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# Determining Optimum Efficiency of using Foliar Spraying with Different Concentrations of Nano Nickel and Copper on Growth Traits, Yield Components and Quality of some Black Cumin (*Nigella sativa* L.) Genotypes Regarding Semi-Arid Regions

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**KEY WORDS:**

Black cumin; Nickel; Copper

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ABSTRACT

A factorial experiment was conducted in two successive seasons (2021-2022 and 2022-2023), according to a randomized complete block design with three replications in fields of Gayyarah, Nineveh Governorate, to study three levels of foliar spraying with nickel and copper at elongation stage in growth and yield of three black cumin genotypes in clay loam soil. Treatments included three concentrations of nickel and copper (0, 3, 6 mg.l$^{-1}$) and three genotypes of black cumin plants (Silingo, Cameli and Kena). Results showed that foliar spraying of nickel and copper at elongation stage with a concentration of (6 mg.L$^{-1}$) for each of nickel and copper achieved a significant increase in each traits of plant height, number of flowering branches per plant, number of capsules per plant$^{-1}$, number of seed per capsulas-1, weight of thousand seeds, seed yield per plant, total seed yield, biological yield, percentage for each of harvest index, fixed oil, protein, oleic acid, linoleic acid, palmitic acid, arachidic acid, stearic acid, eicosadienoic acid and homolinoleic acid compared with comparison treatment in both growing seasons. black cumin genotypes were significantly affected in all studied traits, as Cameli genotype was superior than genotypes of Silingo and Kena by giving highest value for all the studied traits in both growing seasons. It is known that all plants obtain nutrients from soil and the number of these nutrients is 13, which are dissolved in water and absorbed by roots from soil solution. However, these nutrients are not always available in the amounts that plants need and for this reason, foliar spraying process becomes necessary for plant growth and their availability in concentrations less than plant's need leads to poor growth and development of plants. As foliar spray works on distributing nutrients to vegetative systems in a homogeneous manner and this method is also characterized by being economical and reduces need to use large quantities of nutrients as well as speed of response to absorption of nutrients from vegetative parts.

# تحديد الكفاءة المثلى لاستخدام الرش الورقي بتراكيز مختلفة من النيكل والنحاس النانوي في صفات النمو ومكونات الحاصل والنوعية لبعض التراكيب الوراثية من حبة البركة (*Nigella sativa* L.) في المناطق شبه الجافة

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الخلاصة
في الموسمين المتتاليين من العامين (٢٠٢١-٢٠٢٢ و ٢٠٢٢-٢٠٢٣) ، أجريت تجربة عاملية وفق تصميم القطاعات العشوائية الكاملة بثلاثة مكررات في حقول القيارة بمحافظة نينوى، لدراسة ثلاثة مستويات من الرش الورقي بالنيكل والنحاس في مرحلة الاستطالة في نمو وحاصل ثلاثة تراكيب وراثية من حبة البركة في تربة مزيجية طينية. اشتملت المعاملات على ثلاثة تراكيز من النيكل والنحاس (٠ ، ٣ ، ٦ ملغم.لتر-١) وثلاثة تراكيب وراثية من نباتات حبة البركة (سيلينكو، كاميلي وكينا). أظهرت النتائج أن الرش الورقي للنيكل والنحاس في مرحلة الاستطالة بتركيز (٦ ملليغرام. لتر-١) لكل من النيكل والنحاس حقق زيادة معنوية في كل من صفات: طول النبات ، عدد الفروع المزهرة. نبات-١، عدد الكبسولات.نبات-١، عدد البذور.كبسولة-١، وزن ألف بذرة ، حاصل بذور النبات الفردي، حاصل البذور الكلي، الحاصل البيولوجي، النسبة المئوية لكل من دليل الحصاد، الزيت الثابت، البروتين، حامض الأوليك، حامض اللينوليك، حامض البالمتيك، حامض الاراشيدك، حامض الستيارك، حامض إيكوسادينويك وحامض الهومولينوليك مقارنة بمعاملة المقارنة في موسمي النمو. تأثرت التراكيب الوراثية لنبات حبة البركة معنويا في جميع الصفات المدروسة، حيث تفوق التركيب الوراثي كاميلي على التركيبين الوراثيين سيلينكو وكينا بإعطائه أعلى قيمة لجميع الصفات المدروسة في موسمي النمو. من المعروف أن جميع النباتات تحصل على المغذيات من التربة اذ يبلغ عدد هذه العناصر الغذائية ١٣ مادة مغذية تذوب في الماء وتمتصها الجذور من محلول التربة. ومع ذلك ، فإن هذه العناصر الغذائية لا تتوفر دائمًا بالكمية التي تحتاجها النباتات، ولهذا السبب، تصبح عملية الرش الورقي ضرورية لنمو النبات، كما أن توفرها بتراكيز أقل من حاجة النبات يؤدي إلى ضعف نمو النباتات وتطورها. حيث يعمل الرش الورقي على توزيع المغذيات على النظم النباتية بطريقة متجانسة وتتميز هذه الطريقة أيضًا بكونها اقتصادية وتقلل من الحاجة إلى استخدام كميات كبيرة من العناصر الغذائية وكذلك سرعة الاستجابة لامتصاص العناصر الغذائية من الأجزاء النباتية.
الكلمات المفتاحية: حبة البركة، عنصري النيكل، النحاس.

## INTRODUCTION

Black cumin plant (*Nigella sativa* L.), which belongs to Rananculaceae family, is considered one of most important herbaceous winter medicinal plants widely cultivated all over the world for production of seeds and oil and in Iraq it is cultivated on a scale of research and scientific experiments. It is believed to be native plant of southwest Asia and then cultivated in southern Europe, Syria, Egypt, Saudi Arabia, Iran, Pakistan, India, Turkey and Mediterranean region (Shewaye, 2011; Malhotra, 2012 and Ermias et al., 2015).

Black cumin is an upright plant, up to 40 cm high. It has an erect, branched stem and alternately positioned, minute, deep-cut leaves. It is simple, feathery, and deeply lobed. Its flowers are bluish-grey star shaped with white colored cups tinged with green. They are linked at base and lobed at top. They contain 5 petals across. About 2.5 cm, white with blue veins. Mixed pollination is carried out by insects. Small, complete black seeds, bare surface, oily white inside, pyramidal shape, distinct aromatic smell and special taste (Weiss, 2002; Ashraf et al., 2005; Teshome and Anshiso, 2019 and Dessie et al., 2020).

Black cumin is an important medicinal plant that is widely used in many pharmaceutical and food industries. Its seeds and oil are used in treatment of coughs and of chest and respiratory crises. It is also used as a diuretic to treat bladder and kidney diseases (Alemu et al., 2023). oil is used externally on surface of skin to treat of some skin diseases. Seeds of black cumin have a killing effect on bacteria and repellents for intestinal worms and tapeworms. Also increase efficiency of immune system to resist viral diseases and reduce blood sugar. Seeds of black cumin are used alone or mixed with bee honey or garlic to treat nervous tension, it is also used in food industry. It is added as a spice to some baked goods, to improve taste and aroma of cheese and as a natural preservative (Nergiz and Otles, 1993; Matthaus and Ozcan, 2011 and Ali et al., 2015).

Black cumin seeds were used in food industry in preserving canned foods, as well as giving foods a distinctive flavor, in addition to medical benefits such as treating types of paralysis, especially facial and in treating back pain, rheumatism, diabetes, asthma, and malaria. The plant is also used to raise the body's immunity and nigella extracts and oil were used in resistance of various plant diseases in inhibition of fungal growth. Black cumin seeds contain (35%) of fixed oil and approximately (1.4%) of volatile oil (Ramadan, 2007). Black cumin oil contains many unsaturated fatty acids and some saturated fatty acids in addition to a small percentage of steroids (Ustun et al., 1990 and Hendawy et al., 2012; Moradzadeh et al., 2021 and Shiberu et al., 2023).

Black cumin also contains Nigellone, which is a natural antioxidant, as well as (glutathione). Black cumin seeds contain arginine acid. Black cumin oil contains oleic acid 20-24%, linoleic acid 44-56%, linolenic acid 0,6-8,1%, arachidic acid 9,2, eicrazadienoic acid 2,5, palmitolenic acid 3%, palmitic acid 12-14%, stearic acid 2,5-2.3%, myristic acid 0.16% and sterols 0.5% (Sultan et al., 2009 and Ozel et al., 2009). Black cumin oil also contains sterols, most important of which are cholesterol, campestral, stigma sterol, beta-mesosterol and alpha-spina sterol. It also contains vitamins such as vitamin E, B, as well as soapy substances, most important of which is melanin. Black cumin also contains carotene, which has been proven to have effective against cancer cells. In addition to hormones and digestive enzymes and anti-acidity (Sener et al., 1985; Nergiz and Otles, 1993; Khalid et al., 2016 and Kabir et al., 2019). Plant growth depends on many factors such as soil fertility, soil productivity, and environmental factors (light, temperature, humidity and precipitation). Micronutrients play an important role in growth, yield and quality of black cumin plants (Nimet et al., 2015). Nickel and copper are among the most important micronutrients responsible for controlling crop growth and yield. In order to develop cultivation of black cumin in Iraq, it is necessary to determine processes of serving plant and soil, including determining quantities of fertilizers added and time of addition. Plant response to fertilizers varies according to types and quantities of added fertilizers, soil type, plant type, plant density, planting date and fertilization method and time of fertilization.

Nickel is found naturally in all plants and plays an important role in activities of urease enzymes in plants. It is a seventeenth plant nutrient that was recently discovered (Brown 1987). Nickel element is necessary for absorption and transfer of nutrients, especially iron and zinc and a strong competitor for them. It enhances activity of enzymes and peptides, helps in formation of chlorophyll and metabolism process and increases activity of gibberellic acid and indole acetic acid that effect plant growth (Mulrooney et al., 2003 and Aziz and Badran, 2007). Nickel has great importance in formation of anthocyanin pigment (Ragsdale 1998 and Lopez and Magnitskiy, 2011). In a study conducted by (Eskew et al., 1983) on the soybean crop, he noticed a decrease in the level of nitrogen as a result of a decrease in urease enzyme when nickel was not added. Take what was also found by (Ureta et al., 2005 and Zobiole et al., 2009). It was found (Aydinalp and Marinova, 2009 and Sethy and Ghosh, 2013) that increase in nickel concentrations significantly affected most of yield characteristics and caused decrease in the activity of hydrolysis enzymes, amylase and protease this was confirmed by (Seregin et al., 2003) on corn yield and (Kopittke and Menzies, 2007 and Tack, 2010) on cowpea yield, as they noticed that high nickel concentrations had a significant effect on most of growth and yield characteristics. The results of (Kara et al., 2015) in his study on Black cumin crop were identical to the aforementioned study. In a study conducted by (Al-Qurainy, 2009) on bean yield, he noticed a decrease in plant height and total yield when nickel was added at a concentration of 150 $mg.L^{-1}$. It is known that absorption of nickel takes place through root system of a plant with characteristic negative diffusion (Seregin and Kozhevnikova, 2006). Each of (Riesen and Feller, 2005; Page et al., 2006; Panda et al., 2007 and Ma et al., 2009) studied possibility of nickel element availability in valuable form for the plant at the degree of soil reaction 5, as organic acid complexes are formed, which increases availability of nickel element in soil reactivity of acidic reaction.

In addition, it improves root growth and seed filling. Nickel deficiency negatively affects production of flowers and seeds. It has an important role in pollen production, pollen tube growth and increased fertilization and seed production. Its deficiency causes plants to fail to produce seeds. Lack of nickel in soil has been exacerbated by continuous removal of crops in soil, adoption of an intensive cultivation system, use of nickel-free compound fertilizers and the effect of some factors, especially soil pH, whose rise causes inhibition of elements in soil, especially nickel, copper and some trace elements (Rezaei et al., 2018 and Khan et al., 2022). Copper is involved in synthesis of many oxidation-reduction enzymes and contributes to photosynthesis. It is also necessary for the formation of chlorophyll in plants. Copper promotes formation of seeds, that is, it is necessary in growth of reproductive organs, and also helps in functioning of roots (Stehouwer and Roth, 2004). Readiness of copper is greatly affected by the degree of acidity of soil, pH of soil. higher value of pH, lower readiness of this element. Also, copper does not move in soil, but rather it is attracted to organic matter and clay granules. Therefore, increase in organic matter reduces its readiness (Rehm and Schmitt, 2002). Copper is essential for plant growth, but because it is needed in small quantities, it is considered one of micronutrients. The plant contains 2-20% of elemental copper as dry weight (Mahler, 2004). Studies have shown that copper is essential for act of some respiratory enzymes, such as cytochrome oxidase, ascorbic acid oxidase and Monoamine oxidase. Cooper is necessary for acting of tyrosine, which oxidizes various phenolic compounds (Rehm and Schmitt, 2002). Copper is also a basic component of proteins that are involved in synthesis of enzymes that regulate most chemical reactions in plants. This element plays a major role in formation of chlorophyll pigments, although it does not enter the composition of chlorophyll molecules, but its role is in its effect on work of enzymes responsible for formation of chlorophyll (Knezek, 1991). Copper promotes formation of seeds, that is, it is necessary in growth of reproductive organs, and also helps in functioning of roots (Stehouwer and Roth, 2004).

Micronutrients face problems of sedimentation when added directly to base soil, including soil of Iraq, so it has become preferable to use foliar feeding method when adding micronutrients to plants to ensure optimum benefit. As foliar fertilization is considered one of easiest and most suitable ways to absorb nutrients, absorption of nutrients added through foliar fertilization takes place very quickly and plants respond within 2-7 days according to type of plant, its nature and severity of apparent deficiency on plant. Plants respond to addition of micro-nutrients depending on soil characteristics, amount of these added nutrients, soil content of calcium carbonate and soil reactivity (Zheng et al., 2004). Based on the aforementioned, research was carried out to study the effect of spraying elements nickel and copper at elongation stage on growth and some components of yield and quality of some genotypes of black cumin plant.

## MATERIALS AND METHODS

Experiment was conducted during winter season of two years (2021-2022 and 2022-2023) in Gayyarah fields, 100 km south of Nineveh Governorate (within latitude 35° 798 south and longitude 43°291), to study effect of spraying levels of nickel and copper at elongation stage on growth and yield of three black cumin genotypes in clay loam soil with a soil reactivity (pH) 8.4, electrical conductivity 2.62 and its available nickel and copper content 4.86 and 5.40 $mg.L^{-1}$, respectively as average for two growing seasons, traits were estimated according to methods mentioned by Olsen et al., (1954); Black, (1965); Page et al., (1982) and Tandon, (1999) as shown in table (1).

Table 1: Soil texture and its mineral elements content at a depth of 30 cm in Gayyarah location for 2021-2022 and 2022-2023 seasons.

| Traits | | 2021-2022 seasons | 2022-2023 seasons |
|---|---|---|---|
| Sand | (%) | 30.20 | 31.00 |
| Silt | | 31.40 | 29.20 |
| Clay | | 38.40 | 39.80 |
| Soil texture | | Clay loam | Clay loam |
| Soil pH | | 8,6 | 8.2 |
| Organic matter | ($gm.kg^{-1}$) | 2.88 | 3.20 |
| $Caco_3$ | | 27.40 | 25.60 |

| | | | |
|---|---|---|---|
| Nitrogen | Available ($mg.kg^{-1}$) | 4.40 | 4.00 |
| Phosphorus | | 5.60 | 4.40 |
| Potassium | | 30.00 | 46.88 |
| Nickel | | 4.88 | 4.84 |
| Copper | | 5.80 | 5.00 |
| Electrical conductivity | ($dcm.m^{-1}$) | 2.40 | 2.84 |

The experiment included three levels (0, 3, 6 $mg.L^{-1}$) of each of two elements, nickel, which was added in form of nickel sulfate ($NiSO_4.6H_2O$) and copper in form of copper sulfate ($CuSo_4.5H_2O$) at elongation stage and three genotypes of black cumin (Silingo, Cameli and Kena). It was applied according to the design of factorial experiment using randomized complete block design (Factorial Experiment in R.C.B.D.) with three replications and levels of three factors were randomly distributed among experimental units (Duncan, 1955; Steel and Torrie 1980 and S.A.S., 2004).

Soil was prepared for cultivation by plowing it twice perpendicularly, removing weeds. The soil of experiment was divided into 27 experimental units, at a rate of 6 rows for each experimental unit, with dimensions of 0.75 cm between rows and 0.30 cm between plants, to give a plant density of 44.444 $plants.ha^{-1}$. Seeds were sown manually in the first week of October by placing 2-4 seeds in each hole in the upper third of row on one side. 100 $kg.ha^{-1}$ of each potassium sulfate and tri-calcium superphosphate were added during the process of preparing soil for planting. 60 $kg.ha^{-1}$ of urea fertilizer in two batches, first at planting and second about a month later, then soil was irrigated immediately after planting.

Process of replanting the absent seeds was carried out after germination. plants were thinned by leaving one plant in each hole three weeks after start of germination. Weeds around the crop were removed manually 3-4 times throughout growing season.

After plants reached final stage of flowering, ten plants were randomly selected from each experimental unit to measure following characteristics:

1- Plant height (cm).

2- Number of flowering branches per plant.

3- Number of capsules in a plant.

4- Dry weight of plant (g): plants were dried after harvesting in May after seeds ripened and turned yellow, basal leaves dried and before seeds disintegrated and fell on soil at room temperature to prevent evaporation of volatile oils. They were placed in a dark place for two weeks. It should be removed from dirt and dust, in continuous air currents until it is dry and its weight is stable.

5-Number of seeds in each capsule (when harvesting experiment, number of seeds in each capsule was calculated as an average of ten capsules from each experimental unit)

6-Weight of a thousand seeds: randomly selected from each experimental unit.

7-Seed yield per experimental unit (gm.), from which total seed yield ($ton.ha^{-1}$) was extracted.

To estimate qualitative traits (protein and fixed oil): 50 g of seeds were grounded into a coarse powder and extracted using petroleum ether (40-60 °C) in a Soxhlet apparatus. Nitrogen and protein in seeds for both seasons of each treatment were determined using methods described by A.O.A.C. (A.O.A.C., 1984) after grinding seeds using a mortar and pestle. Nitrogen and protein were determined using Micro Kjeldahl method (Agrawal et al., 1980), by taking 1 gm. of ground seed sample, digested in Pyrex and adding 30 ml of carefully concentrated $H_2SO_4$ acid to it, then adding 10 gm. of potassium sulfate and 14 gm. of copper sulfate, heat mixture, leave it to cool, and dilute it with distilled water. Transfer to a 1000 mL Kjeldahl flask, digestion flask was washed, 100 mL of 40% caustic soda was added and the flask was connected to a distillation apparatus. Then take 25 ml of 0.1 sulfuric acid nitrogen into receiving and distillation flask to be tested and complete the reaction process. Flask was titrated against 0.1 caustic soda solution using methyl red index for nitrogen determination to give total protein content. Fatty acids in oil were estimated by dissolving

0.1 gm. of oil in 10 mL. of n-hexane, to form a 0.5 mL. potassium hydroxide/methanol solution and incubated for 30 min at room temperature. Fatty acid content was calculated based on peak area ratio and is expressed as fatty acid In grams / 100 grams of oil (Egan et al., 1980; AL-Kaisey and Hussain, 1995 and Stoffel et al., 1995).

## RESULT AND DISCUSSION

### 1- Effect of foliar spraying with nickel and copper elements and genotypes of black cumin and their interactions on vegetative growth traits:

It is noted from analysis of variance (table 5) that levels of nickel added by spraying on leaves of black cumin plant significantly affected all vegetative growth traits compared to plants not treated with nickel. Maximum significant increase was achieved when spraying plants with nickel at a concentration of 6 $mg.L^{-1}$, with an increase in traits of: number of capsules per plant (81.41 and 77.10%), number of seeds per capsule (22.78 and 21.82%), weight of thousand seeds (72.89 and 64.91%) compared to control treatment and foliar spraying at a concentration of 3 $mg.L^{-1}$ in both seasons 2021-2022 and 2022-2023, respectively. Differences were not significant when spraying nickel with two concentrations of 3 and 6 $mg.L^{-1}$, as percentage of these differences was between highest and lowest values for traits of plant height (35.09 and 38.59%), number of flowering branches per $plant^{-1}$ (23.07 and 21.82%) and seed yield per plant (21.01 and 16.58%), compared to treatment of foliar spray with distilled water in both growing seasons 2021-2022 and 2022-2023, respectively (table 2).

Foliar spraying on leaves of black cumin plants with copper at a concentration of 6 $mg.L^{-1}$ resulted in a significant increase in all vegetative growth traits compared to untreated plants (foliar spraying with distilled water only) and plants treated with a lower concentration (3 $mg.L^{-1}$). Percentage of increase was for: plant height (46.76 and 51.29%), number of flowering branches per $plant^{-1}$ (51.27 and 37.19%), number of capsules per $plant^{-1}$ (35.94 and 34.41%), number of seeds per $capsule^{-1}$ (7.88 and 7.35%), weight of thousand seeds (31.99 and 22.51%) and seed yield per plant (10.2 7 and 8.10%) in both 2021-2022 and 2022-2023 growing seasons, respectively (table 2). Cameli genotype was significantly superior in all vegetative growth traits compared to Kena and Silenko genotypes. Cameli genotype recorded highest rate for all vegetative growth traits with an increase for each traits plant height (28.86 and 31.95%), number of flowering branches per $plant^{-1}$ (33.11 and 31.51%), number of capsules per $plant^{-1}$ (45.46 and 42.23%), number of seeds per $capsule^{-1}$ (12.44 and 11.89%), weight of thousand seeds (89.54 55.33%) and seed yield per plant was (20.42 and 15.01%) compared with two genotypes Kena and Silingo in seasons 2021-2022 and 2022-2023, respectively (table 2). Interaction coefficients among three research factors showed significant differences in a limited number of vegetative growth traits, as interaction between concentrations of nickel and copper elements added by spraying on leaves of black cumin plant was significant for traits of number of capsules per $plant^{-1}$ in two growing seasons 2021-2022 and 2022-2023. Interaction between element nickel and genotypes of black cumin was significant for number of capsules per $plant^{-1}$ during growing season 2021-2022 only. It is clear from table (2) that concentrations of nickel and copper applied spraying on leaves of black cumin plants led to improvement of all studied vegetative growth traits, although effect of concentrations varied among them.

Table (1) shows that nickel and copper in soil of cultivation may not indicate that its concentration is low (Buckman and Brady, 1960) and this does not mean that these elements may be available for absorption by plant, because micronutrients are less available with increase in pH of soil and since soil pH was high in soil of Qayyarah location (8.4 as an average of two growing seasons), so high soil pH may have a negative effect on readiness of these elements for black cumin plants (Mahler, 2004). This may give a clear explanation that spraying black cumin plants with nickel and copper at a concentration of 6 $mg.L^{-1}$ provided all required needs of plants, which was positively reflected in response of plants to these added elements.

**2- Effect of nickel and copper foliar spraying and genotypes of black cumin and their interactions on yield traits, its components and seed quality:**

Results presented in analysis of variance (tables 6 and 7) indicate that spraying plants with nickel had a significant effect on all yield traits, its components and seed quality. Treatment of foliar spraying with nickel at a concentration of 6 $mg.L^{-1}$ gave highest rate for all traits of yield, its components and seed quality, with an increase of each of traits: total seed yield (59.63 and 74.43%), biological yield (2.16 and 2.71%), harvest index (18.06 and 14.06%), fixed oil (33.34 and 36.86%), protein (16.89 and 17.36%), oleic acid (34.67 and 63.90%), linoleic acid (36.50 and 33.87%), palmitic acid (52.03 and 36.36%), stearic acid (70.24 and 30.17%), arachidic acid (94.61 and 73.53%), eicosadienoic acid (59.56 and 71.20%) and homolinoleic acid (35.47 and 84.97%) compared to control treatment and spraying with lower treatment in both growing seasons 2021-2022 and 2022-2023, respectively (tables 3 and 4).

Copper concentrations applied by spraying on leaves of black cumin plants had a significant and clear effect on all yield traits, its components and seed quality. Highest rate of achievement of these traits was when spraying plants with copper at a concentration of 6 $mg.L^{-1}$, with an increase for each of traits: total seed yield (38.95 and 29.16%), biological yield (5.24 and 4.59%), harvest index (7.00 and 2.79%), fixed oil (12.21 and 10.76%), protein (5.51 and 5.49%), oleic acid (18.99 and 19.09%), linoleic acid (12.02 and 10.90%), palmitic acid (19.37 and 13.05%), stearic acid (24.69 and 11.52%), arachidic acid (25.99 and 53.49%), eicosadienoic acid (26.88 and 47.07%) and homolinoleic acid (52.13 and 38.71%) Compared to treatment of spraying with distilled water only and spraying with lower treatment (3 $mg.L^{-1}$) in both seasons 2021-2022 and 2022-2023, respectively (tables 3 and 4).

Genotypes of black cumin plant differed significantly among themselves, as Cameli genotype achieved highest rate for all traits of yield, its components, and quality of seeds, with an increase rate for each of traits: total seed production (43.74 and 31.30%), biological yield (2.00 and 1.63%), harvest index (17.92 and 13.03%), fixed oil (11.41 and 9.43%), protein (8.33. 8.29%), oleic acid (39.61 and 41.43%), linoleic acid (18.05 and 15.96%), palmitic acid (45.95 and 31.64%), stearic acid (57.61 and 23.24%), arachidic acid (27.72 and 58.15%), eicosadienoic acid (28.38 and 50.52%) and homolinoleic (49.05 and 39.85%) compared to Kena and Silingo genotypes in both 2021-2022 and 2022-2023 planting seasons, respectively (tables 3 and 4).

We note from analysis of variance (tables 6 and 7) that some interaction coefficients between levels of foliar spraying with elements nickel and copper had a significant effect on traits: total seed yield, protein, stearic, eicosadienoic and homolinoleic acid in both growing seasons 2021-2022 and 2022-2023. Whereas, interaction between nickel levels and genotypes of black cumin plant was significant for protein percentage, eicosadienoic acid and homolinoleic acid traits in both growing seasons 2021-2022 and 2022-2023. Interaction between copper and genotypes was significant for percentage of protein in seeds, percentage of eicosadienoic and percentage of homolinoleic acid in both growing seasons 2021-2022 and 2022-2023. triple interaction between levels of nickel, copper and genotypes was significant for traits of percentage of protein in seeds, percentage of eicosadienoic acid and percentage of homolinoleic acid in both growing seasons 2021-2022 and 2022-2023 (tables 6 and 7).

It is known that elements nickel and copper are important elements for activity of some respiration enzymes, as well as activity of oxidation enzymes of phenolic compounds. It is also an essential component of proteins that are included in synthesis of enzymes that regulate most chemical reactions in plants, in addition to its role in increasing activity of enzymes responsible for formation of pigments and fatty acids. Several studies have confirmed that deficiency of these elements affects total seed yield, its oil and protein content (Ozguven and Sekeroglu, 2007 and Khan et al., 2022).

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Table 2: Plant height, number of flowering branches per plant-1, number of capsules per plant-1, number of seeds per capsule-1, thousand seed weight (gm.), seed yield (g.plant-1) as affected by aluminum and copper elements, genotypes of black cumin and their interactions in growing seasons 2021-2022 and 2022-2023.

| Treatments | plant height (cm) | | number of flowering branches per plant$^{-1}$ | | number of capsules per plant$^{-1}$ | | number of seed per capsulas$^{-1}$ | | weight of thousand seeds (g.) | | Yield (gm.plant$^{-1}$) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2021-2022 | 2022-2023 | 2021-2022 | 2022-2023 | 2021-2022 | 2022-2023 | 2021-2022 | 2022-2023 | 2021-2022 | 2022-2023 | 2021-2022 | 2022-2023 |
| Nickel levels (mg.l$^{-1}$) | | | | | | | | | | | | |
| Ni1:0 | b37.072 | b33.609 | b2.8380 | b2.9328 | c10.5068 | c11.143 | c81.788 | c84.210 | c1.42400 | c2.21918 | b11.2915 | b14.9804 |
| Ni2:3 | a48.918 | a45.492 | a3.2528 | a3.3317 | b17.9047 | b18.393 | b90.264 | b92.427 | b1.75874 | b2.56504 | a13.1067 | a16.8696 |
| Ni3:6 | a50.079 | a46.579 | a3.4928 | a3.5728 | a19.0600 | a19.734 | a100.422 | a102.585 | a2.86066 | a3.65955 | a13.6637 | a17.4637 |
| Copper levels (mg.l$^{-1}$) | | | | | | | | | | | | |
| Cu1:0 | c35.533 | c32.107 | c1.3033 | c1.3833 | c13.5261 | c14.126 | c87.273 | c89.621 | c1.72374 | c2.51522 | b12.1844 | c15.9104 |
| Cu2:3 | b48.387 | b44.998 | b3.7020 | b3.7894 | b15.5575 | b16.157 | b91.048 | b93.396 | b2.04454 | b2.84714 | b12.4415 | b16.2044 |
| Cu3:6 | a52.149 | a48.575 | a4.5781 | a4.6644 | a18.3879 | a18.987 | a94.153 | a96.205 | a2.27512 | a3.08142 | a13.4359 | a17.1989 |
| Black cumin genotypes | | | | | | | | | | | | |
| Ge1: Silingo | c39.7840 | cb36.284 | c2.7876 | c2.8824 | c12.9164 | c13.553 | c84.914 | c87.336 | c1.38547 | c2.19547 | b11.8059 | b15.5689 |
| Ge2: Cameli | a51.2660 | a47.87700 | a3.7107 | a3.7907 | a18.7879 | a19.276 | a95.480 | a97.717 | a2.62605 | a3.41012 | a14.2170 | a17.9059 |
| Ge3: Kena | b45.0190 | b41.5190 | b3.0852 | b3.1641 | b15.7673 | b16.441 | b92.081 | b94.170 | b2.03187 | b2.83817 | b12.0389 | b15.8389 |
| Interactions | | | | | | | | | | | | |
| Ni*Cu | n.s | n.s | n.s | n.s | ** | ** | n.s | n.s | n.s | n.s | n.s | n.s |
| Ni*Ge | n.s | n.s | n.s | n.s | * | n.s | n.s | n.s | n.s | n.s | n.s | n.s |
| Cu*Ge | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s |
| Ni*Cu*Ge | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s |

Means that share the same letters are not significantly different from each other at 1 and 5% level.

Table 3: Total seed yield (ton.ha-1), biological yield (gm.plant-1), harvest index, ratio of fixed oil, ratio of protein, ratio of oleic acid as affected by elements aluminum and copper, genotypes of black cumin and their interactions in two growing seasons 2021-2022 and 2022-2023.

| Treatments | yield ($ton.ha^{-1}$) | | biological yield ($gm.plant^{-1}$) | | harvest index (%) | | fixed oil (%) | | protein (%) | | oleic acid (%) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2021-2022 | 2022-2023 | 2021-2022 | 2022-2023 | 2021-2022 | 2022-2023 | 2021-2022 | 2022-2023 | 2021-2022 | 2022-2023 | 2021-2022 | 2022-2023 |
| Nickel levels ($mg.l^{-1}$) | | | | | | | | | | | | |
| Ni1:0 | c1.84758 | c2.05957 | c121.6656 | c138.3544 | b9.2464 | b10.8002 | c30.714 | c35.717 | c15.30881 | c14.045 | c20.9541 | c17.8043 |
| Ni2:3 | b2.38912 | b3.11987 | b123.7400 | b140.4659 | a10.5420 | a11.9699 | b37.229 | b42.514 | b16.16630 | b14.866 | b22.7303 | b22.6541 |
| Ni3:6 | a2.94928 | a4.21047 | a124.2970 | a142.0970 | a10.9165 | a12.3192 | a40.954 | a48.881 | a17.89381 | a16.483 | a28.2179 | a29.1809 |
| Copper levels ($mg.l^{-1}$) | | | | | | | | | | | | |
| Cu1:0 | c1.99168 | c2.71768 | c119.4844 | c136.2104 | b9.93240 | b11.6416 | b34.310 | b40.296 | c15.98552 | c14.685 | c21.9764 | c21.2838 |
| Cu2:3 | b2.42690 | b3.16216 | b124.4767 | b141.2396 | ab10.1449 | a12.1310 | ab36.088 | ab42.184 | b16.51759 | b15.217 | b23.7753 | b23.0086 |
| Cu3:6 | a2.76739 | a3.51006 | a125.7415 | a142.4674 | a10.6276 | b11.3167 | a38.500 | a44.6330 | 16.86581 | a15.491 | a26.1506 | a25.3469 |
| Black cumin genotypes | | | | | | | | | | | | |
| Ge1: Silingo | c1.96408 | c2.70304 | b122.2911 | b139.0911 | b9.6069 | b11.1565 | ab34.479 | ab40.575 | c15.83019 | c14.567 | c19.5727 | c18.8060 |
| Ge2: Cameli | a2.82315 | a3.54915 | a124.7393 | a141.3541 | a11.3285 | a12.6099 | a38.4140 | a44.400 | a17.14900 | a15.774 | a27.3263 | a26.5966 |
| Ge3: Kena | b2.39876 | b3.13772 | b122.6722 | b139.4722 | b9.7696 | b11.3228 | ab36.005 | ab42.138 | b16.38974 | b15.052 | b25.0033 | b24.2366 |
| Interactions | | | | | | | | | | | | |
| Ni*Cu | ** | ** | n.s | n.s | n.s | n.s | n.s | n.s | ** | ** | n.s | n.s |
| Ni*Ge | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | ** | ** | n.s | n.s |
| Cu*Ge | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | ** | ** | n.s | n.s |
| Ni*Cu*Ge | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | * | ** | n.s | n.s |

Means that share the same letters are not significantly different from each other at 1 and 5% level.

Table 4: Linoleic acid ratio, palmitic acid ratio, arachidic acid ratio, eicosadienoic acid ratio and homolinoleic acid ratio as affected by elements aluminum and copper, genotypes of black cumin and their interactions in two growing seasons 2021-2022 and 2022-2023.

| Treatments | linoleic acid (%) | | palmitic acid (%) | | stearic acid (%) | | arachidic acid (%) | | eicosadienoic acid (%) | | homolinoleic acid (%) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2021-2022 | 2022-2023 | 2021-2022 | 2022-2023 | 2021-2022 | 2022-2023 | 2021-2022 | 2022-2023 | 2021-2022 | 2022-2023 | 2021-2022 | 2022-2023 |
| Nickel levels (mg.l$^{-1}$) | | | | | | | | | | | | |
| Ni1:0 | c49.436 | c54.139 | c2.93400 | c4.07622 | c1.13738 | c2.67257 | c1.43248 | c0.49411 | c2.14130 | c1.11693 | b1.04130 | c1.13148 |
| Ni2:3 | b57.764 | b62.764 | b3.35430 | b4.45578 | b1.34996 | b2.89255 | b1.99844 | b1.05256 | b2.58867 | b1.54178 | a1.37937 | b1.46222 |
| Ni3:6 | a67.478 | a72.478 | a4.46066 | a5.55844 | a1.93622 | a3.47881 | a2.78770 | a1.84563 | a3.41674 | a2.37011 | a1.41067 | a2.09289 |
| Copper levels (mg.l$^{-1}$) | | | | | | | | | | | | |
| Cu1:0 | c54.773 | c59.699 | c3.23855 | b4.38077 | b1.29305 | c2.83564 | c1.83178 | c0.88970 | c2.36052 | c1.32485 | c0.98871 | c1.28367 |
| Cu2:3 | b58.548 | b63.474 | b3.64454 | a4.75714 | a1.51823 | b3.04601 | b2.07907 | b1.13696 | b2.79119 | b1.75556 | b1.33850 | b1.62233 |
| Cu3:6 | a61.357 | a66.209 | a3.86586 | a4.95253 | a1.61228 | a3.16228 | a2.30778 | a1.36563 | a2.99500 | a1.94841 | a1.50413 | a1.78059 |
| Black cumin genotypes | | | | | | | | | | | | |
| Ge1: Silingo | c52.784 | c57.858 | c2.89547 | c4.03029 | c1.13404 | c2.68404 | c1.83259 | c0.88670 | c2.40474 | c1.36544 | c1.05002 | c1.33011 |
| Ge2: Cameli | a62.313 | a67.091 | a4.22605 | a5.30531 | a1.78736 | a3.30773 | a2.34063 | a1.40230 | a3.08715 | a2.05526 | a1.56507 | a1.86011 |
| Ge3: Kena | b59.581 | b64.432 | b3.62743 | b4.75484 | b1.50216 | b3.05216 | b2.04541 | b1.10330 | b2.65481 | b1.60811 | b1.21623 | b1.49637 |
| Interactions | | | | | | | | | | | | |
| Ni*Cu | n.s | n.s | n.s | n.s | ** | * | n.s | n.s | ** | ** | ** | ** |
| Ni*Ge | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | ** | ** | ** | ** |
| Cu*Ge | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | ** | ** | ** | ** |
| Ni*Cu*Ge | n.s | n.s | n.s | n.s | n.s | n.s | n.s | n.s | ** | ** | ** | ** |

Means that share the same letters are not significantly different from each other at 1 and 5% level.

Table 5: ANOVA table for plant height, number of flowering branches per plant-1, number of capsules per plant-1, number of seeds per capsule-1, thousand seed weight (gm.), seed yield (g.plant-1) in growing seasons 2021-2022 and 2022-2023.

| S.O.V. | D.f | plant height (cm) | | number of flowering branches per plant$^{-1}$ | | number of capsules per plant$^{-1}$ | | number of seed per capsulas$^{-1}$ | | weight of thousand seeds (g.) | | Yield (gm.plant$^{-1}$) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 2021-2022 | 2022-2023 | 2021-2022 | 2022-2023 | 2021-2022 | 2022-2023 | 2021-2022 | 2022-2023 | 2021-2022 | 2022-2023 | 2021-2022 | 2022-2023 |
| Rep. | 2 | 58679.290 | 58840.894 | 4.3435059 | 4.220194 | 120.49047 | 120.52744 | 294.96668 | 310.8454 | 1.7207649 | 1.906600 | 289.85983 | 286.12216 |
| Ni | 2 | 1398.808* | 1397.688* | 2.96304** | 2.82081** | 581.505** | 576.677** | 2350.34** | 2287.69** | 15.2562** | 15.2650** | 41.5468** | 45.4010** |
| Cu | 2 | 2049.70** | 2025.82** | 77.6059** | 77.9430** | 160.990** | 160.540** | 320.507** | 294.677** | 2.07048** | 2.18531** | 11.7953** | 12.3106** |
| Ge | 2 | 892.261* | 910.101* | 5.99437** | 5.83702** | 232.770** | 221.116** | 785.526** | 751.700** | 10.3946** | 9.97004** | 47.7542** | 44.1329** |
| Ni*Cu | 4 | 148.132ns | 158.058ns | 0.15113ns | 0.16014ns | 11.6276** | 9.71486** | 9.95342ns | 7.9182ns | 0.17431ns | 0.18246ns | 2.07984ns | 1.70905ns |
| Ni*Ge | 4 | 179.509ns | 174.900ns | 0.22625ns | 0.18816ns | 6.261776* | 5.52891ns | 0.32373ns | 1.0551ns | 0.01827ns | 0.0232ns | 2.67532ns | 2.91472ns |
| Cu*Ge | 4 | 32.5941ns | 33.633ns | 0.13599ns | 0.14494ns | 1.93904ns | 2.77010ns | 1.09891ns | 1.7663ns | 0.03880ns | 0.04768ns | 1.39773ns | 1.73246ns |
| Ni*Cu*Ge | 8 | 44.4424ns | 46.416ns | 0.18609ns | 0.17790ns | 1.18315ns | 1.38193ns | 1.16726ns | 0.8505ns | 0.03878ns | 0.03259ns | 1.30036ns | 1.12477ns |
| Error | 52 | 397.5930 | 395.9535 | 0.203337 | 0.2061652 | 2.466823 | 2.310215 | 15.70470 | 15.907067 | 0.115343 | 0.1301386 | 1.576133 | 1.5725434 |
| Total | 80 | | | | | | | | | | | | |

*significant at (P< 0.05); **significantly affected at (P< 0.01) and n.s. no significant difference.

Table 6: ANOVA table for total seed yield (ton.ha-1), biological yield (gm.plant-1), harvest index, ratio of fixed oil, ratio of protein, ratio of oleic acid in two growing seasons 2021-2022 and 2022-2023.

| S.O.V. | D.f | yield (ton.ha$^{-1}$) | | biological yield (gm.plant$^{-1}$) | | harvest index (%) | | fixed oil (%) | | protein (%) | | oleic acid (%) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 2021-2022 | 2022-2023 | 2021-2022 | 2022-2023 | 2021-2022 | 2022-2023 | 2021-2022 | 2022-2023 | 2021-2022 | 2022-2023 | 2021-2022 | 2022-2023 |
| Rep. | 2 | 1.0497478 | 0.277008 | 288.91872 | 288.98757 | 152.96937 | 112.95724 | 2542.1313 | 2571.8621 | 1.4997775 | 1.0172583 | 2.2031755 | 2.559623 |
| Ni | 2 | 8.19361** | 31.2300** | 51.9223** | 55.7032** | 20.7367** | 17.0891** | 725.283** | 1170.04** | 46.8081** | 41.5086** | 387.144** | 879.947** |
| Cu | 2 | 4.08184** | 4.25904** | 295.526** | 296.781** | 3.426017* | 4.53609** | 119.372** | 127.694** | 5.30677** | 4.53714** | 118.360** | 112.281** |
| Ge | 2 | 4.98168** | 4.83348** | 46.8509** | 39.6340** | 24.3925** | 17.0859** | 106.309** | 99.8209** | 11.8298** | 9.97130** | 427.518** | 430.893** |
| Ni*Cu | 4 | 0.38496** | 0.43147** | 2.54132ns | 2.33627ns | 1.19306ns | 0.62460ns | 0.53836ns | 0.9724ns | 1.51282** | 1.42691** | 3.22700ns | 2.31051ns |
| Ni*Ge | 4 | 0.14744ns | 0.15135ns | 3.79013ns | 3.96423ns | 1.24239ns | 1.02230ns | 2.27106ns | 3.0308ns | 0.99267** | 1.17177** | 3.19517ns | 2.63129ns |
| Cu*Ge | 4 | 0.11700ns | 0.15333ns | 0.92532ns | 1.13075ns | 0.97538ns | 0.66076ns | 3.92506ns | 3.1115ns | 0.17501** | 0.31066** | 1.17051ns | 0.84512ns |
| Ni*Cu*Ge | 8 | 0.05274ns | 0.06537ns | 1.45887ns | 1.73677ns | 0.86468ns | 0.37436ns | 2.75528ns | 2.3881ns | 0.13349** | 0.24380** | 5.36282ns | 5.35867ns |
| Error | 52 | 0.068611 | 0.0860834 | 1.607714 | 1.789182 | 0.875939 | 0.5823200 | 22.88630 | 22.882123 | 0.05410 | 0.064269 | 5.189010 | 4.679168 |
| Total | 80 | | | | | | | | | | | | |

*significant at (P< 0.05); **significantly affected at (P< 0.01) and n.s. no significant difference.

Table 7: ANOVA table for linoleic acid ratio, palmitic acid ratio, arachidic acid ratio, eicosadienoic acid ratio and homolinoleic acid ratio in two growing seasons 2021-2022 and 2022-2023.

| S.O.V. | D.f | linoleic acid (%) | | palmitic acid (%) | | stearic acid (%) | | arachidic acid (%) | | eicosadienoic acid (%) | | homolinoleic acid (%) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 2021-2022 | 2022-2023 | 2021-2022 | 2022-2023 | 2021-2022 | 2022-2023 | 2021-2022 | 2022-2023 | 2021-2022 | 2022-2023 | 2021-2022 | 2022-2023 |
| Rep. | 2 | 248.38976 | 245.36165 | 1.7854320 | 1.9935936 | 0.0653954 | 0.0535071 | 3.2834340 | 3.1874279 | 0.4569894 | 0.3725484 | 0.3008974 | 0.2564334 |
| Ni | 2 | 2201.59** | 2272.70** | 16.7912** | 16.0060** | 4.6216** | 4.68952** | 12.5094** | 12.4534** | 11.3067** | 10.9669** | 1.13268** | 6.44145** |
| Cu | 2 | 294.671** | 288.459** | 2.73298** | 2.28028** | 0.72655** | 0.74008** | 1.53016** | 1.52968** | 2.83311** | 2.75183** | 1.86949** | 1.74004** |
| Ge | 2 | 650.081** | 609.898** | 11.9904** | 11.0415** | 2.89650** | 2.65413** | 1.75746** | 1.80967** | 3.21808** | 3.30602** | 1.86565** | 1.98382** |
| Ni*Cu | 4 | 6.18825ns | 6.27080ns | 0.25035ns | 0.18116ns | 0.11492** | 0.08463** | 0.23005ns | 0.24197ns | 0.31298** | 0.29590** | 0.18945** | 0.19655** |
| Ni*Ge | 4 | 2.63550ns | 4.82568ns | 0.09564ns | 0.09656ns | 0.06106ns | 0.05702ns | 0.11675ns | 0.10316ns | 0.24978** | 0.22478** | 0.18648** | 0.20503** |
| Cu*Ge | 4 | 6.88234ns | 9.27134ns | 0.04656ns | 0.03918ns | 0.04554ns | 0.04877ns | 0.03508ns | 0.03569ns | 0.06357** | 0.06719** | 0.05245** | 0.04786** |
| Ni*Cu*Ge | 8 | 4.57944ns | 5.92610ns | 0.08334ns | 0.05651ns | 0.05819ns | 0.05708ns | 0.01809ns | 0.01818ns | 0.02976** | 0.03348** | 0.02651** | 0.02695** |
| Error | 52 | 16.67657 | 15.434066 | 0.118516 | 0.1287905 | 0.032921 | 0.0326783 | 0.122019 | 0.1222955 | 0.011086 | 0.0130815 | 0.008819 | 0.0115591 |
| Total | 80 | | | | | | | | | | | | |

*significant at (P< 0.05); **significantly affected at (P< 0.01) and n.s. no significant difference.

## CONCLUSION

From previous results, we conclude that spraying Cameli genotype with nickel and copper at a concentration of 6 mg.$L^{-1}$ gave highest rate for all studied traits, as plants need foliar spraying with microelements in small quantities to avoid deficiency symptoms, as their presence in organic soil is in small quantities especially in surface layers, it leads to its transformation into an insoluble form and emergence of deficiency symptoms on plants.

## CONFLICT OF INTEREST

author declares no conflicts of interest associated with this manuscript.

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