucosinolate. In the case of humic acid application, cv. Jerry by 7.51 μmol/g on average in the density of 40 plants/m^2, and cv. RGS003 by 10.64 and 14.07 μmol/g on average in the densities of 60 and 80 plants/m^2, respectively, had the minimum glucosinolate (Table 2). In the case of humic acid non-application, cv. Jerry by 7.58 μmol/g on average in the density of 40 plants/m^2, and cv. RGS003 by 10.84 and 14.25 μmol/g in the densities of 60 and 80 plants/m^2, respectively, had the minimum glucosinolate. Thus in both treatments of humic acid application and non-application and the various plant densities, glucosinolate content was within the standard range and less than 30 μmol/g of the dry weight of press cake. Increasing glucosinolate reduces the quality and nutritional value of rapeseed press cake (Sulisbury et al. 1987).

The experiment results suggested that the whole experimented cultivars positively affected by the application of humic acid in the different plant densities in particular at the density of 40 plants/m^2. Cv. Jerry captured the maximum seed and oil yield in the case of humic acid application and density of 40 plants/m^2, as well as had the maximum content of oleic and linoleic fatty acids. This cultivar, because of containing a low and standard amount of erucic acid and glucosinolate, is recommendable in the circumstance of the present research.

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