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Original Article

Response of Seed Yield and Essential oil of Black Cumin (*Nigella sativa* L.) Affected as Foliar Spraying of Nano-fertilizers

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Abstract

In order to investigate the effect of micronutrients foliar application on seed yield and essential oil of black cumin, a field experiment was conducted based on Randomized Complete Block Design (RCBD) with three replications at the farm located in Naqadeh city, West Azerbaijan province, Iran, during 2015-2016 growing season. Treatments were separate application of Nano-Iron, Nano-Zinc and Nano-Manganese and double and triple combined treatment of them (Fe+Mn, Fe+Zn, Zn+Mn, Fe+Zn+Mn) and control. Treatments were applied at 2 g/L twice at stem elongation and early flowering stages. Different traits such as plant height, number of follicle per plant, number seed per follicle, 1000- seed weight, biological yield, seed yield, essential oil percentage and yield were measured. The results showed that foliar application of micronutrients had a significant effect on all studied traits and improved yield components and essential oil yield as compared to control. Yield components, seed yield, essential oil percentage and essential oil yield were enhanced by foliar application compared with control. Therefore, combined usage of micronutrients (Nano-Fe+ Nano-Zn+ Nano-Mn) showed the greatest increase in studied traits than their individual usage. The highest seed yield (706.67 $kg.ha^{-1}$) and essential oil yield (8.66%) were obtained from Fe+Zn+Mn treatment. In general, the results of the present study revealed that the foliar spraying of Nano-micronutrients plays a remarkable role in improving the quantitative and qualitative yield of black cumin as a medicinal plant.

Keywords: Black cumin, Components Yield, Medicinal plant, Quantitative and qualitative yield

Introduction

Black cumin (*Nigella sativa* L.) is an annual herbaceous medicinal plant and belonging to the Ranunculaceae family. The origin of this plant is the Mediterranean region, Iran, Pakistan, and India. It is one of the most important medicinal plants cultivated for the essential oils and many other compounds derived from its seeds. The content of essential oil content of seed is ranging between 1 to 1.5%. The main components of Black cumin essential oil are p-cymene (59.5-60.3%), α-thujene (6.9-7.2%), β-pinene (2.4-2.6%), γ-terpinene (3.5-3.8%), terpinene-4-ol (2.1-2.5%), thymoquinone (3.0-3.3%) and carvacrol (2.4-2.7%) [1]. Black cumin seeds due to having hot or spicy characteristics have been using for centuries in culinary arts of different cultures and its antibacterial, antifungal, antioxidant, anti-inflammatory, gastroprotective, anti-diabetic,

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anticancer, hepatoprotective, antihypertensive, immune-enhancing effects are well-known [2].

Because of various reasons such as limy soil, bicarbonate water, drought, and low level of organic substances in arable soil, about 30% of agricultural lands across the world suffer from the severe shortage of micronutrients including iron and zinc [3]. Although the micronutrients are used in a slight amount, they have important consequences. Moreover, deficiencies of micronutrients serve as the limiting factors in the uptake of other nutritional elements, so it requires more attention to their application [4].

Development of agriculture can be achieved exclusively through increasing productivity by effective use of modern technologies. Among these, nanotechnology has the potential to revolutionize the agricultural systems, biomedicine, environmental engineering, safety and security, water resources, energy conversion, and numerous other areas [5]. Nanotechnology is progressively moved away from the experimental into the practical areas. The development of slow/controlled release fertilizers, conditional release of pesticides and herbicides, on the basis of nanotechnology has become critically important for promoting the development of environment-friendly and sustainable agriculture [6].

Iron (Fe) plays important roles in several biochemical and physiological processes in plants [7]. This micronutrient is a key cofactor of certain enzymes involved in plant hormone synthesis and is engaged in many electron transportation reactions [8]. Zinc has a key role in hormone biosynthesis, structural stability of organelles, cytochrome C synthesis, activation and proper function of a number of enzymes, protein synthesis, and stability and integrity of the root cell plasma membrane. In particular, the structure of the thylakoid membranes has been reported to be affected by Zn, with a consequent reduction of the electron transport rates. The inactivation of chlorophyll *a* due to the Mg^{2+} replacement with Zn^{2+}, as well as the substitution of Mn^{2+} with Zn^{2+}at the site of water photolysis, has been also reported [9]. At low concentrations, Zn is required for numerous physiological processes, while it is strongly toxic at high concentrations [10]. Attia [11] reported that foliar application of micronutrients (Fe and Zn) in peanut increased yield and nutrients uptake in comparison to the control treatment also the combination of iron and zinc were more effective than the separate use of them. Foliar application of iron, zinc and iron + zinc and time of their application had significant effects on flower yield, essential oil percentage and essential oil yield of Chamomile (*Matricaria chamomilla* L.) [12].

Manganese (Mn), in turn, is regarded as an activator of many different enzymatic reactions in carbohydrate synthesis and takes part in photosynthesis [13]. Manganese activates decarboxylase and dehydrogenase and is a constituent of complex PSII protein, SOD, and phosphatase. Deficiency of Mn induces inhibition of growth, chlorosis and necrosis, early leaf fall and low fertilization [14]. The results of Bakry *et al* [13] demonstrated that foliar application with Zn +Mn+Fe had a positive effect on seed, oil, and fiber yields of flax plants.

The large-scale use of chemical fertilizers to increase crop productivity is not a suitable option for the long run as these are double-edged swords, which on one end increase the crop production but on the other end disturb the soil mineral balance and decrease soil fertility. Advancement in technology has improved ways for large-scale production of nanoparticles of physiologically important metals, which are now used as “smart delivery systems” in order to improve fertilizer formulation by minimizing nutrient loss and increasing their uptake by plant cells. These “nano-fertilizers” have a high surface area, high sorption capacity, and controlled-release kinetics to targeted sites attributing them as a smart delivery system. However, being an infant technology, the ethical and safety issues surrounding the use of nanoparticles in plant productivity are limitless and must be carefully evaluated before adopting the use of nano-fertilizers. Influence of microelements especially iron, Manganese and zinc on the yield and essential oil of black cumin plants is not well documented. Therefore, the purpose of this research is to investigate the effect of foliar application of iron, Manganese, and zinc on seed yield and essential oil of black cumin (*Nigella sativa* L.).

Material and Methods

This experiment was conducted based on a randomized complete block design with three replications at a research farm located in Naqadeh city (latitude 37.53 ^{0}N, 45.08 ^{0}E, and 1320m above sea level), West Azerbaijan province, Iran, in 2016.

Treatments included Nano iron (50 kg/ha), Nano zinc (40 kg/ha) and Nano manganese (40 kg/ha) and mixed solutions of these elements (Fe+Zn, Fe+Mn, Zn+Mn, Fe+Zn+Mn) and non-application of micronutrients (control). Treatments were applied at 2 g/l twice at stem elongation and early flowering stages. To eliminate the effects of leaf application in control plants, after each spraying, the field was irrigated. Nano-Iron, Nano-Zinc, and Nano-Manganese Chelate obtained from Khazra Company. Soil physicochemical properties are given in table 1.

Seedbed preparation included plowing, disk harrowing and cultivation. The experimental units in each block included 8 rows with 30 cm inter-row and 8 cm intra-row spacing of 2.5 meters long.

For treatments, a basal application of nitrogen and phosphorous was carried out at sowing time, using urea and P_2O_5 fertilizers at the rate of 40 kg ha^{-1} and 100 kg ha^{-1}, respectively. Another 40 kg ha^{-1} urea was also added to the soil when black cumin plants were at stem elongation stage. The remaining urea 40 kg ha^{-1} was applied to the soil when the plants were at flowering stages. Soil moisture was kept at an adequate level to prevent water deficiency stress for good crop growth during the experimental period. The plots were manually weeded to keep them weed free throughout the experiment.

At maturity stage, when the plants turn yellowish in color, yield attributes such as plant height, number of follicles per plant, number seeds per follicle and 1000-seed weight were measured on ten randomly selected plants in each plot. For measuring seed and biological yield we harvested 2-meter squares of experimental plots. Then harvested samples dried at room temperature for 10 days. Thus, biological yield per unit area in plots were determined. Harvest index, the ratio of seed yield to biological yield, was calculated, too.

The seeds of black cumin were shade dried at room temperature, crushed by using a grinder. The essential oil was isolated from 10 g of the fruits with 100ml distilled water by conventional hydro-distillation for 4 h using a Clevenger-type apparatus for determining the oil content [15]. Essential oil content and yield were calculated by the following equation [16]:

Essential oil content (%) = Extracted essential oil (g) / 10 g of peppermint ground sample × 100 (1)

Essential oil yield (gm^2) = biomass yield (gm^2) × Essential oil content (%)
(2)

Statistical Analysis

Analysis of variance (ANOVA) on data was performed using the general linear model (GLM) procedure in the SPSS 16 software. The Duncan's Multiple Range Test was applied to compare treatment means at 5 % probability level.

Results

Analysis of variance showed that foliar application of micronutrients (Fe, Zn, and Mn) had a significant effect on the 1000-seed weight (P≤0.05) and plant height, number of follicle per plant, number seed per follicle, biological and seed yield, essential oil percentage and yield (P≤0.01) (Table 2). The highest plant height of black cumin was obtained from the combined application of micronutrient (Fe+Zn+Mn), while the minimum plant height was observed in control treatment (Table 3).

Also, the highest number of follicle per plant (24 follicles) and number of seed per follicle (39 seeds) were observed with combined application of Fe+Zn+Mn and the lowest numbers of them (14.67 follicles per plant and 29.40 seeds per follicle, respectively) were obtained from control treatment (Table 3).

In addition, results showed that the maximum and the minimum 1000-seed weight (1.92 g and 1.53 g) belonged to the combined application (Fe+Zn+Mn) and control treatment, respectively (Table 3). The seed yield had an increasing trend with the application of nano-fertilizer, so that combined application caused higher amounts of seed yield. Therefore, the highest biological and seed yields (1942.67 and 706.67 kg/ha) were obtained from the application of combined treatment Fe+Zn+Mn and the lowest ones (1443.33 and 515 kg/ha) (were recorded at control treatment, respectively (Table 3).

Table 1 Soil physicochemical properties at 0-30 cm of experimental site.

Soil texture	K (mg/kg)	P (mg/kg)	Fe (mg/kg)	Zn (mg/kg)	Mn (mg/kg)	Organic matter (%)	Total nitrogen (%)	EC×10^3 (dS. m^{-1})	pH
Silty clay	402	14.5	17	1.50	14	1.21	0.99	0.85	7.85

Table 2 Analysis of variance of micronutrients (Fe, Zn, and Mn) effects on seed yield and essential oil of black cumin

S.O.V		Means of Squares (MS)							
		Plant height	Number follicle per plant	Number seed per follicle	1000-seed weight	Biological yield	Seed yield	Essential oil percentage	Essential oil yield
Replication	2	78.79**	1.07n.s	3.99n.s	0.041*	89802.87 **	5676.04**	0.023*	1.953**
Treatment	7	129.40**	34**	13**	0.043 *	88175.13 **	9316.57**	0.033**	4.40**
Error	14	7.60	2.63	2.57	0.010	5064.78	1345.23	0.006	0.26
CV (%)		6.65	7.64	4.54	5.84	4.24	6.12	7.10	7.88

* and ** significant at $P \le 0.05$, $P \le 0.01$, respectively; df, degree of freedom; CV., Coefficient of Variation.

Table 3 Mean comparisons of foliar application of micronutrients (Fe, Zn, and Mn) on seed yield and essential oil of black cumin.

Treatment	Plant height (cm)	Number of follicle per plant	Number of seed per follicle	1000-seed weight (g)	Biological yield (kg/ha)	Seed yield (kg/ha)	Essential oil percentage (%)	Essential oil yield (kg/ha)
Control	32.67 d	14.67 c	29.40 c	1.53 c	1443.33 c	515 c	0.95 c	4.93 f
Fe	36 cd	19.67 b	36.67 ab	1.76 ab	1709.33 b	602.67 b	1.10 abc	6.60 cd
Mn	36.67 bcd	15.33 c	30 c	1.58 bc	1490 c	560 bc	0.99 c	5.54 ef
Zn	47.67 a	19 b	36.10 ab	1.69 bc	1627 b	576.66 bc	1.01 bc	5.84 def
Fe+Mn	38.67 bc	21.33 ab	37 ab	1.78 ab	1632 b	616.67 b	1.13 ab	7 bc
Fe+Zn	47 a	23.33 a	38 ab	1.77 ab	1876.67 a	625 b	1.24 a	7.74 b
Zn+Mn	41.33 b	20 b	35.23 b	1.70 bc	1680 b	590.67 b	1.07 bc	6.35 cde
Fe+Zn+Mn	51 a	24 a	39 a	1.92 a	1942.67 a	706.67 a	1.22 a	8.66 a

Foliar application of Fe+Zn led to 23.38% higher essential oil percentage as compared to control. So that, the highest (1.24%) of essential oil percentage obtained from Fe+Zn treatment and the lowest (0.95%) essential oil percentage related to control (Fig. 1). Furthermore, foliar feeding of combined treatment (Fe+Zn+Mn) produced significantly higher essential oil yield as compared to the other treatments and control. Therefore, the combined application increased the essential oil yield by 43.07% compared to the control (Fig. 2).

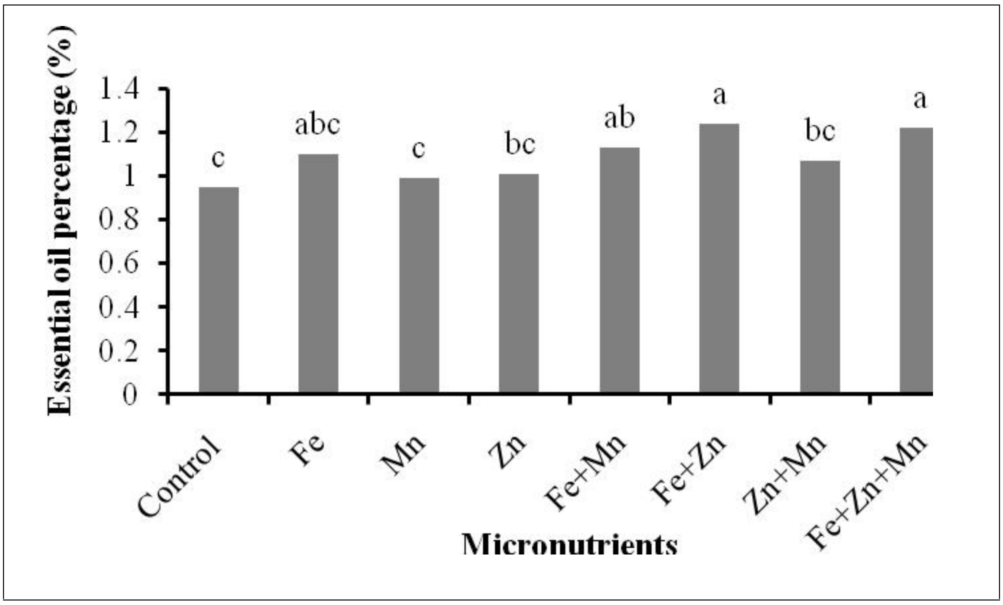

Fig. 1 Mean essential oil percentage of *Nigella sativa* L. under micronutrients application. The dissimilar letters show significant differences at $P \leq 0.05$.

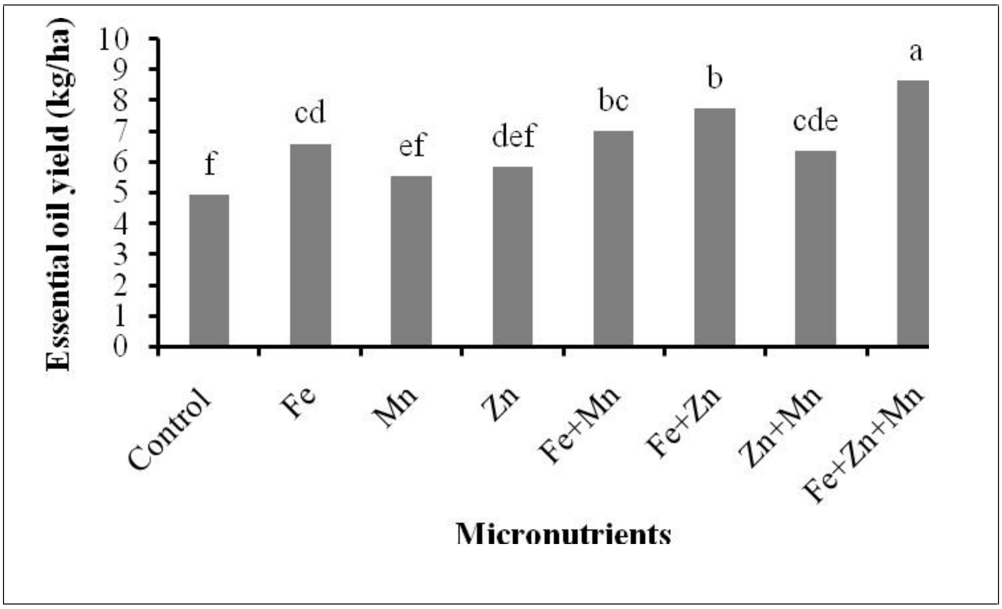

Fig. 2 Essential oil yield of *Nigella sativa* L. under micronutrients foliar application. The dissimilar letters show significant differences at $P \leq 0.05$.

Discussion

Plant height in combined treatments containing zinc was significantly higher compared to non-zinc treatments. The supply of iron compounds increased the number of follicles per plant, number of seeds per follicle, 1000-grain weight, and grain yield and reduced the time for flowering and the passage of the phonological stages of the plant [3]. Keshavarz *et al* [17] stated that the nano-iron chelate fertilizer increased the number of spikes in wheat. Application of nano-fertilizer had significant effect on the number of seeds per follicle in black cumin plants [18]. Nazaran *et al* [19] concluded that number of seeds per spike of wheat was increased due to nano-iron chelated to organic fertilizer. It can be concluded that an increase in the number of seeds per follicle results from the improvement of plant growth conditions and the increase of nutrients as a result of the fertilizers application.

Zn plays an important role in increasing the amount of plant growth regulators, contributing to the metabolism of different substances, participating in the division of meristem cells, and the production of carbohydrates, proteins and their translocation [20]. In the presence of iron, the amount of photosynthetic pigmentation, and photosynthesis increase per unit leaf area. Finally, the production of starches and sugars in the leaves and their storage in the seed, increases the grain weight and yield [20]. Therefore, plant nutrition with zinc and iron increases pollen carbohydrates storage, prolong its longevity and thereby increasing pollination and ultimately increasing the number and weight of seeds. Increasing yields due to the use of nutrients may be associated with increased photosynthetic activity which leads to an increase in the production and accumulation of carbohydrates and the beneficial effect on the growth and maintenance of flowers and seeds. Heidarian *et al* [21] observed that application of Zn+Fe treatment increased the grain yield and 1000-grain weight of soybean (*Glycine max* L.). In *Carthamus tinctorius*, the application of S+Fe+Zn foliar spray significantly increased number of capsules per plant, seed weight per head, 1000-seed weight, and seed yield [22]. Razazi et al [23] reported that nano-iron fertilizer increased stigma yield of saffron. Research on flax showed that foliar spray of all iron, zinc, and manganese increased seed yield more than separate use of them [20]. Naga Sivaiah *et al* [24] indicated that the effect of boron, zinc, molybdenum, copper, iron, manganese, and a mixed treatment of them was significant on 1000-seed weight, seed yield per plant and seed yield per hectare in two tomato cultivars and Mn usage increased seed yield in Utkal Raja cultivar. Ghasemian *et al* [14] reported that Mn application had significant effect on numbers and weight of pods per plant and biological yield of soybean. Naeini *et al* [25] informed that the application of Zn in *Olea europaea* L. increased leaf and root dry mass. Increased biological yield because of nano-fertilizers can be attributed to stimulated vegetative growth, increased straw yield and increased seed yield. The results of the studies conducted by the researchers showed that increases in yield with applying organic fertilizers is resulted froman increase in plant available nitrogen, phosphorus and potassium contents and increase in photosynthesis and growth of the plant [26]. The positive influence of micronutrients application on crop growth may be due to the improved ability of the crop to absorb nutrients, photosynthesis and better sink-source relationship as these play vital role in various biochemical processes [27].

The boost to essential oil yield provided by combined application of Fe+Zn was previously reported by Nasiri *et al* [12] in *Matricaria chamomilla* at field conditions and Said-Al Ahl and Mahmoud [28] in *Coriandrum sativum*. This rise seems to be due to increased flower yield and essential oil percentage as a result of the promotive effects of Fe and Zn. Since the essential oil yield is directly associated with the flower yield and essential oil percentage, any increase in these two traits led to the increase of essential oil yield. It has been observed that application of low Zn concentration alone or in combination with Cu had a positive impact on essential oil yield of Sweet Basil (*Ocimum basilicum* L.). In contrast, high levels of Cu and Zn application to the soil has not significantly affected these parameters [29]. Essential oil of *Mentha piperita* increased by foliar application of 3 ppm zinc chloride compared with the control [30]. Essential oil biosynthesis in basil (*Ocimum sanctum* L.) is strongly influenced by Fe and Zn [31].

Conclusion

This study indicated that the effect of using triple combination of micronutrients (such as Fe+Zn+Mn) on all measured traits was higher than separate application of Fe, Zn, and Mn and double combinations of micronutrient (including Fe+Mn, Fe+Zn, and Zn+Mn). Between the treatments, manganese had minimal impact on measured traits. So that, the manganese effect on the number of follicle per plant, the number of seed per follicle, biological yield, and essential oil percentage was not statistically significant in comparison to control plants. In general, the present study showed that foliar application of nano-fertilizer (such as Fe, Zn, and Mn) can considerably improve the yield and yield components of seed and essential oil of *Nigella sativa* L., particularly if these micronutrients were applied all together. Therefore, this combination could be an interesting option for bio-fertilization. These findings indicated that foliar spraying of some elements, can be an effective way to reduce the effects of nutrient deficiency on the qualitative and quantitative yield of black cumin as a valuable medicinal plant.

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