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Original Article

Evaluation of vermicompost application and amino acid spraying on yield and essential oil of black cumin (*Nigelle sativa* L.)

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ABSTRACT

Intensive cultivation has led to a rapid decline in organic matter and nutrient levels besides affecting the physical properties of soil. The sustainability of production systems has become an important issue throughout the world. Black cumin (*Nigella sativa* L.) is one of the most widely used medicinal and industrial plants. Furthermore, today, using organic and biofertilizers as a tool to achieve sustainable agriculture was considered. The current study was conducted as a factorial experiment at the Forest and Rangeland Research Institute of Iran in 2019 as a randomized complete block design with three replications. The treatments included six vermicompost levels (0, 3, 6, 9, 12, and 15 tons ha^{-1}), and the foliar application of amino acid at 3 levels (no foliar application, foliar application at the beginning of the flowering stage and foliar application at the beginning of the encapsulation stage). The results of the study showed that amino acids, vermicompost, and their interaction had a significant effect on leaf yield. Moreover, the interaction of amino acid × vermicompost significantly affected biological yield and essential oil yield. Comparing the mean oil yield showed that the highest oil yield (73.22 kg ha^{-1}) was related to the 6 tons of vermicompost × no foliar application of amino acids. The results showed that using vermicompost and amino acids could increase the quantity and quality of *Nigella sativa* L. in sustainable cropping systems.

1. Introduction

Black cumin (*Nigella sativa* L.), is a dicotyledonous, herbaceous, annual plant belonging to the Ranunculaceae family (Tavakoli Saberi & Sedaghat, 2005). Black cumin has been reported to have properties such as anti-flatulence, laxative and anti-parasite, anti-epileptic, anti-viral, anti-bacterial, anti-tumor, anti-inflammatory, analgesic and hypoglycemic, strengthening blood circulation, relieving kidney discomfort, miseede, diarrhea, smooth muscle relaxant and immunostimulation (Seyyedi et al., 2017).
In recent decades, using agricultural chemical inputs has caused a number of environmental problems, including pollution of water resources, reduced quality of agricultural crops, and reduced soil fertility (Geng et al., 2019). To reduce these risks, resources and inputs should be employed that, in addition to addressing the plant's immediate demands, also contribute to the long-term sustainability of agricultural systems. The organic matter in the soil is critical to its fertility. To maintain the soil fertility level and productivity, the organic matter content must be maintained at an appropriate level, and using the vermicompost is one of the ways to increase the soil organic matter content. Vermicompost is a rich source of high-consumption elements, trace elements, vitamins, enzymes, and plant growth-promoting hormones (Rahimi et al., 2019).
Vermicompost contains enzymes, such as protease, amylase, lipase, cellulase, and chitinase, and is also able to produce some organic acids, such as oxalic acid, phosphatase, and proteinase (Rekha et al., 2018). Since nutrients are released gradually from vermicompost, the loss of elements is very low in this process. Vermicompost provides a suitable substrate for root growth by improving physical soil conditions (Sajadi Nik et al., 2011). Numerous research have shown the beneficial effects of vermicompost on growth indices, essential oil content, and oil generated by these medicinal plants (Rekha et al., 2018).As the precursors of life, amino acids participate in constructing proteins and peptides and adjust all plant functions, including structural, enzymatic, metabolic and transfer (Asao et al., 2017). Amino acids enhance and correct the physiological parameters and biochemical compositions of plants. Previous research commonly report enhanced yield, increased essential oil content, and better quality

of essential oil generated by spraying amino acids on medicinal plants (Saburi. et al., 2014).The uptake of amino acids in plants increases the nitrogen content, which in turn leads to increased photosynthesis and improved growth and yield (Haj Seyed Hadi & Rezaee Ghale, 2016). Therefore, amino acids increase plant nitrogen storage, which in turn increases essential oil (Golzadeh *et al.,* 2011). They stimulate plant growth and induce systemic resistance in the plants (Thomas *et al*., 2009). The formation of amino acid chelates with micronutrients increases the ability of the plant to uptake nutrients while preventing the loss of nutrients from the roots (Popko et al., 2018).

Since the ongoing approach is to produce medicinal plants through organic cultivation to produce a higher quality product, using organic fertilizers and amino acids has become more important. Moreover, considering the importance of black cumin as a widely used plant in various pharmaceutical and food industries, this study aimed to investigate the effect of the application of different levels of vermicompost and amino acid on the quantitative and qualitative yield of black cumin.

2. Materials and Methods

2.1 Field experiment

The present research was carried out at 2019 in the farm of the Forests and Rangelands Research Institute - Alborz Station, Karaj, Iran, with the longitude of 51° and 19' east and a latitude of 35° and 41' north and an altitude of 1320 meters above sea level. Prior to the experiment, in order to determine the physical and chemical properties of the soil, soil sampling was performed using auger from a depth of 0-30 cm. Soil properties are shown in Table 1.

Table 1. Results of chemical and physical properties of soil for the experiment site

Texture	Clay (%)	Silt (%)	Sand (%)	phosphorus (ppm)	potassium (ppm)	Total nitrogen%	pH
Loam	16	40	44	14.4	178.4	0.08	6.73
T.N.V %	Organic carbon %	Electrical conductivity	Iron(ds / m)	Zinc(ppm)	Copper(ppm)	Manganese(ppm)	boron (ppm)
10.1	0.79	1.33	7.72	0.5	1.34	17.7	0.46

The current study was conducted as a two-factor randomized complete block design (RCBD) with three replications. The dimensions of each plot included 2×3 m, and the distance between the plots and replications was 1.5 and 2 m, respectively. The distance between and on the rows were 35 and 10 cm, respectively. Irrigation was performed using type 18 pipes.

The treatments consist of vermicompost (0, 3, 6, 9, 12, and 15 tons ha^{-1}), and foliar application of Tamarack (no foliar application, foliar application at the beginning of the flowering stage and foliar application at the beginning of the encapsulation stage).Black cumin and vermicompost were prepared from Medicinal Plants Research Center of Isfahan and Soil and Water Research Institute, Soil Biology Research Department, Karaj, Respectively. Amino acid was also prepared from Inagropars Company. Drip irrigation was used to execute the initial irrigation immediately after planting and following irrigations every seven days. Amino acid foliar application was applied at the beginning of flowering and encapsulation at sunset (1 liter/ha). During the growing season, weed control was performed manually three times.

2.2 measurements

The plant was harvested upon yellowing of leaves and capsules after removing the marginal effect (half a meter from both ends of each plot). The harvested samples were placed under the shade for 10 days to dry. After being dried, the seeds were threshed and separated from the straw manually. Plant height, total yield and seed yield, number of sub-stems, number of follicle per hectare, follicle diameter, 1000-seed weight, oil percentage, oil yield, percentage of essential oil, essential oil yield, percentage of essential oil compounds (GC) and identification of essential oil compounds (GC / MS) was measured. Statistical analysis was performed using MSTATC, and SAS software, and curves and graphs were drawn using Excel software. Mean comparison was performed using Duncan's multiple range test at P-value<0.5 %.

3. Results

The results of analysis of variance (ANOVA) showed that the amino acid × vermicompost interaction had a significant effect on flower number, fresh leaf yield, dry leaf yield, fresh stem yield, dry stem yield, fresh root yield, dry root yield, fresh biological yield, dry biological yield, oil content, essential oil yield, oil yield, (Table 2). According to the mean comparison, it was discovered that foliar spray at the start of flowering increased seed follicle by an average of 8 per plant, compared to 6.5 per plant during the encapsulation stage. Also, foliar application of amino acid in the encapsulation stage increased the essential oil percentage (0.43%) compared to the control (0.39%) and foliar application in the flowering stage (0.39%).

Table 2. Analysis of variance of the effect of foliar application of amino acid and vermicompost on black cumin traits.

S.O.V.	df	Plant height	Flowers number	leaf yield Fresh	leaf yield (Dry)	stem yield (Dry)	Follicle yield (dry)	Root yield (dry)
Replication	2	0.03 ^{ns}	17.35^{ns}	25214^{ns}	16306^{ns}	9550^{ns}	24447^{ns}	829^{ns}
Amino acid (A)	2	13.08 ^{ns}	281.02**	436021*	78923*	22489^{ns}	16807^{ns}	2394^{ns}
Vermicompost (V)	5	10.5 ^{ns}	14.67^{ns}	976341**	398075**	74178**	57584^{ns}	4233^{ns}
A×V	10	26.81 ^{ns}	278.25**	902214**	612429**	234308**	126373^{ns}	21225**
Error	34	20.56	17.88	128133	23107	11711	90242	4620
% cv	-	10.66	15.21	17.12	14.74	15.06	16.02	17.79

ns, * and ** indicate insignificant differences and significant differences among treatments at P-value<0.05 and P-value<0.01, respectively.

Continuation of Table 2. Analysis of variance of the effect of foliar application of amino acids and vermicompost on black cumin properties.

S.O.V.	Biological yield) dry)	Follicle per plant	Follicle seed average	Seedyield	Essential oil percentage	Essential oil yield
Replication	10106ns	38.01ns	1.52ns	30149ns	0.002ns	0.57ns
Amino acid (A)	33223ns	33.90ns	10.78××	11323ns	0.01××	1.93ns
Vermicompost (V)	575473××	23.40ns	2.76ns	50911ns	0.004×	3.88ns
A×V	1836877××	124.97ns	3.50ns	97244ns	0.001ns	8.09×
Error	139879	106.01	1.95	48198	0.001	3.40
% cv	9.33	12.91	19.15	16.06	9.82	15.05

ns, × and ×× indicate insignificant differences and significant differences among treatments at P-value<0.05 and P-value<0.01, respectively.

Table 3. Mean Comparison of vermicompost × amino acid foliar application on black cumin traits.

Amino Acid	Vermicompost (Ton ha^{-1})	Flowers number n p^{-1}	leaf yield (Dry) kg ha^{-1}	stem yield (Dry) kg ha^{-1}	Root yield (dry) kg ha^{-1}	Biological yield (dry) kg ha^{-1}	Essential oil yield kg ha^{-1}
Control	0	21.66ef	840.5cdef	619.48fghi	419.30abcd	3306.5f	3.64c
Control	3	42ab	853.3cdef	961.31bc	373.05abcd	4039cde	5.3abc
Control	6	44a	1628.1a	1107.85ab	411.10abcd	5124.7a	5.42abc
Control	9	24.33ef	687.2f	498.55hij	305.08d	3307.6f	4.8bc
Control	12	24.33ef	661.2f	410.94j	361.61bcd	3611.9def	5.81abc
Control	15	32.66cd	1232.3b	935.96bcd	499.88a	4743.6ab	7.41a
Flowering stage	0	33cd	846.9cdef	447.63hij	367.51abcd	3724.5def	4.73bc
Flowering stage	3	22.6ef	611.6f	590.57fghij	376.69abcd	3550.2def	5.79abc
Flowering stage	6	22.53ef	1644.7a	644.03efgh	471.46ab	4447.4abc	5.5abc
Flowering stage	9	39.53abc	1752.3a	1177.72a	463.49abc	5120.7a	5.3abc
Flowering stage	12	25.86de	985.9bcde	606.94fghij	297.06d	3674.8def	5.9abc
Flowering stage	15	25.93de	799.5cdef	822.24cde	289.85d	3716.7def	5.35abc
Encapsulation stage	0	24.6ef	1086.3bc	749.79def	449.19abc	4150.1bcd	5.99ab
Encapsulation stage	3	16.6f	721.5ef	431.11ij	303.39d	3360.4ef	6.07ab
Encapsulation stage	6	16.98f	669.5f	512.96ghij	303.37d	3195.2f	5.82abc
Encapsulation stage	9	25.4de	1016.2bcd	767.52cdef	334.99cd	4103.2bcd	5.63abc
Encapsulation stage	12	36.23bc	1781a	945.34bcd	466.75abc	5070.8a	4.92bc
Encapsulation stage	15	22ef	742.9def	704.71efg	381.24abcd	3866.2cdef	

Means followed by similar letters for each treatment in columns are not significantly different at the 5% probability level.

Table 4. Simple correlation among black cumin traits under the influence of different amino acid foliar application and vermicompost treatments

	Plant height	Root long	Lateral stem	Flowers number	leaf yield	stem yield	Follicle yield	Root yield	Biological yield	Essential oil percentage
	1	2	3	4	5	6	7	8	9	10
1	1									
2	0.36**	1								
3	0.43**	0.49**	1							
4	0.25*	0.47**	0.4**	1						
5	0.34*	0.09ns	0.33*	0.54**	1					
6	0.3*	0.18ns	0.36**	0.7**	0.63**	1				
7	-0.02ns	0.29*	-0.01ns	0.15ns	-0.1ns	-0.01ns	1			
8	0.47**	0.19ns	0.32*	0.39**	0.59**	0.49**	0.006ns	1		
9	0.35**	0.26*	0.35**	0.68**	0.84**	0.78**	0.35**	0.65**	1	
10	-0.09ns	0.19ns	-0.05ns	-0.3*	0.01ns	-0.07ns	0.08ns	-0.04ns	0.01ns	1

ns, * and ** indicate non-significant and the significant differences among treatments at P-value<0.05 and P-value<0.01, respectively

Mean comparison for vermicompost treatments showed that the essential oil and limonene percentage increase compared to the control.

The results showed that the highest dry leaves (1752 kg ha^{-1}) were obtained from 9 tons vermicompost per hectare × foliar application at the flowering stage (Table 5). The maximum dry stem yield per hectare was 1117 kg ha^{-1} that belonged to 9 tons of vermicompost × foliar application at flowering stage.

Mean comparison for dry root yield showed that 15 tons of vermicompost × no foliar application (499 kg ha^{-1}) led to the highest root yield.

The findings of a simple connection between agronomic variables and black cumin production revealed a substantial positive relationship between seed yield and root length, fresh and dry follicle yield, and fresh and dry biological yield (Table 6). The amount of essential oil and the number of flowers had a strong negative correlation. There was no significant correlation between the essential oil percentages with any of the measured traits. Thousand-seed weight showed a significant positive correlation with fresh and dry Follicle yield, fresh and dry biological yield, essential oil percentage and seed yield (Table 7).

4. Discussion

The results of ANOVA revealed that vermicompost and amino acid had little influence on growing plant height, which might be related to the plant's short growth period. Amino acids independently and combined to organic fertilizer had no effect on increasing plant height, which could be in terms of the insufficient uptake by the plant.

In addition to increasing the bioavailability of soil minerals via the biostabilization of nitrogen, solubilization of phosphorus and potassium, and inhibition of pathogens by producing growth-regulating hormone, fertilizers affect crop yields. The application of biofertilizers was reported as the most important strategy in integrated plant nutrition management for a sustainable agricultural system with sufficient input (Geng et al., 2019). The insignificant effect should be further investigated depending on the conditions, place and time of the experiment. Ghanepasand and Haj Seyed Hadi (2016) investigated the effect of biofertilizers on black cumin and reported similar results to the present study.

The flower numbers data indicated that there was a statistical difference between amino acids and the interaction amino acid × vermicompost. In general, biostimulants are substances that stimulate metabolism and metabolic processes to increase plant efficiency. The formulation of biological and growth stimulants in new inputs of amino acids or amino acids in combination with nutrients, hydrolyzed proteins, humic acid, algae and medicinal plant extracts and other metabolites. As life's antecedents, amino acids aid in the formation of proteins and peptides, which are responsible for all of the plant's structural and enzymatic activities. They have often been reported as pioneers to combine many types of small molecules with a very important biological role (Faten et al., 2010).

Given its effect on soil factors such as microorganisms, element absorption, and the production of growth stimulating hormones and biologically active factors in the soil, vermicompost in black cumin appears to increase vegetative growth and thus the number of lateral branches and capsules. The results of yield and yield components showed that leaf yield, stem yield, root yield, and biological yield were significantly changed under the influence of treatments used. These changes can be in terms of the role of fertilizer nutrients on the plant and their role in the soil and the ability to absorb the elements as well as the effect of foliar application on the plant. Improving soil structure (Sajadi Nik et al., 2011) and improving physicochemical conditions of the soil (Anwar et al., 2005) are among effects of vermicompost fertilizers.

Khorramdel et al. (2010) studied the impact of biofertilizers on black cumin production and yield components. They reported a significant difference in the number of follicles per plant, similar to the present study. In the study of the effect of nitrogen on black cumin, some studies have shown that vermicompost can improve plant growth by increasing water storage capacity, nutrient supply and production of plant hormones (Rahimi et al., 2019). Vermicompost has a high potential for absorption and storage of water and nutrients, and as a result, high porosity, proper ventilation and drainage and its use in sustainable agriculture, in addition to help increase the population and activity of beneficial soil microorganisms provides nutrient requirements, such as soluble nitrogen, phosphorus and potassium and improves plant growth and yield (Arancon et al., 2005). The fact that auxins enhance cell division and gibberellin and its derivatives increase cell longitudinal growth supports this notion. When vermicompost organic fertilizers were utilized, the biological yield of black cumin increased due to an increase in certain features as well as an increase in plant development rate. Contrary to previous research, amino acids had a significant effect on biological yield, which could be due to insufficient soil elements for black cumin, as a result of which the used amino acid was significantly consumed.

The quantity of organic matter in the soil must be kept at an optimal level to sustain soil fertility and production. Organic fertilizers, such as vermicompost, are one way to boost soil organic matter. Vermicompost is a rich source of high-consumption elements, trace elements, vitamins, enzymes and plant growth-promoting hormones (Rahimi et al., 2019). Considering its high porosity, proper ventilation and drainage, vermicompost has a high power of absorption and storage of water and nutrients and its use in addition to increasing the population and activity of soil microorganisms such as mycorrhizal fungi and phosphate solubilizing microbes (PSMs), provides plant nutrients such as soluble nitrogen, phosphorus and potassium and improves plant growth and yield (Rezvani Moghadam et al., 2013). Vermicompost contains enzymes such as protease, amylase, lipase, cellulase and chitinase, which play an effective role in the breakdown of soil organic matter and thus the availability of nutrients required by plants. Vermicompost also includes bacteria that may produce oxalic acid, phosphatase acids, and proteinases, among other organic acids (Rekha et al., 2018). These microbes create acid, which dissolves nutrients like N, K, and P in the soil (Candellas et al., 2002). The element loss in this procedure is decreased since the nutrients from vermicompost are released gradually. Vermicompost provides a suitable substrate for root growth by improving physical soil conditions (Sajadi Nik et al., 2011). The positive role of vermicompost in improving growth indices, the essential oil percentage, essential oil compounds, and the amount of oil produced by medicinal plants has been reported in many studies. Providing plant nutrients and improving physical soil and biological conditions is the main reason for this improvement (Rekha et al., 2018).

Khorramdel et al. (2010) stated that biofertilizers had a significant effect on black cumin seed yield, which were consistent with the present study. The enhanced seed yield in black cumin seems to be due to additional nutrients being provided to the plants, which has boosted the production of photosynthetic material. It was observed that, among the three traits of the number of follicle per plant, average seed per follicle, and mean follicle diameter, only amino acid had a significant effect on the average number of seeds per follicle. Amino acids play an important role to reduce the negative effects of environmental stresses such as salinity, drought and pests on plants and improving their growth quantitatively and qualitatively under these conditions. Proline, tryptophan, and beta-aminobutyric acid, for example, minimize the damage caused by environmental stress in plants by strengthening cell walls (Yan et al., 2015), stimulating plant growth, and inducing systemic resistance in plants (Wu et al., 2011; Yan et al., 2015). Formation of amino acid chelates with micronutrients while preventing the loss of nutrients from the roots increases its uptake capacity for the plant (Popko et al., 2018).

Ayatollahi et al. (2012) showed that inoculation of biofertilizers with black cumin seed and interaction of treatments caused a significant difference in 1000-seed weight, but similar to the present research, foliar application of amino acids had no significant effect on 1000-seed weight of black cumin.

Comparing the mean interaction of foliar application of amino acid × vermicompost showed that the highest essential oil yield belonged to vermicompost × no foliar application treatments. It was observed that the essential oil percentage increased when amino acids were used in the seedling stage.

The negative correlation between root length and flower number demonstrates that as long as vegetative growth conditions and non-stress conditions are provided, the plant will tend to show vegetative growth and the reproductive organs will be reduced, resulting in the plant with longer root length absorbing more water and nutrients and fewer flowers. Therefore, it seems that paying attention to flowering treatments and the application of environmental stresses in different stages of the black cumin, which is an annual plant with a short growth period, can help produce more seeds. According to the correlation results, seed yield, which is one of the most important traits of black cumin, had a significant positive correlation with plant leaf yield, which indicates that the higher the leaf area of the plant, the higher the seed yield, probably due to more photosynthesis. The positive correlation between seed yield and biological yield indicates that plants with more sub-stems can have higher seed yield due to higher photosynthesis. The positive correlation between seed yield and number of flowers indicates that the number of seeds plays a significant role in the seed yield. Moreover, the positive relationship between seed yield and 1000-seed weight produced, indicates the plant's sufficient ability to establish the relationship between source and sink and high photosynthetic power in the seed-fill period. The positive correlation between 1000-seed weight and biological yield indicates that as the number of leaves and sub-stems increases, the rate of photosynthesis increases as well. Additionally, in plants with more sub-stems and leaves, leaf surface durability is increased, resulting in an increase in 1000-seed weight and follicle diameter.

5. Conclusion

The results showed that the application vermicompost alone or combined to amino acids, improved the yield of fresh and dry parts, such as leaves, stems, roots, seeds and biological yield. The essential oil percentage and yield improved in some treatments and was significantly different from the control. In general, using 6 ton vermicompost per hectare and foliar application of amino acids in different stages, especially in the final growth stages showed an acceptable result.

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