

Comparative Study on Black Cumin (*Nigella sativa*, L.) Plants, Grown Under Different Plant Spacing and Fertilization treatments

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Abstract:

Field experiment was performed during the 2004-2005 and 2005-2006 seasons at the Floriculture Nursery, Experimental Farm, Faculty of Agriculture, Assiut University to investigate the effects of plant spacing (15 and 30cm) and various fertilizer treatments; cattle manure (15m³/feddan), NPK fertilization [ammonium nitrate (33.5% N) 60, calcium superphosphate (15.5%P₂O₅) 45, and potassium sulphate(48%K₂O) 48kg/feddan] and bio-fertilizers [Biogen (500g) and phosphorein (300g/Kg seeds) were added either individually or in combination].

A complete randomized block design in a split-plot arrangement with four replicates was used in this experiment. Plant spacing was randomly distributed in the main plots and fertilizer treatments in the sub plots.

Data obtained showed that significant increases were found in branch number, seed production, yields of volatile and fixed oil in seeds in relation to plant spacing of 30cm comparison to that of 15 cm. Moreover, leaf contents of carbohydrates, nitrogen, phos

phorous and potassium recorded similar trend. All fertilizer treatments significantly increased plant height and branch number per plant compared to unfertilized plants. However, cattle manure was more effective in this concern. Cattle manure produced higher yields of seeds and volatile oil than other treatments. In addition, it significantly increased leaf contents of carbohydrates, N,P and K. The interaction among treatments cleared that the space of 30 cm along with cattle manure yielded the best results.

Introduction:

Black cumin plant, *Nigella sativa*, L.; Family Ranunculaceae is one of the most promising medicinal and aromatic plant. It is widely cultivated in middle and upper Egypt regions, for seed yield and oil production. The whole seeds contain 30-35% of oil (fixed and volatile) which has several uses in pharmaceutical and food industries (Ustun *et al.*, 1990).

The effect of plant spacing on growth, seed and oil production of *Nigella sativa*, L. has been reported by numerous investigators. Emad EL-Deen (1997)

Received on:16/10/2012

Accepted for publication on: 15/11/2012

Referees:Prof.Abd-Elrazk.Elngar

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reported that plant height and number of branches per plant were not significantly affected by spacing (20, 30 and 40 cm), while stem diameter was increased with increasing spacing between plants up to 40cm. He also found that plant spacing at 30 cm increased number of fruits and seed yield, herb dry weight, and volatile and fixed oil yield per plant in comparison with either 20 or 40 cm. **Ahmed et al; (1998)** stated that number of branches, fresh and dry weights per plant of black cumin were increased at the space of 45 cm compared to those of either 15 or 30 cm. Meanwhile, oil percentage was increased by increasing plant space from 15 to 45cm. **Matter (1997)** found that 10 cm plant spacing increased height of *Nigella sativa, L.* plants, while 20 cm spacing increased number of branches, fresh and dry weights, number of capsules and seed yield per plant.

The beneficial influences of organic manure on growth, seed and oil production of some medicinal plants were investigated by **Hammam (1996)** through supplying 40m³ per feddan to *Pimpinella anisum, L.*, **Abdel-Kader (1999)** added 50 m³ per feddan to *Foeniculum vulgare, L.*, **Abd-EL-Latif (2002)** used 12m³ per feddan on *Carum carvi, L.*, and **Yuonis et al (2004)** applied 25 m³ per feddan to *Ami visnaga, L.* **El-Gendy et al. (2001)** showed that increasing organic fertilizer addition as compost at rates of 15, 25, 35,

45, 55 and 65 m³ per feddan significantly increased plant height, number of branches, fresh and dry weights per plant of *Ocimum basilicum, L.* The best organic addition was 45 m³ per feddan.

Some studies reported the effect of NPK-fertilization on growth characteristics and production of seeds and oil content of *Nigella sativa, L.* and other medicinal plants. **Das et al (1992)** concluded that application of 60 Kg N, 30 Kg P₂O₅ and 30 Kg K₂O per hectare resulted in the best vegetative growth and increased seed and oil yield of black comin. **Munshi et al., (1990)** treated *Carum carvi, L.* with N at 0-80, P₂O₅ at 0-40 and K₂O₅ at 0-25 Kg per hectare and found that plant height, branch number, seed yield and oil production were increased with increasing the rates of NPK. **Vghreja and Chundawat (1994)** applied N, P and K each at 0,30 or 60 Kg per hectare to *Coriandrum sativum, L.* They found that plant height and number of branches per plant were increased with increasing N or P, while K treatment did not affect plant growth. **Tomar et al (1995)** reported that seed yield of *Coriandrum sativum, L.* was the highest by fertilizing with N 80 + P₂O₅ 40 + K₂O 30 Kg per hectare. **Amin and Abd EL-Wahab (1998)** demonstrated that 450 Kg ammonium sulphate, 100 Kg calcium superphosphate and 100 Kg potassium sulphate per feddan increased plant height, number of branches, seed yield,

oil percentage and oil yield of *Cuminum cyminum*, L. **Bhuvaneshwari et al.,(2002)** stated that addition of N at 80 Kg, P and K each at 60 Kg per hectare to *Pimpinella anisum*,L.increased plant height, number of leaves, number of fruits, size of umbels, fruit yield and essential oil yield. **Abd EL-Kader and Ghaly (2003)** concluded that supplying *Coriandrum sativum*, L. plants with 300 Kg ammonium sulphate (20.5% N), 300Kg calcium superphosphate (15.5% P₂O₅) and 50 Kg potassium sulphate (48% K₂O) per feddan increased volatile oil percentage and volatile oil yield. **Kandeel et al (2001)** reported that application of 300Kg ammonium sulphate, 300Kg calcium superphosphate and 80 Kg potassium sulphate per feddan increased oil yield of *Foeniculum vulgare*, L.

The favourable effect of bio – fertilizers on growth, seed production and oil yield have been reported by some investigators. **Shaalan (2005)** inoculated seeds of *Nigella sativa*, L. with Biogein, Nitrobein and Phosphorein either individually or in combination and found that all bio-fertilizer treatments increased plant height, number of branches, number of capsules, seed yield per plant, percentage and yield of volatile and fixed oils. **Abd EL-Latif (2002)** concluded that plant height, number of branches, plant fresh and dry weights, number of umbels, fruit yield per plant and oil content were increased by inoculating the seeds of *Carum*

carvi, L. with 1 Kg Nitrobein + 1Kg Phosphorein per feddan.

Therefore, the major objective of this work on black cumin is to reveal the best plant spacing and the suitable fertilizer treatments for producing the highest yield and best quality of seeds and volatile oil as well as alternating considerable consumption of chemical fertilizers with organic manure and bio-fertilizers.

Materials and methods:

The present study was carried out during the 2004-2005 and 2005-2006 growing seasons at the Floriculture Nursery, Experimental Farm, Faculty of Agriculture, Assiut University to investigate the effects of organic manure, mineral NPK and bio-fertilizers under two different plant spacing (15 and 30cm) on growth, seed yield, oil production and chemical analysis of black cumin (*Nigella sativa*, L.).

A complete randomized block design in a split-plot arrangement with four replicates was used in this experiment. Plant spacing was randomly distributed in the main plots and fertilizer treatments in the sub plots. On 1st November, during the two growing seasons, seeds of black cumin were sown in the experimental units; each was 1.0 x 1.5 meter including three rows with 50 cm distance The thinning was done at six weeks after planting leaving two seedlings per hill (each experimental unit contains 30 or 60 plant).

The treatments were:

- **Control:** (unfertilized).

- **Cattle manure** 15 m³/fed.
- **NPK fertilizer:** [ammonium nitrate (33.5% N) 60, calcium superphosphate (15.5% P₂O₅) 45, and potassium sulphate (48% K₂O) 48kg/feddan.
- Bio-fertilizers:

Biogein {containing N- fixing bacteria *Azospirillum brasilense* (2.1×10^9 cell/cm³)} at 500 g/kg seeds.

Phosphorein {containing phosphate solubilizing bacteria"; *Bacillus megatherium* var. phosphaticum (4.1×10^9 cell/cm³) + *Bacillus polymyxa* (3.8×10^9 cell/cm³)} at 300 g/kg seeds.

Biogein at 250 g + phosphorein at 150 g/kg seeds. The seeds were sown in clayey loam soil at 15 and 30 cm. spaces. Plants were harvested in the mature stage on April. Samples of plants were randomly chosen from each experimental unit in both seasons.

Data recorded:

1. Vegetative growth: Plant height; Number of main branches per plant.
2. Seed production: seed yield (g) per plant.

3. Oil production:

3.1. Essential oil percentage

was determined in the seeds of black cumin using 100 g crushed (just before distillation). Volatile oil in the seeds was extracted by water distillation according to **Egyptian Pharmacopoeia (1961)**. Distillation was continued for three hours after boiling to complete the oil extraction. The oil was then left to stand undisturbed to assure complete separation and determined according

to **Guenther (1961)**. The volatile oil percentage was calculated as ml of oil per 100 g of dried seeds.

3.2. Fixed oil percentage, estimated by Soxhlet apparatus using petroleum ether (40 – 60 C°) as a solvent according to **A.O.A.C, 1990**.

4. Determination of carbohydrates (**Hansen and Moller 1975**)
- 5- nutrient contents: N (**Black et al., 1965**), P and K (**Jackson (1978)**) percentage in leaves.

The obtained data were statistically analyzed according to **Dowdy and Stanley, (1983)**

Results and Discussion:

1-Plant height and branch number:

Plant height and branch number of *Nigella sativa*, L. as affected by plant spacing and different fertilizer treatments are presented in Table (1). It was observed that plant height was increased by decreasing plant spacing from 30 to 15 cm. In contrast; the space at 30 cm produced higher number of branches per plant than space at 15 cm. These results are in agreement with findings of **Matter (1997)** who found that 10 cm plant spacing increased height of *Nigella sativa*, L plants while 20cm spacing increased number of branches. **Ahmed et al., (1998)** on black cumin, stated that number of branches, was increased at the space of 45 cm compared to that of 15 and 30 cm

An antagonistic relationship was found between vegetative growth and plant spacing. **Kandeel et al., (2001)** attributed the

increments in vegetative characteristics to less competition among plants for the environmental conditions necessary for building up more metabolites and producing more lateral branches at wider spaces. On the contrary, **El-Gendy et al., (2001)** reported that the competition among plants for solar energy trapping in the narrow spacing pushed plants to grow higher.

All fertilizer treatments significantly increased plant height compared with untreated control. Cattle manure produced the tallest plants followed by mineral (NPK) and the combined biofertilizers. However, the differences among cattle manure, and NPK-fertilizer were not-significantly different.

Regarding branch number, application of cattle manure, mineral NPK and bio-fertilizers resulted in a significant increase compared with unfertilized plants. Cattle manure increased branch number up to 13.15 and 10.26 compared to the control (9.03 and 6.91) for the first and second seasons, respectively. Similar results were reported on organic manure by **Hammam (1996)** on *Pimpinella anisum, L.*, **Abdel-Kader (1999)** on *Foeniculum vulgare, L.*, **Abd-El-Latif (2002)** on *Carum carvi, L.*, **Mohamed and Ahmed (2002)** on fennel plants and **Yuonis et al (2004)** on *Ammi visnaga, L.*

Khattab and Helmy (2003) reported that the maximum values of fennel plant height and number of branches per plant were

obtained with mineral NPK fertilizer. **Shaalan (2005)** reported that the combination of Biogein, Nitrobein and Phosphorein was the most effective treatment for increasing plant height and branch number of *Nigella sativa, L.*

The favorable effects of fertilization treatments on vegetative growth characteristics can be attributed to the important roles in the different physiological processes within plant growth. **Burns (1982)** recorded that cattle manure is an excellent source of element nutrients for crop production and improving physical and chemical properties of soil. **Mahmoud (1988)** reported that organic matter contained the principal nutrient elements needed for plant growth and has a great water holding capacity.

On the other hand, **Subba Rao et al., (1985)** attributed the increase in branch number to the increase in nitrogen content in the soil as a result of N₂ fixation or availability of phosphorus in soil from phosphate dissolving bacteria as well as the beneficial effects of growth promoting substances such as indole acetic acid (IAA) and gibberellins which were produced by all micro organisms used.

The interaction between plant spacing and the different fertilizer treatments were not significant for vegetative growth parameters.

2. Seed yield:

Seed yield per plant was significantly increased as plant spacing increased. The wider space (30cm.) increased seed yield by 44.12 and 45.84% over

space at 15 cm for the first and second seasons, respectively. These results are in line with those obtained on *Nigella sativa*, L. plants by **Das et al., (1992)**, and **Matter (1997)**. They found that increasing plant spacing increased seed yield per plant. **Malav and Yadav (1997)** on *Coriandrum sativum*, L. also cleared that seed yield was increased with the wider space which enabled plants to receive more light, water and nutrients compared to narrower one. Cattle manure, mineral NPK and bio-fertilizers significantly increased seed yield per plant compared to unfertilized plants. However, cattle manure treatment gave the highest values compared to bio-fertilizers. The obtained data are in agreement with those obtained by **Abed El-Latif (2002)** on *Carum carvi*, L., **Mohamed and Ahmed (2002)** on fennel, and **Shaalán (2005)** on *Nigella sativa*, L. Other authors found that seed yield per plant were increased with mineral NPK; **Emad El-Deen (1997)** and **Singh and Sardar (1999)** on *Nigella sativa*, L. found a positive relationship between vegetative growth characteristics and seed yield.

The interaction between plant spacing and fertilizer treatments showed that the highest seed yield was resulted from plant spacing at 30 cm. combined with cattle manure, followed by the same space with NPK-fertilization. Some investigators found that the wider plant spac-

ing with mineral NPK increased seed yield, according to **Kandeel et al., (2001)** on *Foeniculum vulgare*, L. and **Nofal et al., (2001)** on *Ammi visnaga*, L. plants.

3. Oil production

Volatile and fixed oil yields were significantly increased with increasing plant spacing. The space of 30cm gave the maximum values than that of space at 15 cm (Table 1) these results were similar to that observed in branch number and seed yield. These results are in accordance with the findings of **Emad El-Deen (1997)** and **Ahmed et al., (1998)** on black cumin.

Concerning fertilization, volatile and fixed oil yields were significantly increased as a result of treating *Nigella sativa*, L. plants with cattle manure, mineral NPK and the combination of bio-fertilizers in comparison with individual bio-fertilizers and control plants. Using cattle manure gave the greatest values for yield of volatile oil. Many authors found that organic fertilizer was more effective than chemical one for improving oil production. Increases in oil yield as a result of organic manure are recorded by **Shaalán (2005)** on *Nigella sativa*, L. Other reports declared that mineral NPK and bio-fertilizers increased volatile and fixed oil percentages and yields; according to **Awaad (2004)** on lavender and **Shaalán (2005)** on *Nigella sativa*, L. plants. However, **Salem et al., (2001)** cleared that volatile and fixed oil produc-

tion had a greatest positive relationship with seed yield.

The interaction among the different treatments showed that plant spacing at 30cm. with cattle manure gave the maximum values for volatile oil yield. Besides, the same spacing (30cm) with the mineral NPK or cattle manure resulted in the maximum values of fixed oil yield per plant. These results may be attributed to the higher efficiency in synthesizing biochemical metabolites due to available levels of fertilization, water, and light at wider plant spacing; **El-Gendy et al., (2001)** on sweet basil, demonstrated that the increase in oil yield per plant resulted when plants received the high level of organic manure (35 or 45 m³/fed.) and the wider space (45cm.).

4- Plant constituents

4.1. Total Carbohydrates

The wider plant spacing produced the highest total carbohydrates percentage in leaves of *Nigella sativa*, L. in comparison to that of the closer one (Table 2). This result agreed with the findings of **Kandeel et al., (2001)** on fennel plants and **Nofal et al., (2001)** on *Ammi visnaga*, L. plants.

Results obtained on fertilizers showed that cattle manure was more effective than other treatments. It produced the highest carbohydrates percentage. **Heikal (2005)** revealed that cattle manure increased total carbohydrates in *Thymus vulgaris*, L. plants. Other authors reported that mineral NPK application

increased total carbohydrates in herb and seeds of *Cuminum cyminum*, L. plant; Additionally, **Massoud et al., (2004)** found that nitroben + phosphorein promoted total carbohydrates in dry leaves of thyme plant in comparison to the control. In this regard, positive relationship was found between total carbohydrates content in leaves and either seed or oil yield (volatile and fixed).

The interaction among plant spacing and fertilizer treatments cleared that the wider plant spacing (30cm) with cattle manure increased total carbohydrates in leaves followed by the same space with mineral NPK. The obtained results are in agreement with those obtained by **Yuonis et al., (2004)** on *Ammi visnaga*, L. Meanwhile, **Emad El-Deen (1997)** on *Nigella sativa*, L. found that the interaction among plant spacing and fertilizer treatments showed non-significant effect on total carbohydrates.

4.2. Nutrients

Percentages of N, P and K in leaves of *Nigella sativa*, L. were increased as plant spacing increased from 15 to 30cm. (Table 2). Many investigators stated that the wider space produced higher percentage of N, P and K in leaves of different plants; **Matter (1997)** and **Ahmed et al., (1998)** on *Nigella sativa*, L. and **Nofal et al., (2001)** on *Ammi visnaga*, L.

Concerning the effect of fertilizers on N, P and K percentages in leaves, results showed that leaf nutrients were increased with any

each fertilizer used. The highest values resulted from cattle manure as reported by; **Mohsen (2002)** on sweet basil plant, **Abdou and Mahmoud (2003)** on *Foeniculum vulgare*, L. and **Yuonis et al., (2004)** on *Ammi visnaga*, L. plants. The effect of cattle manure on improving the growth is correlated with increasing the amounts of nutrient elements (N, P, K, Ca, Mg and SO₄) which become available to the plant according to **Burns (1982)**. Besides, **Soliman (2005)** reported that the application of organic manure to soil progressively increased growth and NPK uptake as a result of improvement in the physical and chemical properties of the soil.

The interaction among the different treatments showed that the wider space (30cm) along with cattle manure gave the maximum values for increasing leaf contents of N, P and K, in

most cases. The next treatment was the same space with mineral NPK. Similar trend was obtained by **Kandeel et al., (2001)** on fennel and **Yuonis et al., (2004)** on *Ammi visnaga*, L. plant. This result could be attributed to less competition between plants in the wide space as well as organic manure promoted the uptake of nitrogen, phosphorus and potassium by plant.

It could be recommended to cultivate *Nigella sativa*, L. plants at the space of 30cm and to be fertilized with cattle manure at the rate of 15m³ per feddan to produce better growth, increase seed yield and to maximize volatile oil production. The next effective treatment was the same spacing with either NPK fertilization or the combination of bio-fertilizers (biogein + phosphorein).

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**"دراسة مقارنة لنباتات حبة البركة المنزرعة على مسافات زراعة ومسمدة
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جمال طه موسى، محمد مصطفى جاد، جمال عبد الحفيظ احمد،
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أجريت هذه التجربة بمزرعة أبحاث نباتات الزينة بجامعة أسيوط خلال موسمي 2004-2005، 2005 - 2006 على نباتات حبة البركة لدراسة تأثير مسافات الزراعة (15 و 30 سم) والسماذ العضوي (سماذ الماشية بمعدل 15 م³/فدان)، السماذ المعدني (ن، فو، بو) والسماذ الحيوي (بيوجين، فوسفورين والخليط بينهما) بهدف الحصول على أعلى القيم لصفات النمو الخضري، محصول البذرة، نسبة ومحصول الزيت محتوى النبات من الكربوهيدرات الكلية وعناصر ن - فو - بو. ومعرفة المسافة المثلى للزراعة وكذلك الحد من الاعتماد على استخدام الاسمدة الكيماوية بكثرة والتي لم تعد مرغوبة هذه الأيام.

تم إضافة سماذ الماشية بمعدل 15 م³/فدان أثناء إعداد التربة للزراعة وإضافة السماذ المعدني ككترات امونيوم (33.5 % ن)، سوبر فوسفات الكالسيوم (15 % فو 2 أ 5) وسلفات البوتاسيوم (48 % بو 2 أ) بمعدل 45، 48 كجم ن، فو 2 أ 5، بو 2 أ/فدان على الترتيب كما تم تلقيح بذور حبة البركة بخلطها بالبيوجين بمعدل 500 جرام، او الفوسفورين بمعدل 300 جرام أو الخليط ببيوجين 250 جم + فوسفورين 150 جم لكل كجم بذرة.

صممت التجربة بنظام القطاعات العشوائية المنشقة وكررت المعاملات أربع مرات، تم استخدام مسافات الزراعة في القطع الرئيسية واستعمال الأسمدة العضوية والكيماوية والحيوية في القطع المنشقة، والتي تبلغ مساحة كل منها 1.5 متر مربع محتوية على ثلاث خطوط، المسافة بينهما 50 سم وعلى ريشتي كل خط زرعت البذور في جور المسافة بينها 15 او 30 سم يحتوى كل منها على نباتين (يحتوى كل حوض على 30 او 60 نبات تقريبا). وقد أوضحت نتائج التجربة ما يأتي:

- زراعة نباتات حبة البركة على مسافة (30سم) أعطت زيادة معنوية في عدد الأفرع، محصول البذرة للنبات ومحصول الزيت الطيار والثابت في بذور النبات ومحتوى الأوراق من الكربوهيدرات الكلية والنيتروجين، الفوسفور والبوتاسيوم مقارنة بمسافة الزراعة 15سم.

- أظهرت المعاملة بسماذ الماشية أعلى القيم في صفات المحصول مثل محصول البذرة للنبات، المحصول للزيت الطيار، كما أعطت زيادة معنوية في محتوى الأوراق من الكربوهيدرات الكلية والنيتروجين، الفوسفور والبوتاسيوم مقارنة بالتسميد المعدني والأسمدة الحيوية ومعاملة المقارنة.

- أوضح التأثير المتبادل بين مسافات الزراعة والأسمدة المستعمله ان مسافة 30سم مع السماذ العضوي (سماذ الماشية) أفضل المعاملات حيث أظهرت أعلى القيم للصفات المدروسة ولذا يمكن النصح بها عند زراعة نباتات حبة البركة في منطقة صعيد مصر (مصر الوسطى).