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Effects of Biostimulants on Cold Stress in Late Sowing of Rape (*Brassica napus ssp. oleifera* L.) under Arak Province Conditions in Iran

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ABSTRACT

Cold stress limits the yield of rapeseed (*Brassica napus spp. oleifera L.*, Okapi) in late sowing. The experimental design was a split plot with three replications in two years (2018-2019). The main plot had two sowing dates (the first sowing on 18 September and the second one on 10 October). Biostimulants treatment included: Blank (fertilizers according to the soil test without biostimulants); Blank with humic acid fertigation; Blank with fulvic acid; Blank with amino acid; Blank with seaweed extract; Mixed treatment consisted of humic acid, fulvic acid, seaweed, and amino acid. Humic acid was used at the second irrigation and six-leaf stage (5 kg ha^{-1}). Fulvic acid, amino acid, and seaweed extract were used as foliar at the six-leaf stage and stem elongation (0.5% and 0.3% w/v in mix treatment). Based on the results, the main effect of biostimulants on grain yield, nitrogen (N), and phosphorus (P) uptake was significant. The difference between the two sowing dates on shoot dry weight (DW) and grain yield (3458 vs. 3048 kg ha^{-1}) was significant. The seaweed improved the grain yield in delayed cultivation by 34% compared to the control and had the highest profit-to-cost ratio in normal cultivation (15.1) and in delayed cultivation (13.7).

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INTRODUCTION

The widespread use of canola in the food industry has increased its position from the 6th oilseed crop to the 2nd in the world after soybean (Gaber et al., 2018; Chew, 2020). In 2021, canola was cultivated in more than 66 countries, and the total world cultivated area consisted of 36.8 million hectares with seed production of 71.3 million tons with an average of 1940 kg ha^{-1} yield (FAO, 2023). Iran's cultivation area of canola was 112,729 hectares with a mean yield of 1,910 kg ha^{-1} and a seed production of 215,291 tons in 2021 (FAO, 2023).

In plants, stress is a condition that leads to the disruption of the plant's biological balance. Cold (chilling) and frost abiotic stresses, (Hasanuzzaman et al., 2018) occur in autumn in cold and moderately cold regions of canola sowing areas and cause damage to the plant and a decrease in grain yield (Lei et al., 2019). Chilling stress damage depends on the intensity and duration of the cold, and the amount of damage increases as the duration of stress increases. The speed of temperature reduction is another factor affecting the damage amount (Raza et al., 2023). The desired temperature for the growth and development of canola is 25 ºC to 30 ºC (Secchi, 2022). Exposure to very low temperatures reduces the efficiency of photosynthesis and causes destruction to the cell membrane, which leads to electrolyte leakage and overall damage to the plant (Megha et al., 2018; Elferjani and Soolanayakanahally, 2018). Cold also causes oxidative stress and accumulation of reactive oxygen species such as superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH). These oxygen-reduced species destroy nucleic acids, carbohydrates, and proteins and induce unusual cell signals. Combining these factors intensifies stress damage (Hasanuzzaman et al., 2018). For complete vital growth stages, the canola plant needs to obtain a certain amount of heat from the environment in each period, which is expressed as the growth degree date (GDD) (Canola Watch, 2013). The growth degree date for canola in the germination stage is 120, in the vegetative period, is 360, in the reproductive stage is 400, and in the maturity stage is 1200 (Canola Watch, 2013). These values are for five degrees Celsius as the base temperature (the physiological base temperature that most researchers consider for autumn canola) (Begna and Angadi, 2016; Derakhshan et al., 2018). But in some sources, the base temperature is considered zero degrees (Kirkegaard et al., 2012), and in this condition, the GDD numbers will be different because it is dependent on the minimum daily temperature, maximum daily temperature, and base temperature (GDD) = ($(T_{max}+T_{min})/2-T_{base}$). The use of nutrients in the proper amount and the suitable recommendation of fertilizers can be effective in increasing the tolerance of this plant to cold stress (Waraich et al., 2012; Evans et al., 2016; Lohani et al., 2020). Application of plant growth biostimulants is suggested for increasing the tolerance of this plant to abiotic stress (Van Oosten et al., 2017; Gavelienė et al., 2018). A plant growth biostimulant is any substance or microorganism that increases nutritional efficiency, abiotic stress tolerance, or increases crop quality, regardless of its nutrient content (Du Jardin, 2015). General Growth stimulants are in three main groups of humic compounds, seaweed extracts, and amino acid compounds that are added to the soil or plant to regulate or strengthen plant physiological processes (Rouphael and Colla, 2020).

Amino acids prevent protein breakdown and save energy resources in plants. These compounds increase the accumulation of osmotic molecules in cold stress conditions by stimulating biosynthetic pathways, which leads to the production of cold protective materials. Also, these compounds increase the thermal stability of the membrane and reduce the damage caused by cold (Bulgari et al., 2019). Gaveliene et al., (2016) observed resistance of wheat and canola plants to -5 ºC with the application of free amino acids. Wheat plants showed more tolerance than canola. The survival percentage of the plants compared to the control was 14-28%. The concentration of the used compound was effective in response of plants.

The seaweed extract can increase the regulation of cold tolerance genes. It increases proline synthesis, induces osmotic regulation, and protects membrane integrity (chloroplast membrane stabilization) and carbohydrate metabolism (Bhupenchandra et al., 2022). These compounds also regulate stress-responsive genes such as Na/K transporters and late embryogenesis-abundant proteins including dehydrins and aquaporins (Deolu Ajayi et al., 2022). In one of the researches, these compounds increased the cold resistance of canola seedlings at -5 °C temperature more effectively than wheat seedlings (Gavelienė et al., 2018), while in the case of amino acids, the effect on wheat was greater than canola plants.

Humic acids are another plant growth stimulant. Investigations showed that humic acid affects plant growth and yield indices such as dry weight, plant height, stem diameter, leaf surface, and chlorophyll index value (Hemati et al., 2022). These substances can be effective in the absorption and metabolism of nutrients. Humic acids affect membrane fluidity and plasma membrane pyrophosphatase activity. The activation of this enzyme facilitates the absorption of N, P, K, and other plant nutrients such as S, Ca, Mg, Mn, and Fe (Bhupenchandra et al., 2022).

In cold and moderately cold regions, due to the late harvesting of the previous crop (mainly corn), canola cultivation is delayed and it is outside of the time frame suggested by the Seed and Plant Improvement Institute (SPII), Karaj, Iran (Seed://www.spii.ir/en-US/DouranPortal/1/page/Home)) for regions. This issue causes a delay in the growth of the plant and increases the possibility of chilling stress in the cultivated areas because the plant reaches the stage of 6 to 8 leaves in a delayed time when temperature is reduced. In the spring, after the regrowth of the plant (in the stem elongation and flowering stages), the second time of cold stress occurs because of the climatic changes and reaching the temperature to lower amounts. Due to the lack of information regarding the use of plant growth biostimulants on the reduction of cold stress in delayed cultivation of canola yield and yield components, nutrient uptake, and economic assessment for application of these compounds, this research was conducted over two years.

MATERIAL and METHOD

Soil and Climatic Characteristics of The Implementation Area

The Arak agricultural research station is located at 34.092 °N and 49.692 °E (Markazi province of Iran). The average annual temperature is 13.9 °C. The average annual rainfall is 342 mm and 1718 m altitude from sea level. The weather is middle cold and semi-arid based on the De Martonne classification. To carry out this experiment, a soil

sample was taken before sowing. The studied soil is Calcixerollic Xerochrepts, Loamy Skeletal over Fragmental, Carbonatic and Thermic (based on the USDA taxonomic classification).
Soil texture was measured according to Gee and Bauder (1986), organic carbon according to Nelson and Sommers (1996), available micronutrients according to Lindsay and Norvell (1978), calcium carbonate equivalent according to Loeppert and Suarez (1996), pH and electrical conductivity (EC) according to Rhoades (1982), available potassium (K) according to Sparks et al., (1996); available P according to Olsen and Sommers (1982). The soil and water properties are presented in Tables 1 and 2. The soil was non-saline and the physical condition of the soil was favorable with a silty clay loam texture and a saturation percentage of 34% (Table 1).

Table 1. Analysis of field soil before sowing canola at a depth of 0-30 cm

Cu	Fe	Zn	Mn	K	P	O.C	pH	EC	TNV
			(mg kg^{-1})			(%)	-	dS m^{-1}	%
0.97	4.1	1.8	11.3	270	9	0.4	7.8	1.2	20

Calcium carbonate was 20% and the soil was calcareous with poor organic carbon. The amount of available P and Fe (9 and 1.4 mg kg^{-1}, respectively) was lower than the sufficiency range (Orlovius, 2003). The amounts of other nutrients in the soil were in the sufficient range. The results of the water analysis showed that the used irrigation water was non-saline (Table 2).

Table 2. Results of analysis of irrigation water used in rapeseed cultivation

SAR	Na^+	Mg^{2+}	Ca^{2+}	SO_4 meq.l$^{-1=}$	Cl^{-1}	H CO_3^{-1}	pH	EC dS m^{-1}
0.7	1.2	5.3 (sum)		2.1	0.95	3.6	7.8	0.68

Used Compounds and Description of Treatments

The experiment was carried out on a randomized complete block design as a split plot with three replications in two years (2018-2019). Two factors of the experiment included the cultivation date as the main plot and plant growth biostimulants as the subplot (sowing in normal time in September and delayed sowing time in October). The treatments of plant growth biostimulants included 6 treatments as follows:
1- Blank treatment (application of chemical fertilizers according to the soil test without biostimulants),
2- Blank + humic acid fertigation at the rate of 5 kg ha^{-1} in the early stages of growth (at the second irrigation time, decimal code of 01, Bleiholder et al. 2001) and at the six-leaf stage (growth stage of 16),

3- Blank + foliar application of fulvic acid (0.5% w/v) at the six-leaf stage and stem elongation (growth stage of 30),
4- Blank + treatment one + foliar application of amino acid (0.5% w/v) at the six-leaf stage and stem elongation,
5- Blank + foliar application of seaweed extract (0.5% w/v) at the six-leaf stage and stem elongation,
6- The mix treatments of 2 + 3 + 4 + 5 (foliar spraying concentration for fulvic, seaweed and amino acid was (0.3% w/v)).

The biostimulants were checked for effective material concentration in the laboratory of the Soil and Water Research Institute. Compounds had 52.95% humic acid, 36.79% free amino acid, and 22.1% fulvic acid and the seaweed extract had 10% alginic acid. Surfactant was added to the foliar spray composition in the amount of 80 cc for 400 liters of water in each tank.

The length of each plot was 6 meters with 2.4 meters width. The 60 cm stacks were used for cultivation (four lines for each plot) and two rows of canola were planted on each stack. The drip tape irrigation system was used in the middle of each stack. The canola cultivar (Okapi) was prepared by the Seed and Plant Improvement Institute (SPII). It is non-hybrid (open pollinated) with an autumn growth type and a growth period of 260-280 days. The average height is 160-180 cm.

Characteristics Measured in The Experiment

For measuring the concentration of N, P, and K nutrients with the method of Chapman and Pratt (1962), 5 plant samples were taken at the pre-bud stage (before decimal code 58, according to Bleiholder et al., 2001), as a whole aerial part and its dry weight (DW) was measured too. Uptake of nutrients was obtained from the product of the concentration in the dry weight of the plant (DW). Concentrations were compared with reference concentrations provided for canola (Jones and Olson-Rutz, 2016; Nourgholipour et al., 2022). At the end of the growing season, indices such as plant height (cm), and number of silique per plant (number/plant), were measured in each plot. In the maturity stage, grain yield was measured (code 89 of growth).

Normal sowing was made on 18th September and delayed sowing on 7th October. At the second irrigation (23rd September and 29th October, respectively for normal and delayed sowing), humic acid was applied in humic acid treatments. The foliar application was made at the 6-leaf stage (29th October and 9th November, respectively for normal and delayed sowing) and stem elongation (30th March and 10th April, respectively for normal and delayed sowing) in all treatments except the blank treatment. Nitrogen from Urea (200 kg ha^{-1} N) was used in three steps (1/3 in second irrigation, 1/3 in stem elongation on 15th March, and 1/3 in budding on 30th March). Canola flowering began on 10th March. Plants were sprayed against wax aphids with Primor pesticide on 15th May. Yield and yield components were measured on 8th June.

Meteorological Characteristics of The Region in Two Years of the Experiment

Arak region has cold and wet winters and hot and dry summers. Figure 1 shows the comparison of meteorological characteristics of the Arak region in two years. The total rainfall was 545 mm in the first year and 496 mm in the second year (Figure 1b). This amount of precipitation was higher than the long-term average annual precipitation of the region (342 mm). Autumn and spring in the first year and winter in the second year were better in terms of precipitation. The minimum temperature below zero occurred in the first year corresponding to three winter months and in the second year from December to February. The minimum temperature in January and February in the second year was lower than in the first year (Figure 1 a, c, and d).

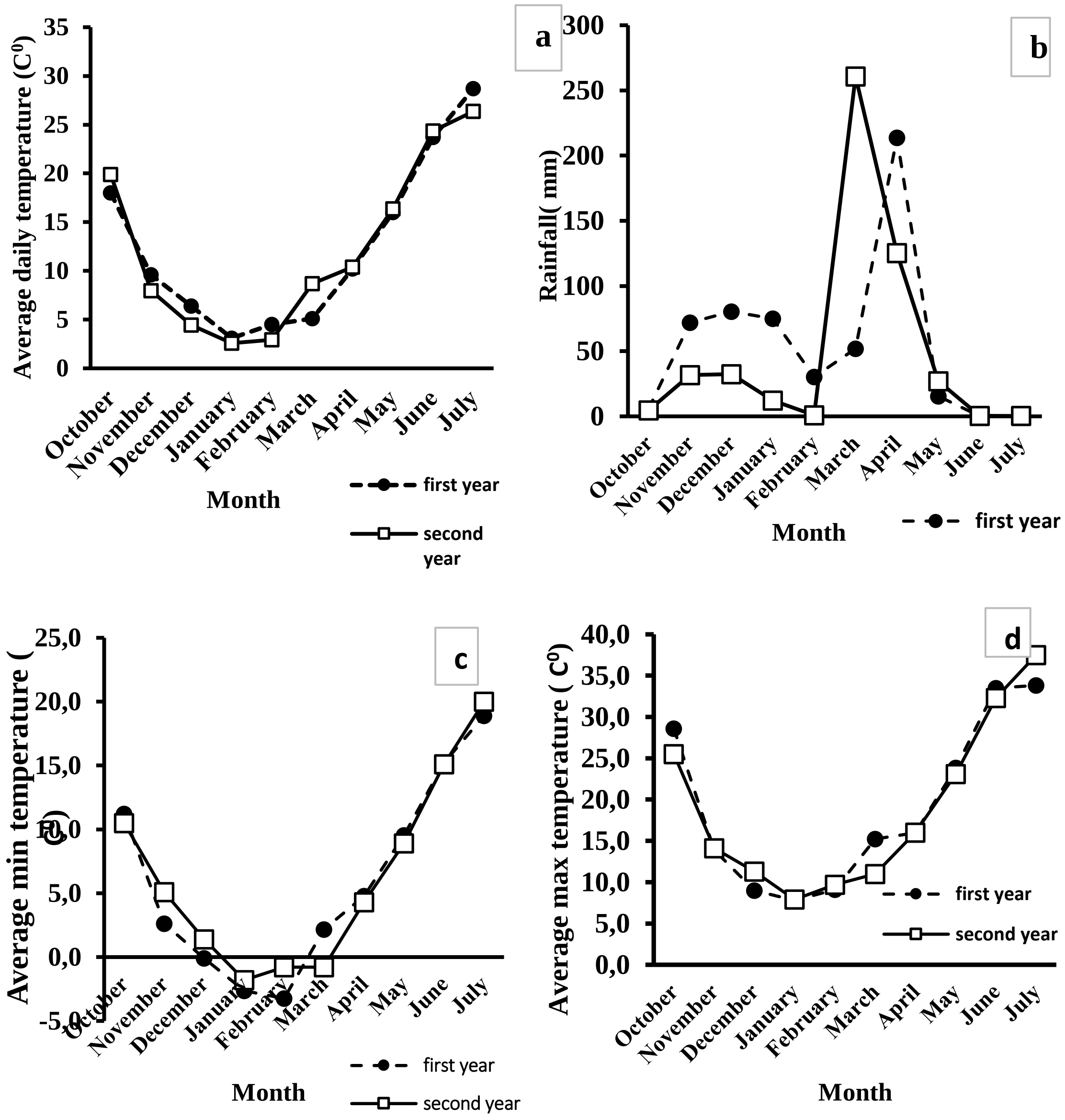

Figure 1. Meteorological condition of the Arak region during the experimental time in two years. a) Average daily temperature (Cº), b) rainfall (mm), c) Average min temperature (Cº), d) Average max temperature (Cº)

Statistical Analysis

The results of shoot dry weight (DW), grain yield and yield components, nutrient concentration, and uptake were statistically analyzed with SAS software (version 9.3, SAS Inst. Inc., Cary, NC) using Duncan's multiple range test at 95% confidence.

RESULTS and DISCUSSION

Effects of The Sowing Date and Biostimulants on The Growth Indices of The Canola

Variance analysis of results for two years showed that the effect of year on plant height was significant ($p < 0.01$) (Table 3).

The main effect of sowing date on seed yield was significant at $p < 0.01$, and the interaction of the year and the date of sowing on plant height and the number of siliques per plant was significant at $p < 0.05$, and on the DW at $p < 0.01$. The main effect of biostimulant treatment on grain yield and the number of siliques per plant was significant at $p < 0.01$.

Table 3. Composite analysis of variance for treatments on canola growth indices in two years

Source of variation	df	Silique per plant	Shoot dry weight (DW)	Height	Grain yield
Year	1	24.5	48.4	4521.3**	105188
Date	1	10272*	299.8*	6222.1**	3027440**
Year*date	1	3784.5*	481.3**	1173.5*	1298735
Bst	5	1287**	25.7	60.5	1905083**
Year*bst	5	307.2	19.8	119.2	212656
Date*bst	5	425.9	33.3	74.4	289433
Date*bst*year	5	377.1	22.8	49.1	326640
CV (%)	-	13.5	14.3	6.0	17.9

*,**, significantly different at $p < 0.05$ and $p < 0.01$, respectively. Biostimulants: Bst

In the first year, there was no significant difference between the two sowing dates in terms of DW, but in the second year, the DW of the September sowing date was significantly higher (Figure 2c). In the average of two years, its amount was higher in September (24.4 gr) than on the sowing date of October (19.3 gr) (Table 4).

Table 4. Mean effect of sowing date on the growth indices of canola in two years

	Silique per plant Number		Height cm		Shoot dry weight (DW) gr plant^{-1}		Grain yield kg ha^{-1}	
Sowing date	October	September	October	September	October	September	October	September
Mean	127^{b}	151^{a}	131^{b}	150^{a}	19.3^{b}	24.4^{a}	3048^{b}	3458^{a}

Similar letters indicate no significant difference at p<0.05 by Duncan's method*

The maximum yield of 3775 kg ha^{-1} was obtained from seaweed extract foliar application (Table 5).

The minimum yield was obtained from the control treatment (2806 kg ha^{-1}). Another treatment that had a significant effect compared to the blank treatment was mixed treatment. Other biostimulants had no significant difference with the blank treatment. The grain yield on the normal sowing date (3458 kg ha^{-1}) was significantly higher than the delayed sowing date (3048 kg ha^{-1}) (Table 4). The results showed that there was no statistically significant difference between the biostimulants in each sowing date on the height of the plant. However, there was a significant difference between the two sowing dates in terms of plant height (150 cm in the normal sowing date and 131 cm in the delayed sowing date) (Figure 2a).

Table 5. The main effect of growth stimulants on grain yield, number of siliques per plant, P concentration, and uptake

Biostimulants	P uptake kg ha^{-1}	P concentration gr 100gr^{-1}	Silique plant^{-1} Number	Grain yeild kg ha^{-1}
Amino acid	0.067^{ab}	0.313^{ab}	132b^{c}	3093^{c}
Seaweed extract	0.073^{a}	0.31^{ab}	146a^{b}	3775^{a}
Humic acid	0.061^{ab}	0.283^{b}	146a^{b}	3223^{bc}
Fulvic acid	0.067^{ab}	0.319^{a}	131^{bc}	2931^{c}
Mix	0.066^{ab}	0.298^{ab}	151^{a}	3690^{ab}
Blank	0.054^{b}	0.285^{b}	125^{c}	2806^{c}

abc: Similar letters indicate no significant difference at the five percent probability level by Duncan's method, P: phosphorus, mix: contains amino acid, fulvic acid, humic acid, and seaweed extract

The results showed that in the first year, there was no significant difference between the two sowing dates in terms of silique per plant, but in the second year, the number of siliques per plant was significantly higher in September. In the average of two years, the number of siliques per plant was significantly higher on the first sowing date compared to the second sowing date (151 numbers in normal cultivation and 127 numbers in delayed cultivation) (Figure 2b).

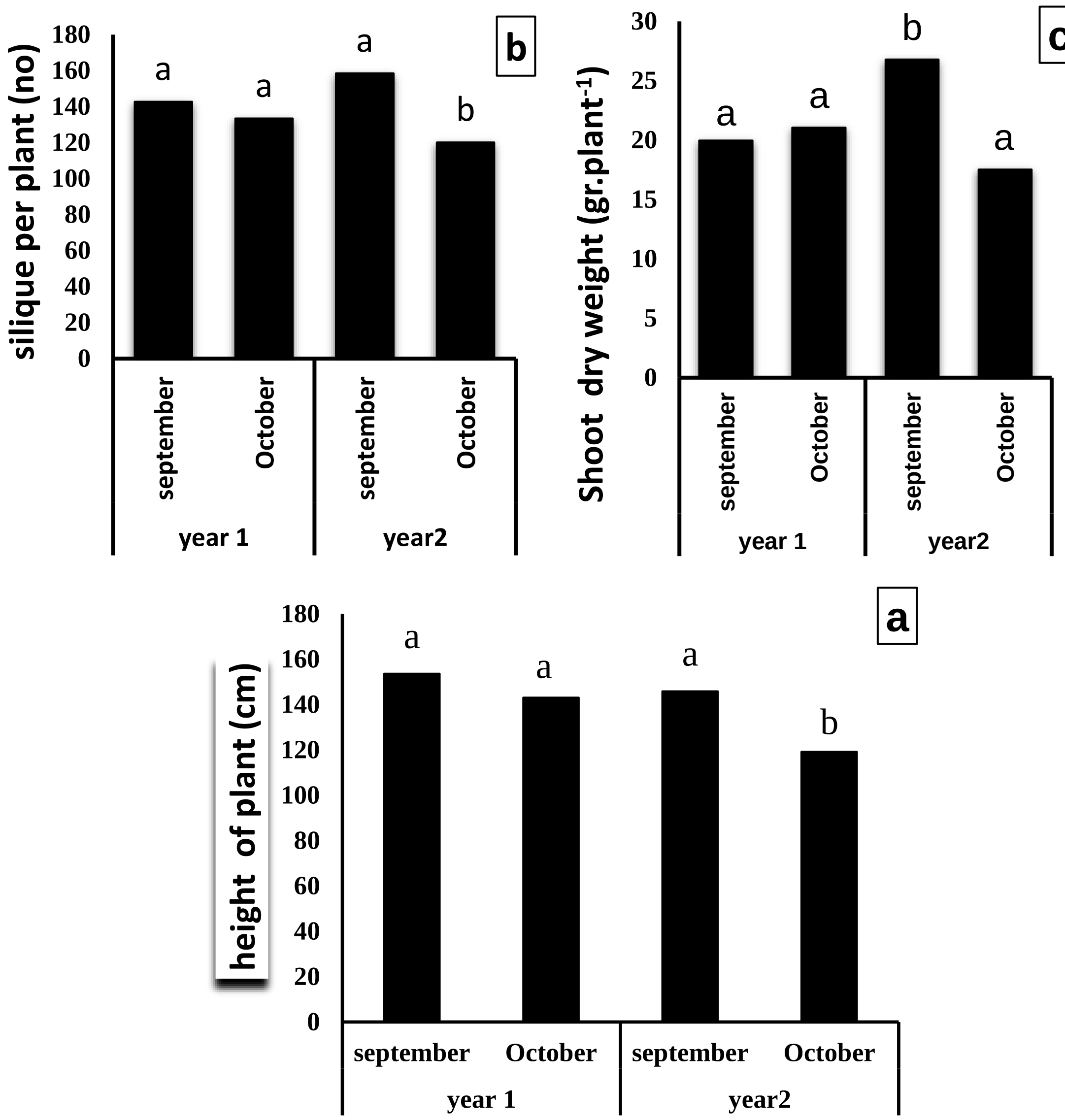

Figure 2. The interaction of the year and the sowing date on plant height (a), number of silique per plant (b) and shoot dry weight (DW) of canola plant (c). September and October (first and delayed sowing, respectively)

The Mean Effect of Different Treatments on The Concentration and Uptake of N, P, And K at The Stem Elongation Stage

Analysis of variance showed that (Table 6) the effect of sowing date on the N and P concentration was significant at $p < 0.01$ and $p < 0.05$, respectively. The effect of plant growth biostimulants on the concentration of P was significant at $p < 0.05$. The effect of cultivation date and biostimulants on plant K concentration was not significant (Table 6).

Table 6. Composite analysis of variance for the effect of treatments on the concentration and uptake of nutrients in canola

Source of Variance	DF	K	P	N	K	P	N
		Uptake (gr plant^{-1})			Concentration (%)		
Year	1	0.033	0.0005	0.02	0.003	5.55	0.76
Date	1	0. 24*	0.005*	0.9*	0.247	0.008*	2.2**
Year*date	1	0.24*	0.005*	0.77*	0.028	0.0002	0.07
Bst	5	0.02	0.0005*	0.09	0.240	0.003*	0.55
Year*bst	5	0.005	0.0002	0.37	0.007	0.0003	0.14
Date*bst	5	0.016	0.0002	0.06	0.09	0.0006	0.14
Date*bst*year	5	0.013	0.0003	0.26	0.003	0.0001	0.06
CV (%)	-	26	26	28.7	17.3	11.2	15

*,**: significantly different at $p<0.05$ and $p<0.01$, respectively. Biostimulants: Bst.

The main effect of cultivation date on the uptake of N, P, and K was significant at $p<0.05$, and the main effect of biostimulants on P uptake was significant, but the interaction effect of cultivation date×biostimulant on the uptake of these three nutrients was not significant (Table 6).

Results of N percentage showed that there was a significant difference between the two sowing dates (Table 6) and its amount in the normal sowing date (4.3%) was significantly higher than the delayed sowing date (3.9%). (Table 7). Results of P concentration showed that the maximum concentration of P was obtained from the fulvic acid treatment (0.319%) (Table 5), which was significantly different from the blank treatment (0.285%). The difference between the two cultivation dates was also significant (0.311% vs. 0.291%) (Table 6). The results of K concentration showed that there was no statistically significant difference between the used biostimulant treatments at the stem elongation stage. The difference between the two cultivation dates was not significant for K concentration (2.18% vs. 2.06%) (Table 6).

There was a significant difference between the two cultivation dates on N, P, and K uptake. The amount of N, P, and K uptake was 0.987, 0.073, and 0.509 gr plant^{-1} in September and 0.744, 0.056, and 0.394 gr plant^{-1} on October cultivation date, respectively (Table 6). The amount of P uptake in the seaweed extract was significantly higher than the control treatment (0.073 gr plant^{-1} in the seaweed compared to 0.054 gr plant^{-1} in the control treatment). The difference between the other treatments and the control was not significant for P uptake. The difference between biostimulant treatments for N and K uptake was not significant.

Table 7. Mean effect of sowing date on the concentration and uptake of nutrients in canola plants (composite of two years)

	K		P		N	
Sowing date	October	September	October	September	October	September
Concentration (%)	2.06[a]	2.18[a]	0.291[b]	0.311[a]	3.9[b]	4.3[a]
Uptake (gr plant^{-1})	0.394[b]	0.509[a]	0.56[b]	0.073[a]	0.764[b]	0.987[a]

a,b:Similar letters indicate no significant difference at $p<0.05$ by Duncan's multiple range test

Economic Analysis for The Application of Biostimulants

The effect of biostimulant treatments on the benefit-cost ratio resulting from the use of these substances in two conditions of normal and delayed cultivation is presented in Tables 8 and 9 as one of the economic evaluation criteria of the treatments**.**
The results showed that in the conditions without cold stress (Table 8), the combined application of growth stimulants had the greatest increase in grain yield compared to the control treatment (1319 kg ha^{-1}) and fulvic acid had the lowest increase in grain yield among the used biostimulants. After the mixed treatment, the application of seaweed extract was a superior treatment. Seaweed had the highest benefit-to-cost ratio (15.1), followed by amino acid (9.57) and humic acid (9.28) (Table 8)**.**

Table 8. The benefit-cost ratio of used biostimulants in normal cultivation date

Biostimulants	Grain increase compared to blank	Net income of grain	Expense of treatment	BCR
	Kg ha^{-1}	(US$ ha^{-1})	(US$. ha^{-1})	-
Humic acid	554	371.18	40	9.28
Fulvic acid	239	160.13	5+24	5.52
Amino acid	357	239.19	5+20	9.57
Seaweed	1017	681.39	5+40	15.1
Mixed	1319	883.73	5+90.4	9.26

The price of each kg of amino acid, humic acid, fulvic acid, and seaweed extract was 5, 4, 6, and 10 US$, respectively, and the price of canola was 0.67 US$ kg^{-1}, and the cost of foliar application of biostimulants was 5 US$ ha^{-1}. A 400-liter tanker was used for 0.5% (w/v) foliar application. Therefore, of each seaweed, fulvic acid and amino acid 4 kg ha^{-1} were used. 10 kg ha^{-1} of humic acid was used. In the mixed treatment, 10 kg ha^{-1} of humic acid, and 2.4 kg ha^{-1} of each of seaweed, amino acid, and fulvic acid were used. The seed yield of the control treatment was 2877 kg ha^{-1}. Benefit-cost ratio (BCR).

In the conditions of delayed cultivation and faced with cold stress (Table 9), the application of seaweed extract had the greatest increase in grain yield compared to the control treatment (922 kg ha^{-1}) and fulvic acid showed the least increase in grain yield among the used biostimulants (11 kg ha^{-1}). After the seaweed extract, mixed treatment (450 kg ha^{-1}) was better for increasing grain yield (Table 9). Seaweed had the highest benefit-to-cost ratio (13.7), followed by the amino acid treatment (5.8). Fulvic acid had a benefit-cost ratio of less than one in delayed cultivation**.**

Table 9. The benefit-cost ratio of used biostimulants in delayed cultivation

Biostimulants	Grain increase compared to blank	Net income of grain	Expense of treatment	BCR
	Kg ha^{-1}	(US$ ha^{-1})	(US$. ha^{-1})	-
Humic acid	218	146.06	40	3.7
Fulvic acid	11	7.37	5+24	0.25
Amino acid	218	146.06	5+20	5.8
Seaweed	922	617.74	5+40	13.7
Mixed	450	301.5	5+90.4	3.16

The price of each kg of amino acid, humic acid, fulvic acid, and seaweed extract was 5, 4, 6, and 10 US$, respectively, and the price of canola was 0.67 US$ kg^{-1}, and the cost of foliar application of biostimulants was 5 US$ ha^{-1}. A 400-liter tanker was used for 0.5% (w/v) foliar application. Therefore, of each seaweed, fulvic acid and amino acid 4 kg ha^{-1} were used. 10 kg ha^{-1} of humic acid was used. In the mixed treatment, 10 kg ha^{-1} of humic acid, and 2.4 kg ha^{-1} of each of seaweed, amino acid, and fulvic acid were used. The seed yield of the control treatment was 2877 kg ha^{-1}. Benefit-cost ratio (BCR).

Although on the date of normal cultivation, the application of mixed treatment was better than seaweed in terms of increasing grain yield, in delayed cultivation time, the amount of yield increase was higher in seaweed application. In the average of two sowing dates, seaweed application had the highest benefit-cost ratio for the studied region in the tested period of time. The price of canola seed was extracted from Tridge (2023). The prices of biostimulants are extracted from the Alibaba website (https://www.alibaba.com/trade)**.**

DISCUSSION

The Effect of Cultivation Date and Biostimulants on Canola Growth Indices

Delayed cultivation (19 days) decreased the dry weight of the shoot by 26% compared to normal date (Table 4). For each day of delay in cultivation, a 1.37% growth reduction occurred. In the current experiment, the dry weight of the shoot (DW) had a significant difference from the normal cultivation date, and this effect also was seen in grain yield in the maturity stage (Table 4). The decrease in DW of plants before the budding stage in the delayed sowing can be due to the reduction of the vegetative growth period before the occurrence of cold stress. This causes a decrease in the production of photosynthetic compounds and assimilates when cold stress occurs in plants (Barekati et al., 2019; Ren et al., 2022). Canola needs to obtain a certain amount of heat (GDD) from the environment in each period to complete its vital growth stages (Canola Watch, 2013). The growth degree date amount is 120 in the germination period and 360 in the vegetative period. Delayed cultivation causes a delay in the completion of

these periods (Dolatparast et al., 2012).
There was no statistically significant difference between the used biostimulants in terms of DW in the stem elongation stage. It seems that up to the plant sampling stage (stem elongation), the biostimulants had no significant effect on the DW.
Based on the results, delayed cultivation (19 days) caused a decrease in grain yield by 13.5% compared to cultivation on a normal date (Table 4). For each day of delay in cultivation, 0.711 percent of grain yield reduction occurred.
On the normal sowing date, the application of mixed treatment improved the grain yield of canola in the amount of 46% compared to the blank treatment and foliar spraying of seaweed extract, which improved it by 35%. The amount of increase in fulvic acid, amino acid, and humic acid treatments was 8, 12, and 19%, respectively compared to the blank treatment (Table 5).
In the delayed cultivation, the use of seaweed extract improved the grain yield by 34% compared to the blank treatment and reduced the damage caused by cold stress in the tested period time (with a 19-day delay in cultivation) (Table 5). The increasing amount for the mixed treatment, fulvic acid, amino acid, and humic acid was 16, 0.4, 8, and 10%, respectively compared to the blank treatment. Therefore, in the conditions of cold stress in delayed cultivation, the application of seaweed extract was a better suggestion. In the mean of two years and two sowing dates (Table 5), only the effects of seaweed application and mixed treatment were significant on canola grain yield. Seaweeds are used to produce plant growth stimulants which contains cytokinin and auxin or other hormone-like substances. These substances contain many active inorganic and organic compounds including complex polysaccharides such as laminarin, fucoidan, and alginates. So they can help the plant grow better and increase yield (Battacharyya et al., 2015; Ma et al., 2022). Increasing the integrity of the cell membrane and protecting chlorophyll are other mechanisms of the effect of these compounds on increasing plant tolerance to cold stress (Borella et al., 2023). In the present research, the application of fulvic acid had a small effect compared to the other biostimulants in cold stress conditions. It may be due to the concentration of material used or the concentration of active ingredients in these compounds. Based on Gaveliene et al. (2018) the concentration of used biostimulant is important in its effectiveness.
In this research, the effect of sowing date on plant height was more effective than the effect of plant growth stimulants on plant height, which can be argued that sowing date has a great effect on the phenological, morphological, and yield characteristics of canola cultivars (Dolatparast et al., 2012). According to the results of this research, the change in the sowing date caused the plant to face different environmental stress factors, and the growth of the plant was affected by these factors. The number of siliques per plant was significantly higher on the first sowing date compared to the delayed sowing (Table 4). According to the references, the number of siliques per plant is one of the important factors in increasing grain yield in canola (Barekati et al., 2019).

Increasing the number of siliques in the plant provides more photosynthetic surface for the plant, as a result of which a greater amount of production materials are produced in the plant and transferred to the seeds (Barekati et al., 2019). It can be due to the effect of optimal growth before the winter dormancy and the creation of a stronger rosette due to the availability of more growth time in the September sowing date compared to the October sowing. The correlation of seed yield with the number of seeds per plant (R^2=0.17*) (data not shown) and with silique per plant (R^2=0.23**) was positive and significant. In this research, the increase in the grain yield of mixed and seaweed treatments can be affected by the increase in the number of siliques in the plant. Grain yield had no significant correlation with other indices.

The Effect of Treatments on The Concentration and Uptake of Nutrients

Phosphorus uptake in the delayed cultivation date was significantly lower than the normal cultivation (6.9% decrease in concentration and 30.4% in P uptake). The effect of cultivation date on N concentration and uptake as well as K uptake was also observed (10.3% decrease in concentration 29.2% in N uptake and 5.8% decrease in concentration and 29.2% in K uptake). It may be due to the reduction of plant growth characteristics such as height and leaf area (data not shown) and root growth on the second sowing date, which can cause a reduction in the absorption of nutrients, including P (Berbara and García, 2014). Considering that N is effective in the vegetative growth of the plant, therefore, in the normal cultivation date, more N absorption can lead to an increase in the height and yield of the plant. The norm amount for N, P, and K concentrations of the canola plant is presented for the Arak region as 3.62, 0.39, and 2.37% before the budding stage (Nourgholipour et al., 2020).

According to the obtained results, the concentration and uptake of P in the aerial parts of the canola were affected by biostimulants (Table 5). The effect of fulvic acid on P concentration and seaweed extract on the uptake of this nutrient was significant (Table 5). The specific structural properties of growth-promoting compounds allow them to chelate nutrients and be effective in plant nutrition (Berbara and García, 2014). The positive effect of growth stimulants can be related to the improvement of plant growth. As can be seen, the application of seaweed extract improved the uptake of P in the canola plant (Table 5), which can be one of the reasons for the increase in the grain yield of the canola plant in this region, and this mechanism is separate from the effect of the hormonal compounds in the seaweed extract. These compounds can be effective on root growth by activating the antioxidant defense system, although they are used as foliar sprays. The presence of marine bioactive substances in seaweed extract improves stomatal uptake efficiency in treated plants compared to untreated plants (Rathore et al. 2009).

Meteorological Characteristics of The Region in Two Years of The Experiment

One of the most important variables in repeated field experiments is the effect of climate change, including the amount of precipitation and temperature fluctuations. According to the meteorological results (Figure 1), the amount of rainfall was greater between the months of October and February in the first year, and considering that the initial stages of growth take place during this period, therefore, the first-year cultivation had better conditions due to the higher relative humidity of the air for establishment. In the two years of the experiment, an increase in rainfall in March and April was observed in compare to the previous months, and the amount of rainfall was higher in the second year. These months are very important to complete root growth and vegetative and reproductive growth. The minimum temperatures, which usually lead to chilling damage, were observed further in the second year than in the first year. Therefore, the model of temperature changes in the first year was more suitable for plant adaptation to chilling than in the second year. The high air humidity due to suitable rains also significantly reduced the possibility of frost. Therefore, no significant damage from frost was observed in the two years of the experiment.

Economic Estimation of Biostimulants Application

The results showed that the application of biostimulants can be effective in increasing the grain yield, reducing the cold stress, and increasing the economic advantage of the important canola crop (Tables 8 and 9). The economic evaluation of applied treatments showed that in both normal and delayed conditions, seaweed extract had the highest benefit-cost ratio (15.1 and 13.7, respectively). Vigorous plant growth and high nutrient uptake improve the translocation of photosynthates for increasing grain yield and higher grain yield results in higher BCR (Tahir et al., 2015). The application of mixed treatment had priority for producing more grain yield in normal conditions, but due to the amount of used materials the cost of them, and the price of canola grain, this treatment (mix) did not have priority for economic evaluation. Application of all biostimulants (except fulvic acid) in delayed cultivation had a benefit-cost ratio greater than one, but with considering the cost of used materials and the price of canola in the experimental period, the application of seaweed extract for the studied region can be recommended. Results show that with the application of seaweed extract for every 1.0 US$ invested in a normal sowing date, farmers can obtain an additional 15.1 US$ and 13.7 US$ for delayed condition.

CONCLUSION

A significant difference was observed between the two sowing dates in terms of shoot dry weight, plant height, the number of siliques per plant, grain yield, the concentration and uptake of N and P, and the uptake of K. For each day delay in cultivation time, a 1.37% growth reduction occurred for shoot dry weight. In the normal sowing date, the effect of mixed treatment on grain yield was more, but in the late sowing time, foliar application of seaweed extract was more preferable. One of the reasons for the increase in grain yield of seaweed treatment may be the higher uptake of P and increase in silique per plant. It seems that the application of these materials can be considered in reducing the cold stress and increasing the economic efficiency of the canola crop. The use of all the growth-promoting substances (except fulvic acid) in the delayed condition had a benefit-cost ratio greater than one. The economic evaluation of the treatments showed that in both normal and delayed conditions, the use of seaweed extract had the highest benefit-cost ratio and had a higher economic efficiency than other treatments.

Conflict of Interest

The authors confirm that there is no conflict of interest between them.

Authors' Contribution

FN presented the idea, managed the project, and wrote the manuscript. MAK carried out the project in Arak province. SN co-worked with FN to write the manuscript. JG co-worked with MAK in carring out of the project.

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