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Assess the Regression and Correlation Relationships between the Traits Affecting the Seed Yield of Black Cumin (*Nigella sativa* L.) Cultivars Affected Different Planting Dates and Combined Nutrition

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ABSTRACT

BACKGROUND: Choosing the right planting date, managing plant food sources and identifying traits that increase yield are the most important factors of success in increasing agricultural production.

OBJECTIVES: To investigate the regression and correlation relationships among the factors affecting the yield of black cumin under the influence of planting date and different sources of nitrogen supply, a study was done.

METHODS: This research was conducted according factorial split plots experiment based on randomized complete block design (RCBD) with three replications. Seven levels of nitrogen sources (including urea, manure fertilizer, Azotobacter, urea + manure fertilizer, urea + Azotobacter, Azotobacter + manure fertilizer, and urea + Azotobacter + manure fertilizer) as the first factor in the main plots. Additionally, two planting dates (November 22 and February 14) and two black cumin cultivars (Arak and Semirom) were considered as the secondary factors and were factorially assigned to the sub-plots.

RESULT: The number of seeds per plant showed the highest explanatory coefficient with a justification of 93.16% of the changes in seed yield, indicating its significant influence on seed production. Moreover, the correlation coefficients between seed yield and the number of seeds per plant, biological yield, and number of pod per plant were found to be 0.997, 0.932 and 0.816, respectively. These strong correlation values suggest close relationships between these factors and seed yield. The Arak cultivar, planted on November 22 and treated with urea fertilizer, exhibited the highest seed yield with an average of 498.8kg.ha^{-1}. This yield was not significantly different from the Semirom cultivar, which showed an average yield of 460.8 kg.ha^{-1} when subjected to combined nutrition (Urea + Azotobacter + manure fertilizer) and planted on February 14.

CONCLUSION: It can be concluded the first planting date and traits such as number of seeds per plant, number of pod per plant and biological yield are essential factors that researchers should consider to enhance seed yield in black cumin cultivation.

KEYWORDS: *Black seed, Genotypes, Nitrogen, Stepwise regression, Urea.*

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1. BACKGROUND

Black cumin is an annual and herbaceous plant. Considering the advantages of medicinal plants, their proper use can play an important role in society's health, employment and non-oil exports (Sabet Teimouri *et al*., 2010). Black cumin is one of the most useful and valuable plants in the Middle East and North Africa. Due to the characteristics of the components in the oil, it is considered as an important herbal plant (Iqbal *et al*., 2010). The management of nutrients required by plants, such as fertilizers, is one of the most important methods of long-term plant production sustainability (Ning *et al*., 2017). In sustainable soil management, it is important to pay attention to maintaining the balance of nutrients and soil fertility and should nutrients uptake from the soil by plant organs should be returned to the field through organic and chemical fertilizers (Martin *et al*., 2006). The use of chemical fertilizers as the fastest way to compensate for the lack of soil nutrients has expanded significantly In addition to environmental pollution and ecological damage, the use of chemical fertilizers also increases the cost of production (Moradi *et al*., 2011). Nitrogen is one of the most important food elements that plays an important role in various physiological and morphological processes such as photosynthesis, the expansion of the green area of the plant and determines the seed yield (Raei *et al*., 2016). Nitrogen consumption in an optimal amount and choosing the planting date on time increases the plant size and plants branching (Moosavi, 2014). Mollafilabi *et al*. (2010) in a research using different amounts of nitrogen (0, 50, 100 and 150 ha^{-1}) with the aim of investigating the correlation of yield and yield components, reported that except for the traits of the number of follicles per plant and the number of seeds per follicle, other traits had a significant correlation with seed yield. Considering the negative effect of various fertilizers on the quantitative and qualitative characteristics of medicinal plants, the use of organic inputs in agricultural soils improves soil structure, organic matter content and soil fertility (Jha *et al*., 2011). After being decomposed, animal manure helps to provide nutrients to the soil and acts as an energy source for living organisms and increases the population dynamics and diversity of soil microorganisms and also increases the cation exchange capacity of the soil, absorbs more nutrients, prevents from leaching and increases the rate of water penetration (Moradi *et al*., 2018). In the study of Salehi *et al*. (2014), it was found that the highest amount and production of essential oil in black cumin plant was obtained in the application of manure fertilizer and the combination of animal manure + undivided urea chemical fertilizer, and through a positive effect on the yield components, it increased the yield of essential oil. The researchers concluded that in areas with calcareous soils, the use of organic fertilizers along with the use of biosulfur biological fertilizer can be useful in reducing the problems caused by the high use of chemical fertilizers (Rezvani Moghaddam *et al*., 2017). Nitrogen biological fertilizers increase nitrogen. It becomes available for the root of the plant and nitrogen increases the speed of cell division and increases the strength of different parts due to its role in the production and export of cytokinin hormone from the root to the shoot (Fathi *et al*., 2013). Biofertilizers such as nitrogen-fixing bacteria, in addition to providing nitrogen needed by plants, do not have the negative effects of chemical fertilizers (such as soil salinization) and animal fertilizers (such as increasing the weed seed bank). Bromand Sivieri *et al*. (2020) reported that inoculation with biological fertilizers increased the growth and yield of black cumin. Another factor affecting the yield potential of plants is the right planting date because choosing the right planting date provides enough time to go through each of the stages of growth and development. The planting time of crops is determined based on the matching of the ambient temperature with the optimal temperature of each of the phonological stages of growth, as well as the non-coincidence of sensitive growth stages with environmental stresses. Each seed yield components are stabilized at a certain stage of growth, and providing enough time to go through these stages increases seed yield (Siadat *et al*., 2013). Planting date is one of the most important agricultural factors in improving the yield and quality of umbelliferous family plants; So that in early planting, number of lateral branches decreases and in late planting, in addition to the number of lateral branches, the volume of roots also decreases (Khosh-Khui and Bonyanpour, 2006).

2. OBJECTIVES

To assess the regression and correlation between factors affecting the yield of black cumin under the influence of planting date and different sources of nitrogen supply, a study was done.

3. MATERIALS AND METHODS

3.1. Field and Treatments Information

This research was done during the 2017 and 2018 cropping seasons in the experimental field of Islamic Azad University, Shushtar, Iran. The geographical location are 48˚35´E longitude and 32˚ and 26´ N latitude, with an altitude of 150 meters above sea level. The average annual rainfall of 240.6 mm, with an average temperature of 25.3°C. To determine the physical and chemical characteristics of the soil at the experimental site, soil samples were collected from five points within the field at a depth of 0-30 cm before planting. The results of the soil analysis are presented in table 1. The study was consisted of a split-plot factorial experiment, using Randomized Complete Block Design with three replications. The sources of nitrogen supply in seven levels [including urea (C_1), manure fertilizer (C_2), Azotobacter (C_3), urea + manure fertilizer (C_4), urea + Azotobacter (C_5), Azotobacter + manure fertilizer (C_6), urea + Azotobacter + manure fertilizer (C_7)] and two planting dates [November 22 (A_1) and February 14 (A_2)) as the first factor were placed in the main plots and black cumin cultivars (including Arak (B_1) and Semirom (B_2)] as the secondary factor were also placed factoriallly in the sub-plots.

Table 1. Chemical and physical characteristics of the experimental soil

Characteristic	Soil texture	pH	EC ($dS.m^{-1}$)	N (%)	P ($mg.kg^{-1}$)	K ($mg.kg^{-1}$)
2017 (year)	Silt loam	7	2	1.6	193	281
2018 (year)	Silt loam	7	2	1.8	201	289

EC: electrical conductivity: Nitrogen; P: phosphorus; K: potassium.

Each experimental plot was designed to have 9 rows of black cumin plants arranged in a stacked formation. Each row was 2 meters in length, with a spacing of 40 cm between the rows. The distance between individual plants within a row was set at 2.5 cm, resulting in a plant density of 100 plants per square meter. For sowing, the amount of seed used was $12.5kg.ha^{-1}$, and in each experimental plot, 9 grams of seeds were used. The distance between repetitions (replicates) was set at 2 meters, while the distance between the main treatments (factorial treatments) was 4 meters. Lastly, the distance between the sub-plots (secondary treatments) was maintained at 50 cm.

3.2. Farm Management

After preparing the land and tilling the soil according to the plan, the black cumin seeds were planted at a depth of 1 cm in the furrows on the rows. Following the planting, the first irrigation was immediately carried out and three days later, second irrigation was performed to aid germination. For urea fertilizer application, the basis of consumption was 120 $kg.ha^{-1}$. For each experimental plot, 86.4 grams of pure urea were used in two stages. During the tillage operation, manure fertilizer was mixed with the soil at a rate of 20 $ton.ha^{-1}$ (equivalent to 14.4 kg for each plot). To utilize the Azotobacter biofertilizer, it was applied as a seed inoculant based on 100 grams per 12.5 kg of seeds used for one hectare, which is equivalent to 72 mg per plot. At the 2-6 leaf stage, a thinning operation was performed manually after ensuring the health and greenness of the plants. Mechanical methods were employed for weed control. At the physiological maturity stage of the plant, characterized by yellowing of the plants and before the pod split, the black cumin plants were harvested from an area of one square meter at the center of each plot.

3.3. Measured Traits

3.3.1. *Number of pod per plant and the number of seeds per pod*

To calculate the number of pod per plant, a specific stage was chosen when the pods were fully ripe, characterized by yellowing leaves and black, hardened seeds, just before the pod open. From each experimental plot, 10 plants were randomly selected. The number of pod on each of these 10 plants was counted, and then the average number of pod per plant was calculated (Babaie Abarghoie, 2015). To determine the number of seeds per pod, the number of seeds from the same 10 selected plants was counted. Then, this total number of seeds was divided by the average number of pod per plant, as reported by Abbaspour *et al*. (2017).

3.3.2. *Number of seeds per plant*

After the black cumin plants reached full maturity, 10 plants were randomly selected from each plot to assess the total number of seeds per plant. The number of seeds from each of the 10 selected plants was counted individually. To obtain the average number of seeds per plant, the total number of seeds counted for the 10 plants was divided by the number of plants (which is 10 in this case). This calculation allowed for determining the average number of seeds produced by each plant in the experimental plots. The consideration of marginal effects helps in obtaining a more accurate and representative estimation of the average seed yield per plant (Abbaspour *et al*., 2017).

3.3.3. *1000 seed weight*

To determine the 1000-seed weight, Initially, 500 seeds were randomly selected from the seed lot and the weight of these 500 seeds was measured precisely using a scale with an accuracy of 0.001 grams. Then, a second sample of 500 seeds was taken and weighed using the same method as the first sample. If the difference in weight between the two samples was less than 5%, the total weight of the two samples was considered as the 1000-seed weight (Babaie Abarghoie, 2015).

3.3.4. *Seed yield*

At the maturity stage of the black cumin plants, the seed yield was calculated by considering the marginal effect. All plants within the final harvest area were harvested. The harvested plants were then processed to separate the seeds from the pod. After the seeds were separated, they were dried to ensure accurate measurements. The seeds harvested from each plot were weighed separately using a precise scale, and the weight of the seeds was recorded. To calculate the seed yield, the total weight of seeds harvested from each plot was converted to kilograms. The seed yield was then expressed in kilograms per hectare, providing a standardized measure of the productivity of black cumin in each experimental plot. This comprehensive approach accounts for the combined effects of planting dates, combined nutrition, and other factors on the final seed yield per hectare (Abbaspour *et al*., 2017).

3.3.5. *Biological yield*

To calculate the biological yield of black cumin, the plants from each subplot were harvested from a one square meter area at the center of the plot, excluding the margins. After harvesting, the plant samples were collected and subjected to drying in an oven at a temperature of 70°C for 48 hours to remove all moisture content (Farhoudi and Modhej, 2018).

3.3.6. *Harvest index*

The harvest index is a crucial indicator that reflects the efficiency of photosynthetic materials distribution in the plant towards seed production. To calculate the harvest index, the seed yield is divided by the biological yield, and the resulting value is expressed as a percentage (Abassi, 2015).

3.4. Statistical Analysis

Analysis of variance was done via SAS (Ver.8) software. Mean comparison was done by LSD test at 5% probability level. For doing statistical calculations, identifying correlation coefficients and regression analysis, Minitab (Ver.15) software was utilized.

4. RESULT AND DISCUSSION

4.1. *Seed Yield and its components*

The results of variance analysis of the data showed that the planting date and the combined effects of planting date × nitrogen and planting date × nitrogen × cultivar were significant at the 1% of probability level, and the effect of planting date × cultivar at 5% of probability level was significant on the number of pod per plant of black cumin cultivars (Table 2). The results of the comparison of the triple effects of planting date × nitrogen × cultivar showed that the maximum number of pod per plant was related to pure urea fertilizer treatments (with an average of 10.8), urea + Azotobacter (with an average of 9.6) and Azotobacter + manure fertilizer (with an average 6/9) in Arak cultivar, and the planting date of November 22 (Table 3). The number of seeds per pod was also affected by planting date, nitrogen, planting date × nitrogen and nitrogen × cultivar and the triple effects of planting date × nitrogen × cultivar treatments at the 1% of probability level (Table 2). The results of mean comparison of the combined effects of planting date × nitrogen × cultivar were determined that the planting date of February 14, had the highest number of seeds per pod under treatment of urea fertilizer + Azotobacter + manure fertilizer (with an average of 36.56) in Semirom cultivar. The lowest number of seeds per pod (with an average of 12.7) in Semirom cultivar was obtained by applying urea on the planting date of February 14 (Table 3). Planting date, nitrogen, planting date × nitrogen, nitrogen × cultivar and combined effects of planting date × nitrogen × cultivar had a significant effect of at 1% of probability level on the number of seeds per plant (Table 2). The results of the mean comparison of the combined effects of planting date × nitrogen × cultivar, showed that the highest number of seeds per plant was related on the planting date of December 22 in Arak cultivar with the use of pure urea and urea + manure fertilizer treatment (with an average of 284.7 and 249.7, respectively) and the lowest number of seeds per plant, with the use of azotobacter on the planting date of February 14 (with an average of 76 numbers) in the Semirom cultivar were obtained. The effects of planting date, nitrogen, planting date × nitrogen, and planting date × nitrogen × cultivar and the effects of planting date × cultivar and nitrogen × cultivar on the 1000 seed weight were significant (Table 2). The mean comparison of the combined effects of planting date × nitrogen × cultivar showed that the highest 1000 seed weight with an average of 1.8 g on the planting date of December 22 with the feeding of azotobacter + manure fertilizer treatment fertilizer was assigned to Arak cultivar and the lowest one was 1.2 g on planting date of February 14 with Urea + Azotobacter fertilizer feeding was in Arak cultivar (Table 3).

Table 2. ANOVA for studied traits (2017-2019)

S.O.V	df	No. pod per plant	No. seeds per pod	No. seeds per plant	1000-seed weight
Year (Y)	1	0.006^{ns}	0.843^{ns}	86.43^{ns}	0.001^{ns}
Repetition (R)	4	2.315	30.96	908.57	0.036
planting date (A)	1	255.054**	1265.55**	406874.18**	0.991**
Y×a	1	0.006^{ns}	5.75**	287.83^{ns}	0.001^{ns}
Cultivar (B)	1	0.006^{ns}	76.141^{ns}	5608.68^{ns}	0.021^{ns}
Y×b	1	0.006^{ns}	4.18^{ns}	55.201^{ns}	0.001^{ns}
a×b	1	5.006*	0.41^{ns}	4518.83^{ns}	0.065*
Y×a×b	1	0.006^{ns}	10.251^{ns}	615.252^{ns}	0.001^{ns}
Error I	12	1.038	78.695	6204.009	0.059
Nitrogen (c)	6	1.151^{ns}	258.20**	12564.06**	0.074**
a×c	6	4.04**	340.43**	12058.35**	0.108**
b×c	6	1.464^{ns}	231.97**	14279.53**	0.03*
a×b×c	6	3.242**	205.89**	9585.081**	0.063**
Y×c	6	0.02^{ns}	0.883^{ns}	44.391^{ns}	0.001^{ns}
Y×a×c	6	0.02^{ns}	1.841^{ns}	62.466^{ns}	0.001^{ns}
Y×b×c	6	0.048^{ns}	1.79 ^{ns}	65.52^{ns}	0.001^{ns}
Y×a×b ×c	6	0.048^{ns}	3.76^{ns}	193.74^{ns}	0.002^{ns}
Error II	96	0.947	32.54	2355.25	0.013
CV (%)		12.586	25.080	27.166	6.888

^{ns}, * and **: Not-significant and significant at 5% and 1% probability levels, respectively.

It seems that the longer period of vegetative growth on the first planting date (December 22) and the effectiveness of the nitrogen supply method, as well as the genetic superiority of the black seed mass of the Arak cultivar in using these conditions compared to the Semirom cultivar, cause suitable vegetative growth and the production of more photosynthetic materials. In this plant, the use of nitrogen fertilizer along with biological fertilizer provides adequate supply of nutrients and improves the environmental conditions of plant growth to form more pod (Mazloumi *et al*., 2018). Researchers believe that the reason for this increase in yield in the integrated systems of plant fertilizer feeding is due to the greater compatibility between the available nitrogen of the soil and the needs of the plant (Mooleki *et al*., 2004). The number of seeds per pod actually determines the capacity of the sink, so with more seeds number, the plant has a larger and more storage to receive the produced photosynthetic materials and the increase of this feature leads to an increase in yield. The positive effect of urea and manure fertilizer treatments on the number of seeds in black cumin plant was also reported by Rezvani Moghadam *et al*. (2017).

Continue table 2.

S.O.V	df	Seed yield	Biological yield	Harvest index
Year (Y)	1	1025.14^{ns}	19500.6^{ns}	12.815^{ns}
Repetition (R)	4	865.60	32021.28	8.0717
planting date (A)	1	149333.8**	22866192.8**	73.604^{ns}
Y×a	1	1104.69^{ns}	11833.93^{ns}	0.126^{ns}
Cultivar (B)	1	8677.46^{ns}	157872.02^{ns}	387.661**
Y×b	1	1.269^{ns}	6192.86^{ns}	3.259^{ns}
a×b	1	29706.88*	222214.88^{ns}	34.2^{ns}
Y×a×b	1	1893.42^{ns}	7735.71^{ns}	1.844^{ns}
Error I	12	7712.26	61948.26	30.096
Nitrogen (c)	6	22728.34**	222738.5^{ns}	243.451**
a×c	6	37870.68**	562628.5**	375.139**
b×c	6	40883.03**	343891*	186.099**
a×b×c	6	27693.13**	226967.74^{ns}	203.006**
Y×c	6	95.12^{ns}	9590.54^{ns}	1.623^{ns}
Y×a×c	6	100.91^{ns}	9133.32^{ns}	1.296^{ns}
Y×b×c	6	196.42^{ns}	7696.97^{ns}	2.371ns
Y×a×b ×c	6	357.08^{ns}	6345.94^{ns}	2.443ns
Error II	96	6482.53	137690.8	25.168
CV (%)		27.02	28.909	21.369

^{ns}, * and **: Not-significant and significant at 5% and 1% probability levels, respectively.

Considering that the highest number of seeds per plant was obtained with the use of urea, but no significant difference was observed between the integrated feeding method and the use of urea. Black cumin is a terminal flower plant and limited growth, and flowers and fruits are formed only at the end of each branch, so the number of seeds per plant depends on the number of flowering branches. Probably, the reason for the increase in seed yield in the planting date of December 22 can be attributed to better vegetative growth, canopy development and, as a result, more appropriate use of solar radiation and high photosynthesis. The 1000 seed weight is one of the factors that is mostly under genetic control and has high heritability and is less influenced by environmental factors, but the use of combined fertilizer sources that provide nitrogen can effective in increasing the 1000 seed weight from other treatments. The increase in the 1000 seed weight can be justified due to the increase in the length of the seed filling period on the first planting date (December 22), which was 85 days more than the second planting date (February 14), and it can indicate the effect of bacteria that increase plant growth through increasing the amount of stored photosynthetic materials during the seed filling period (Babaei Abargoui, 2015).

Table 3. Comparison of the average effects of planting date × Cultivar × sources of nitrogen supply for the investigated traits of black seed plant

Planting date	Cultivars	Sources of nitrogen	No. pod per plant	No. seeds per pod	No. seeds per plant
22 November	Semirom	C1	8.167	20.1	164.033
		C2	8.333	24.533	201.55
		C3	8.333	25.75	206.85
		C4	8.833	28.117	251.583
		C5	9.333	25.067	232.317
		C6	9.5	24.733	236.467
		C7	9	25.067	225.467
		LSD 5%	1.589	9.222	78.458
	Arak	C1	10.833	26.217	284.717
		C2	7.833	27.817	216.283
		C3	8.333	28.933	234.55
		C4	9	27.75	249.75
		C5	9.667	24.233	236.917
		C6	9.667	23.5	226.117
		C7	8.667	25.033	223.433
		LSD 5%	1.589	9.222	78.458
14 February	Semirom	C1	7	12.717	89.033
		C2	7	17.933	125.367
		C3	6	12.833	76.9
		C4	7	13.45	93.983
		C5	6.667	23.133	146.583
		C6	6	19	114
		C7	7	36.567	256.033
		LSD 5%	1.589	9.222	78.458
	Arak	C1	6	19.283	115.533
		C2	6.667	19.917	134.733
		C3	7	13.6	95.25
		C4	6.333	19.733	126.317
		C5	6	41.333	247.9
		C6	6	13.817	82.767
		C7	6.333	16.683	107.683
		LSD 5%	1.589	9.222	78.458

*The means that their differences are larger than LSD are significantly different at the 5% level.

Urea (C1), manure fertilizer (C2), azotobacter (C3), urea + manure fertilizer (C4), urea + azobacter (C5), azotobacter + manure fertilizer (C6), urea + azotobacter + manure fertilizer (C7).

Continue table 3.

Planting date	Cultivars	Sources of nitrogen	1000-seed weight (g)	Seed yield ($g.m^{-2}$)	Harvest Index (%)
22 November	Semirom	C1	1.767	290.783	21.167
		C2	1.667	336.5	22.833
		C3	1.767	364.167	26
		C4	1.783	439.667	24.333
		C5	1.733	400.833	21.667
		C6	1.7	399.167	23.5
		C7	1.65	371.083	22
		LSD 5%	0.186	131.479	8.192
	Arak	C1	1.75	498.833	22.617
		C2	1.817	391.267	26.333
		C3	1.717	397.967	28.333
		C4	1.7	424.117	25.333
		C5	1.733	399.133	24.333
		C6	1.8	404.833	24.333
		C7	1.667	372.833	25.167
		LSD 5%	0.186	131.479	8.192
14 February	Semirom	C1	1.533	136.433	17.667
		C2	1.783	223.5	22.5
		C3	1.5	115.333	13.667
		C4	1.433	124.5	13.5
		C5	1.55	207.833	26.75
		C6	1.667	190	20.167
		C7	1.8	190	31.667
		LSD 5%	0.186	460.833	8.192
	Arak	C1	1.6	131.479	24.833
		C2	1.7	184.833	19.333
		C3	1.55	233.833	21.833
		C4	1.567	147.55	23.333
		C5	1.25	200.667	48.5
		C6	1.567	309.167	17.167
		C7	1.6	129.667	18.5
		LSD 5%	0.186	177.167	8.192

*The means that their differences are larger than LSD are significantly different at the 5% level.
Urea (C1), manure fertilizer (C2), azotobacter (C3), urea + manure fertilizer (C4), urea + azobacter (C5), azotobacter + manure fertilizer (C6), urea + azotobacter + manure fertilizer (C7).

Investigating the effect of 5 planting dates on the yield and yield components of black cumin plant showed that the planting date has a significant effect on all traits, including the 1000 seed weight (Vaseghi *et al*., 2014). In a similar study, the consumption of manure fertilizer significantly affected the number of umbrellas and the seed weight in black cumin (Khorramdel *et al*., 2010). Salehi *et al*. (2014) also observed that combined black cumin feeding is more effective than separate nitrogen feeding on seed weight. The results of the combined variance analysis showed that the simple, reciprocal, double and triple effects of all investigated treatments, except for the simple effect of cultivar, were significant at the statistical probability level of 5% on the seed yield (Table 2). The Arak cultivar had the highest seed yield (with an average of 498.8 $kg.ha^{-1}$) in the treatment of the planting date on December 22 and the application of urea (Table 3). Therefore, according to the results, it is recommended to select the Arak cultivar, the date of first planting and combined nutrition to achieve more seed yield in the region. In a similar study, Safai (2012) showed that the highest and lowest seed yield in Black cumin plant was obtained from the planting dates of November 8 and April 29, respectively. The higher seed yield for the black cumin plant in the application of integrated treatment was also reported in the research of Salehi *et al*. (2014) and Abid *et al*. (2018). They also observed in the research on black cumin that delayed planting caused due to the lack of the plant has enough opportunity for vegetative growth and the lack of proper and sufficient absorption of nitrogen has a lower height. The highest yield of black cumin is related to the biofertilizer treatment at the rate of $20.54 g.m^{-2}$, which was 30% higher than without fertilizer application (Pirzad *et al*. 2018). Probably, the reason for the increase in seed yield on the planting date of November 1 compared to the delayed planting date of February 25 can be attributed to better vegetative growth and canopy development, and as a result, more appropriate use of solar radiation and high photosynthesis. Najafi *et al*. (2014) in a similar research observed that the use of fertilizer treatments significantly affected the growth characteristics, yield components and seed yield and essential oil of the medicinal plant fennel, so that the best results for the combined treatment of chemical fertilizers and animal manure was obtained. Animal manure as an organic input improved the physical and chemical characteristics of soil moisture content and the availability of nutrients in the soil, improved the vegetative characteristics, and finally, increased the seed yield and essential oil. In this way, the consumption of organic inputs such as animal manure can be taken into consideration in combination with other fertilizer treatments. The highest seed yield in black cumin plant was the result of combined feeding (Salehi *et al*., 2014). In a research on Coriander plant, Aqhhavani Shajari *et al*. (2015) observed that the use of integrated nutritional treatments was superior compared to individual treatments. Also, the result of the research by Tashakorifard *et al*. (2018) on the fennel plant showed that the highest seed yield is the result of 75% chemical fertilizer treatment along with biological fertilizer and vermicomposting.

4.2. *Step-by-step regression*

The final model obtained from the step-by-step regression analysis can identify the independent variables affecting the dependent variable and rank them in terms of importance. In order to predict the relationship between yield and its components and to eliminate less important variables, regression analysis was done step by step, seed yield was analyzed as dependent variable and other measured traits as independent variables. Seed yield of black cumin, the number of seeds per plant was the most important trait affecting seed yield, which accounted for 93.16% of the variation in seed yield (Table 4). After the number of seeds per plant, the 1000 seed weight was entered into the model and had the greatest impact on seed yield with 6% and explained the changes in seed yield along with the number of seeds per plant (99.33%). According to the results of the third and fourth steps, the number of seeds per pod and the number of pod per plant had a significant effect on the seed yield, but since these two variables play a role in calculating the percentage of the number of seeds per plant, the effect of each of them on the coefficient of explanation is much less and in the third and fourth steps, these two variables have increased only 0.2% of the coefficient of explanation (Table 4).

Table 4. Step-by-step regression analysis of traits affecting seed yield

Fixed variable	1	2	3	4
Intercept	-0.01538	-31.86468	-29.64605	-22.54102
number of seeds per plant	0.00167**	0.00159**	0.00170**	0.00207**
1000-seed weight		20.04**	19.08**	19.57**
the number of seeds in a capsule			-0.110**	-0.361**
number of capsules in a plant				-1.01**
R^2 (%)	93.16	99.35	99.47	99.55

ns, * and **: Not-significant and significant at 5% and 1% probability levels, respectively.

The number of pod, the 1000 seed weight and the biomass of the plant are important traits that determine seed yield. (Rezvan Bidekhti *et al*., 2012). According to the mentioned materials, it can be concluded that the components that determine the seed yield of black cumin cultivars include the number of pod per plant, biomass, 1000 seed weight and 50% flowering, so the selection of these four can lead to an increase in seed yield and cultivar production. Of course, this is possible due to the compensatory role of these components as long as the balance between the source and the sink is established. Therefore, it is very likely that the selection based on these traits that are included in the model can lead to the creation of plants with high yield. The trait of seed number per pod had the most direct and positive effect on sesame plant yield and had a high correlation with seed yield (Shadan *et al*., 2022). Mostafavi *et al*. (2019)

announced that biomass and seed yield have a positive and significant correlation with the number of pod per plant. The result of stepwise regression showed that the highest direct effect on seed yield in sesame plant was related to the number of pod per plant (Mostafavi *et al*., 2019). Pezeshkpour *et al*. (2015) reported that the 1000 seed weight, the number of fertile pods and biomass as the most important components of chickpea yield had the largest contribution to the final formation of seed yield.

4.3. *Results of correlation analysis between traits*

In order to study the relationship between seed yield and other measured traits, the correlation between traits was calculated and interpreted. According to the results of correlation analysis between traits, seed yield has a positive and significant correlation at 1% probability level with number of pod per plant, 1000 seed weight, number of seeds per plant, number of seeds per pod and biological yield, respectively (with coefficients 0.816, 0.757, 0.997, 0.777, and 0.874), So that with the improvement of these traits, the seed yield also increased (Table 5). Shadan *et al*. (2022) reported that in rapeseed, seed yield has a significant positive correlation with the number of seeds per pod and the number of pods per plant. The number of branches per plant is considered one of the most important yield components in rapeseed (Anjadi *et al*., 2003).

Table 5. Correlation test among investigated traits in (*Nigella sativa* L.)

Traits	No. pod per plant	1000 Seed weight	Seed yield	Biological yield	Harvest index	No. seeds per plant
Seed weight-1000	0.757**					
Seed yield	0.816*	0.632*				
Biological yield	0.932*	0.761*	0.874*			
Harvest index	0.030^{ns}	-0.0890^{ns}	0.451^{ns}	0.0806^{ns}		
No. seeds per plant	0.766	0.464^{ns}	0.997*	0.803*	0.587**	
No. seed per pod	0.338^{ns}	-0.203^{ns}	0.777*	0.424^{ns}	0.870*	0.851*

^{ns}, * and **: Not-significant and significant at 5% and 1% probability levels, respectively.

5. CONCLUSION

Based on the findings of this study, the combination of seed inoculation with biofertilizer containing beneficial bacteria and the application of manure fertilizer alongside urea chemical fertilizer during the first planting date (December 22) resulted in enhanced plant growth by promoting nutrient uptake, ultimately leading to an increased yield of black cumin. Moreover, the first planting date (December 22) exhibited improved vegetative growth, canopy development and more efficient utilization of solar radiation, leading to higher photosynthesis. The highest seed yield of 498.8 $kg.ha^{-1}$ was achieved in the Arak cultivar when planted on December 22 and treated with urea fertilizer, which was significantly different from the combined nutrition treatment (urea + azotobacter + manure fertilizer) yielding an average of 460.8 kg.ha^{-1} in the Semirom cultivar on the planting date of February 14. Based on these results, it is recommended to choose the Arak cultivar, select the first planting date, and consider combined nutrition practices to attain higher seed yield in the region.

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FOOTNOTES

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