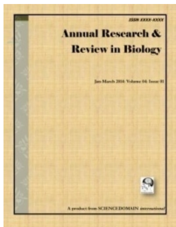

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Effects of Nitroxin and Nitrogen Fertilizers on Grain Yield and Essential Oil from Seeds of (*Anethum graveolens* L.).

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Author's contribution

The whole work was carried out by the author FNB.

Original Research Article

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ABSTRACT

Aims: Dill (*Anethum graveolens* L.) is a perennial herbaceous plant belonging to the (Apiaceae or Umbelliferae) family, with high essential oil content, and dill leaves and and seeds are used as seasoning. This study was conducted in 2011 and 2012 at Iran County, aiming to determine the effects of biological and chemical nitrogen fertilizers on grain yield, essential oil yield, and essential oil composition of dill.
Methodology: The treatments included Nitroxin bio-fertilizer (0% and 100%), and nitrogen (0, and 100 kg ha^{-1} (NH_4NO_3)), 50% nitroxin×50 kg ha^{-1}nitrogen and control. A completely randomized block design with four replications was adopted.
Results: Results showed highest essential oil content detected in biological fertilizer and chemical fertilizer. Identification of essential oil composition showed that content of carvone increased with application of Nitroxin biofertilizers**.**
Conclusion: Results indicated, application of Nitroxin biofertilizers enhanced yield and essential oil content in this plant.

Keywords: Bio-fertilizer; GC/MS analysis; essential oil content.

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1. INTRODUCTION

Dill (*Anethum graveolens* l.), a biennial or annual herb of the parsley family (Apiaceae or Umbelliferae), is native to south-west Asia or south-east Europe and cultivated since ancient times [1]. It grows up to 90–120cm tall and has slender branched stems, finely divided leaves, small umbels (2–9cm diameter) of yellow flowers, and long spindle-shaped roots. In general, dill leaves (dill weeds) and seeds (small fragrant fruits) are used as seasoning. The leaves could be used in eggs, meats, salads, sea foods and soups; the seeds could be used in bread, and flavouring pickles and soups. Dill essential oil, extracted from both leaves and seeds, could also be used in chewing gums, candies and pickles [2,3]. Literature demonstrates that dill leaf consumption could lower the risk of cancer [4] and reduce the level of cholesterolaemia [5]. Moreover, dill leaf, seed and their essential oil could provide good antioxidant activities [6,7] Many reports indicate that plant flowers have remarkable antioxidant activity [8,9,10].

Therefore, nitrogen application becomes a necessary practice for oil-producing plants. Although it is a long time that biologic fertilizers are used in agriculture, their scientific application is novice. Although their application has reduced in recent years, because of problems in indiscriminate use of chemical fertilizers, using them in agriculture is reviewed again [11]. In this respect, the goal is to use potential of organic materials in soil to increase the quality and quantity of productions and saving the environment [12]. In recent days, biological fertilizers are good replaces for chemical fertilizers to increase the fertility of soil and production of agricultural products in sustainable agriculture. These kinds of materials unlike chemical fertilizers do not make toxic and microbial materials in food cycle as well as they have this capability to do auto proliferation that causes reform in physiochemical characteristics of soil [13].

Biological fertilizers release active precursors like gibberellin, auxin, cytokinine, vitamins, amino acids, polypeptides, anti-bacteria and anti-fungi especially exo polysaccharides to have a positive effect on yield of crops. Applied microorganisms as biological fertilizers have effects on growth of the plant to provide food elements by colonization in rhizosphere environment or in cooperation with symbiotic [14]. On the other hand, these bacteria can produce fungi complexes that they can be used against plant diseases and improvement of germination and at last growth of the plant. These bacteria can reinforce performance of the plant by fixation of nitrogen and producing of materials causing growth stimulation, root growth and as a result water absorbent, reforming acidity of the soil and absorbing of food elements [15]. Biological fertilizers stimulate the development of plant root systems that increase the rate of seed germination [16]. Combined application of organic fertilizer and urea fertilizer or the combination of urea fertilizer and polyamines significantly increases yield, vegetative growth and evaluations on the chlorophyll index [17]. Our study aimed to determine the effect of Nitroxin and Nitrogen on grain yield, essential oil content and essential oil yield in *Anethum graveolens.*

2. MATERIALS AND METHODS

The experiment was conducted under field conditions during 2011 and 2012, at the Experimental field of Islamic Azad University, Khoy, Iran (38°33' 38"N, 44°58' 24"E, and 11.3m of altitude). The climate is classified as semiarid climate, with an average annual temperature of 13.6°C. The soil is loamy clay texture [18].

Table 1 presents information on the chemical characteristics of the soil from the experimental area. The experiment was conducted in a completely randomized block design with four replications. Nitrogen doses were applied at sowing (N_1=0 and N_2=100kg ha^{-1} (NH_4NO_3)), Nitroxin bio-fertilizer (0%, and 100%) of the recommended amount (1 liter of biological fertilizer for 30Kg of seed), 50% Nitroxin×50 kg ha^{-1}nitrogen and control respectively. Based on soil analysis, fertilization was carried out applying 9 kg ha^{-1} P_2O_5 (triple superphosphate) and 30kg ha^{-1} K_2O (potassium oxide). Seeds of the Dill were provided by Institute of Seed and Plant, Karaj. Plots consisted of six rows 5m long, spaced 0.25m. The experiment was planted manually on April, 2011 and 13 May, 2012, at a rate of 800,000 plants per hectare. During the crop cycle manual weeding was performed, with no chemicals applied to control pests throughout the crop cycle.

Table 1. Soil chemical characteristics from in experimental area at 0–20 cm depth (Khoy, 2011/2012).

pH	P	O.M.	Ca	K	N	Ec	C.C.C.	V
$CaCl_2$	mg dm^{-3}	%	$cmol_cdm^{-3}$			ds/m		%
7.77	9.52	1.14	1.40	0.46	18	2.85	7.02	33.21

O.M. = organic matter. C.C.C. = cationic change capacity. V = basis saturation.

At around 80-120 days in both agricultural years, a manual harvest was conducted of the two central rows in each plot. Impurities were removed using sieves, the grain then cleaned, Essential oil content and yield determined, and data converted to kg ha^{-1}.

For the evaluation of Essential oil content, the extraction was performed under laboratory conditions according to the methodology proposed by IAL – Adolfo Lutz Institute, with Soxhlet extraction [19], through the use of petroleum ether solvent. Extraction was carried out in all replicates of each treatment with 500g of milled plant material. Thus, it was possible to obtain the results in percentage, by sample weight differences. The contents of (+)-carvone were analysed in 1g seed samples using gas chromatography in a hexane extract essentially as described by using isobutylbenzene and camphor as internal standards [20]. Hydrodistillation of the samples was carried out using all glass apparatus (Clevenger apparatus) [20]. The distillation was continued for two hours to yield 3.2% oil, based on dried weight of sample. Heavier fractions collected in the final stages were kept separately and it is reported to contain dillapiole only [20]. The first fraction of the distillate (devoid of dillapiole) was analysed by GC and GC-MS spectroscopy. The oil was dried over anhydrous sodium sulphate and stored at 4°C in the dark.

The GC analysis of the oil was performed on a Varian 3300 gas chromatogram, using a fused capillary column (30m×0.25mm i.d., film thickness 0.25µm), coated with dimethylsiloxane (BP-1). The oven temperature was programmed at 80-225°C at 4°C/min, and then held isothermal at 250°C, detector used FID, detector temperature 300°C, injection volume 0.1µl and carrier gas nitrogen was used.

The GC-MS data were obtained on a Shimadzu QP 2000 instrument at 70eV and 250°C. GC column: Ulbon HR-1, fused silica capillary column 0.25mm×50m i.d., film thickness 0.25µm. The initial temperature 100°C for 6 minutes and then heated at 10°C/min to 250°C. Carrier gas helium at a flow rate of 2ml/min was used.

Statistical analyses were performed with the SPSS computational program.

3. RESULTS AND DISCUSSION

There was a significant effect of treatments on yield and essential oil content. Treatments with higher Nitroxin and nitrogen levels resulted in increased yield Fig 1. Essential oil yield, achieved agree with the results obtained by makkizadeh et al. [21], who also noted a significant increase in (*Anethum graveolens* L.). Nitrogen is essential for increasing yield, as part of the production of Protein [22]. In 2011-2012, the yield increased from 1005 kg ha^{-1} without fertilization to 1320kg ha^{-1} when 100% of nitroxin was provided.

In this condition the yield values obtained were below the vegetable specie production capacity [21]. It is about 1323kg ha^{-1}, due the occurrence of freezing temperatures limited plant growth. Increased essential oil yields are achieved only with Nitrogen supply in quantities compatible with crop demands, thus excessive NH_4NO_3 applications are required, since no other nutrient limits production [21]. This explains the high essential oil yields from higher NH_4NO_3 rates applied, as occurred in studies conducted by Chen et al. [16] and Makkizadeh et al. [21], where the authors observed average yields around 1183kg ha^{-1} in Dill plants. It becomes evident that Nitroxin and Nitrogen fertilizations is responsible for higher yield essential oil, as is confirmed by several studies performed with other species and environmental conditions, as described by Gewaily et al. [23]. For essential oil production, there was significant effect due to nitroxin and nitroxin×nitrogen rates in Fig 2. Combined application of bio- fertilizer and urea fertilizer or the combination of urea fertilizer and polyamines significantly increases yield, vegetative growth and evaluations on the chlorophyll index [17]. In studies with Makkizadeh et al. [21], and Marotti et al. [24] find a significant difference between Nitrogen rates applied in relation to essential oil content and increase leaf yield per plant. Values observed for essential oil production were within the potential oil production of Dill, which according Makkizadeh et al. [21], ranges from 2.11 to 3.03%, suggesting similar values 2.96 to 4.01% obtained in this work, and agreeing with Kapoor et al. [25], who found a range in essential oil content from 2.47 to 3.61%. Higher Nitrogen and nitroxin rates may not act directly on essential oil yield in leaves, but does result in higher grain yield and consequently higher essential oil production per hectare, as shown in Fig 3. Increased Nitrogen and nitroxin rates resulted in higher essential oil production per hectare, and this effect was observed in 50kg ha^{-1} of NH_4NO_3 and 100% of nitroxin. Suggesting the importance of bio-fertilizer for the development of this species, as observed in studies conducted for Chen [16] where lower manure rates limited plant development of D-Carvone and Carvacrol, resulting in inferior oil yield, the most important characteristic of this crop.

Other authors have investigated Dill's response to fertilizers rates. Mandal et al. [26] evaluated manure and fertilizer on biological and biochemical activities in soil. They observed that Nitrogen and bio-fertilizers increased dry matter yield in superior parts of the plants.

The oil obtained by the hydro-distillation method from the seeds of (*Anethum graveolens* L.) was analyzed by GC-MS Table 2. Five compounds were isolated and identified. The major constituents were carvone, limonene, α–Phell- andrene, Dill-ether and Trans-Dihydro-carvone (Kruger and Hammer, 1996). Nitroxin×Nitrogen resulted in increased carvone (56.34%) and Nitroxin treatments increased other constituents such as limonene, α–Phell-andrene, Dill-ether and Trans-Dihydro-carvone in this plant.

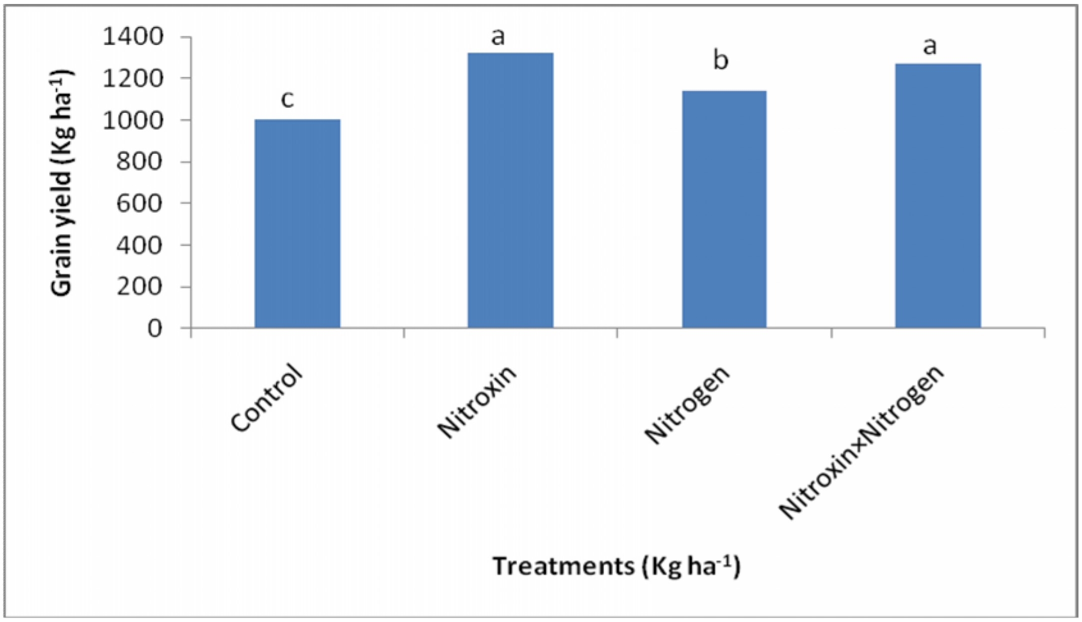

Fig. 1. Grain yield under different treatments on *Anethum graveolens* L.

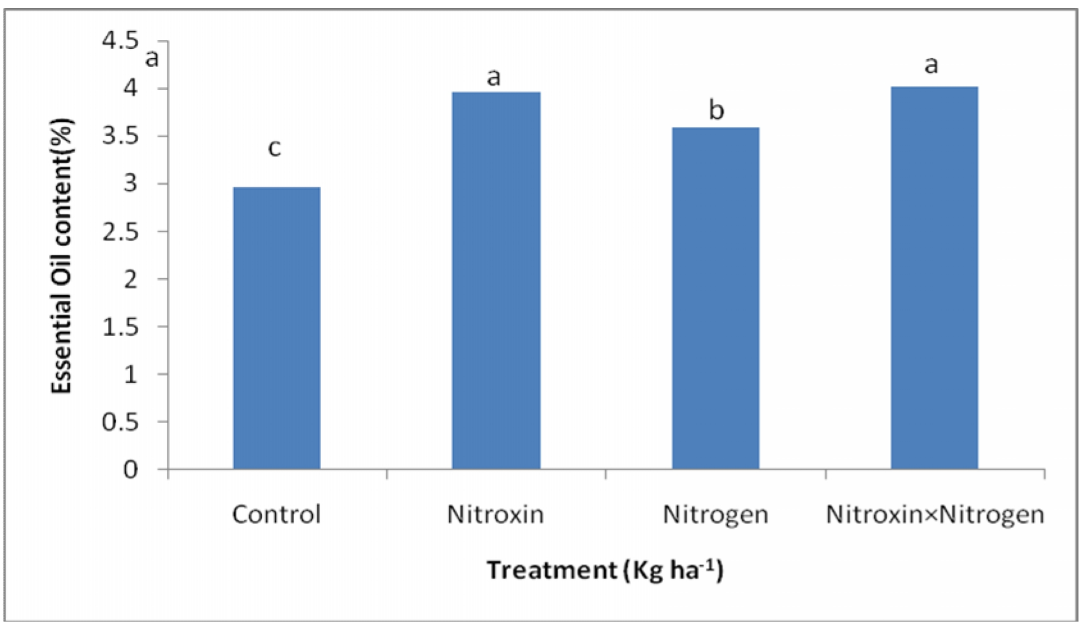

Fig. 2. Essential Oil content (%) under different treatments on *Anethum graveolens* L.

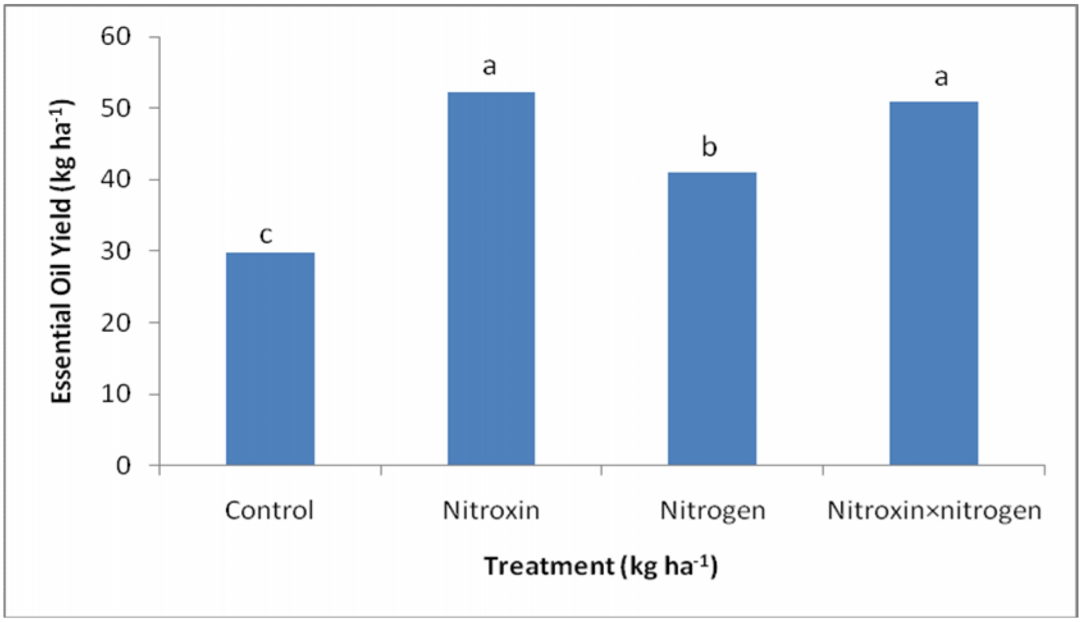

Fig. 3. Essential oil yield (kg ha^{-1}) under different treatment on *Anethum graveolens* L.

Table 2. Oil composition of dill seeds affected by different fertilizers.

Compound (%)	Nitroxin×Nitrogen	Nitrogen	Nitroxin	Control
Carvone	56.34a	54.00b	56.00a	54.31b
Limonene	26.10c	28.00b	31.30a	31.04a
α–Phell- andrene	3.31b	3.01b	4.21a	2.22c
Dill-ether	2.00a	1.01b	2.02a	1.01b
Trans-Dihydro- Carvone	4.00b	4.01b	4.02b	5.01a

5. CONCLUSION

Data obtained revealed that increased rates of NH_4NO_3 and nitroxin resulted in higher grain yield and higher essential oil production per hectare. Based on results it can be said application of NH_4NO_3 and nitroxin, it can be an important role in increasing essential oil content in this study.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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