Research Article

Effect of Nitrogen Fertilizer and Salinity on Essential Oil Yield and Bioactivity of Oregano (*Origanum vulgare*)

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Abstract | Application of nitrogen fertilizer improves the nutritional status of plants as plants utilize these nitrogenous compounds for growth and as nutrients for different metabolic processes. Salt stress is an important environmental factor that affects plants vegetative growth and other metabolic processes. The objective of the current study was to explore the effects of nitrogen fertilizers and salinity on herbage yield, essential oil and bioactive compounds of oregano under local conditions. N-fertilizers (NPK:Urea) in four different combination (1:1,1:2, 2:1, 2:2) and salinity treatments (5dSm^{-1}, 10dSm^{-1}, 17.5 dSm^{-1}, and 25 dSm^{-1}) were applied. The plant height, diameter, plant fresh and dry weight, essential oil yield, total phenols and total flavonoids were recorded for each treatment. Results showed reduction in plant height, diameter, plant fresh and dry weight with increasing the dose of salinity. Maximum yield was achieved with fertilizer application at 2:2 (NPK:Urea). An increase was observed in total phenols and flavonoids at 25 dSm^{-1} of salt stress and (2:2) N-fertilizers. Essential oil yield was decreased at 25ds/m^{-1} while, highest essential oil yield (1.4%) was observed at 2:2 (NPK:Urea). Antioxidant activity of essential oil of *Origanum vulgare* increased with increasing the salinity and fertilizer doses. Higher antioxidant activities were observed at higher concentrations of essential oil.

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Keywords | Oregano, Essential oil, Antioxidant activity, Polyphenols, Flavonoids, Nitrogen fertilizer, Salinity

Introduction

Origanum vulgare L. is a highly valued medicinal herb and belongs to family Lamiacea, mainly produced in France, Greece, Spain, Turkey, Chile, Mexico and Peru (Barreyro *et al.*, 2005). Oregano possesses ample range of bioactive and pharmacological compounds in which the major constituents are mono and sesquiterpenes (Levya-Lopez *et al.*, 2017). The prime terpenes reported in the different species of oregano are carvacrol, thymol, γ-terpinene and p-cymene and β-caryophyllene

giving a peculiar fragrance to oregano essential oil. These compounds impart oregano essential oil potent anti-bacterial, anti-fungal, antioxidant, anti-inflammatory, anticancer and antidiabetic properties (Govaris *et al*., 2010; Figiel *et al*., 2010; Zheljazkov *et al*., 2012; Wei *et al*., 2015).

Plant nutrition is an important factor that increases plant yield. Nitrogen is present in the structure of nucleic acids and protein molecules. The nitrogen containing porphyrin structure is metabolically important for chlorophyll pigments essential for photosynthesis and respiration. Medicinal plants are severely affected by different environmental stress such as salinity and drought. Plant health plays vital role in herbage yield and ultimately higher essential oil contents. Essential oil constituents and concentration of the compounds present in the essential oil usually vary due to a great diversity of factors such as species, pests, soil conditions, harvest season, geographical location, climatic and growth conditions (Azizi and Honermeier, 2009; Baranauskiene *et al*., 2013; Gerami *et al*., 2016). Salinity reduces plant growth due to accumulation of Na^+ or Cl^- to toxic levels in cells, creates ionic imbalance and induces oxidative stress (Munns and Tester, 2008). The biosynthesis of secondary metabolites even though regulated genetically but environmental influences, particular growing region, agronomic conditions and harvesting time effect both quantity and quality of essential oil of aromatic plants (Bernstein *et al*., 2009; Miguel, 2010; Baydar *et al*., 2004). Decline in nitrogen promote volatile oil production in annual herbal plants. Robret (1986) reported decrease in total oil yield in *Juniperus horizontalis* (creeping juniper) While increase in thyme (Thymus *vulgaris* L.) was reported by Baranauskienne *et al*. (2003). An increase up to 2% in essential oil in different oregano species have also been reported by researchers (Karamanos and Sotiropoulou, 2013).

Oregano is gaining popularity in Pakistan for its typical flavor as culinary herbs in pizza, pasta and different sauces and its essential oil possesses strong antioxidant compounds making it valuable as medicinal herb for treatment of different ailments of respiratory and gastrointestinal tract. Studies on agronomic factors and nitrogen fertilization of different medicinal herbs are scarce in Pakistan. A glasshouse pot trial was aimed to evaluate the effect of nitrogen fertilizer and salinity on the essential oil contents and bioactivity of *Origanum vulgare* L. This will help in generating information for profitable cultivation of oregano under local conditions. Cultivation of medicinal herbs will also help in conservation of biodiversity and stoppage of collection of wild herbs without replenishing them.

Materials and Methods

The experiment was conducted National Medicinal, Aromatic Plants and Herbs Program (NMAP and HP), PGRI, NARC in growing season of 2022-23. After germination of seeds, plants were transplanted into the earthen pots having diameter of 20cm and depth of 25cm.

Four treatments [1:1, 1:2, 2:1, 2:2] of Nitrogenous fertilizer (NPK and Urea) with three replications for each dose were applied. Fertilizer applications were measured by assuming that one hectare land having $2x10^6$ kg soil. NaCl was applied in four treatments [5ds/m, 10ds/m, 17.5ds/m, 25ds/m] in three replications for each dose. The salinity concentrations were measured by the formula.

$$\text{Amount of salt} = \frac{\text{Saturation \%} \times \text{TSS} \times \text{M. weight of salt} \times \text{Weight of soil}}{100} \times 1000$$

Plant material (leaves and branches) was harvested and essential oil was extracted through hydro-distillation according to the method outlined in the Ph. Eur. Suppl. 11.8. Strasbourg, France: Council of Europe; 2025.

Percentage yield was calculated as:

$$\text{Percentage yield} = \frac{\text{Amount of essential oil (ml)}}{\text{Amount of dried plant material (g)}}$$

Total phenols were determined spectrophotometrically by following Marinova *et al*. (2005). Gallic acid was used as standard. Total flavonoids were determined by following method described by Marinova *et al*. (2005). Catechin was used as standard. DPPH assay was used to determine the antioxidant activity of oregano essential oil obtained from each treatment. The experimental layout was factorial in a complete randomized design (CRD), with three replications. Each replication contained three pots.

Results and Discussion

Four different salt (NaCl) concentrations (5dS/m, 10dS/m 17.5dS/m and 25dS/m) were used to check

the tolerance level of oregano plant. The results presented in Figure 1 depicts significant reduction in plant height, diameter, plant fresh and dry weight with increased level of salt concentrations. As the salt concentration increased gradually, these parameters decreased. At 5dS/m oregano herbage yield was statistically sound 170g PFW, 69g PDW, 4.9inchs PH and 17.35 inches PD thereafter significantly reduction was noticed up to 25 dS/m. Caliskan *et al.* (2010) reported decreased plant growth in sweet basil with increasing the salinity levels from 0.4 to 8.0 dS/m. The effect of salinity was more visible in roots than in leaves which ultimately led to retarded plant growth and reduced dry plant material. Kumar *et al.* (2021) also reported reduction in plant height and number of branches in water dropwort (*Oenanthe javanica*) cultivars when treated with 50, 100 and 200 mm NaCl.

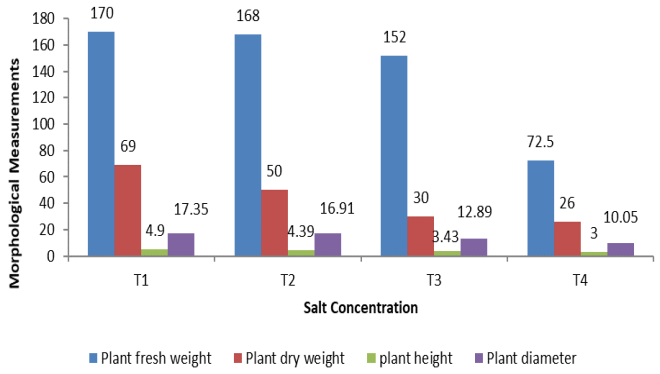

Figure 1: *Effect of salinity on morphological characters of Origanum vulgare.*

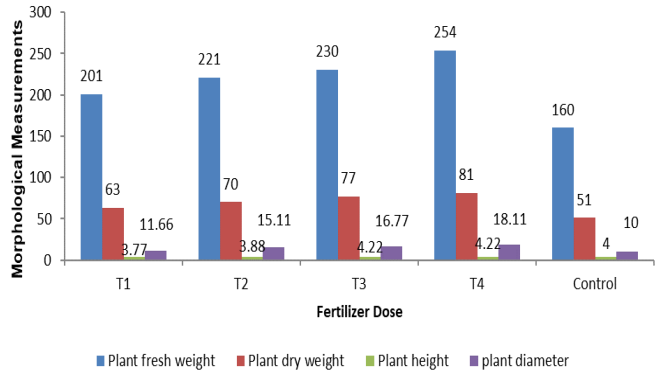

Figure 2: *Effect of fertilizer dose on morphological characters of Origanum vulgare.*

Optimization of plant nutrition in medicinal crops under field conditions is a challenge because nutrient supply may promote plant growth but production of secondary metabolites might be compromised (Liu *et al.*, 1999). The balance diet of nitrogen is of special importance for biomass production and secondary metabolites like alkaloids (Lambers *et al.*, 2008; Palumbo *et al.*, 2007). In disparity, N-deficiency can support the production of essential oils (Martins *et al.*, 2007). Four different doses (1:1, 1:2, 2:1 and 2:2) of NPK and urea were applied to assess the plant height, diameter, fresh and dry plant weight during present study. The results indicate maximum plant growth at 2:2 (NPK:Urea) treatment as shown in Figure 2. Higher doses of NPK and urea resulted in increased plant growth which ultimately yielded increased fresh and dry plant material. Our results are supported by Al-Humaid (2004) who narrated that the supply of NPK fertilizer favored both biomass and alkaloid production in *Datura stramonium* plants. Deficiency of P and K can stimulate the production of alkaloids and essential oils (Gremigni *et al.*, 2003; Martins *et al.*, 2007).

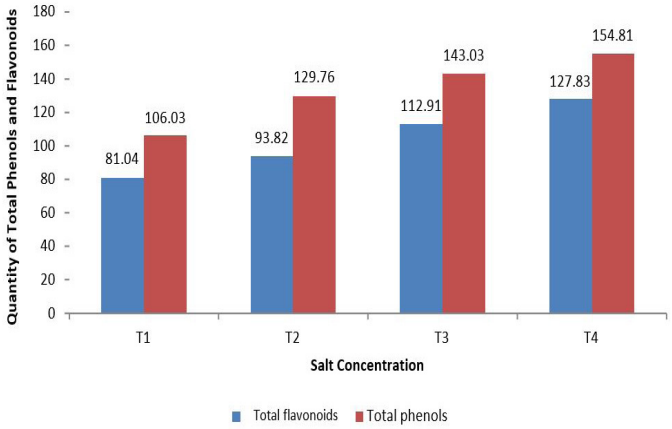

Figure 3: *Effect of salinity on total phenols and flavonoids in Origanum vulgare.*

Total polyphenols and flavonoid contents of methanolic extract of dried oregano leaves were determined under different salinity levels. Results revealed that the amount of total phenolic contents were higher than total flavonoids contents in oregano (Figure 3). With increasing the salinity level, polyphenols and flavonoids were increased which indicate that abiotic stress induces the secondary metabolites in medicinal herbs. Highest amount of total phenolic and total flaovonids contents were recorded (154.81mg GAE/100g DW, 127.83 mg CE/100g DW), respectively when salt concentration was kept 25dS/m, while lowest total phenolics and total flavonoids content were observed in 5dS/ml treated plants. Kasera *et al.* (2018) reported higher levels of alkaloids and phenolics in *Sida cordifolia* and *Boerhavia diffusa* in higher salt concentration but with a reduced biomass yield. This may be due to response of plants to abiotic stress which triggered

the production of secondary metabolites. Various environmental stresses may alter the behavior and structure of secondary metabolites (Waller and Nowacki, 1978).

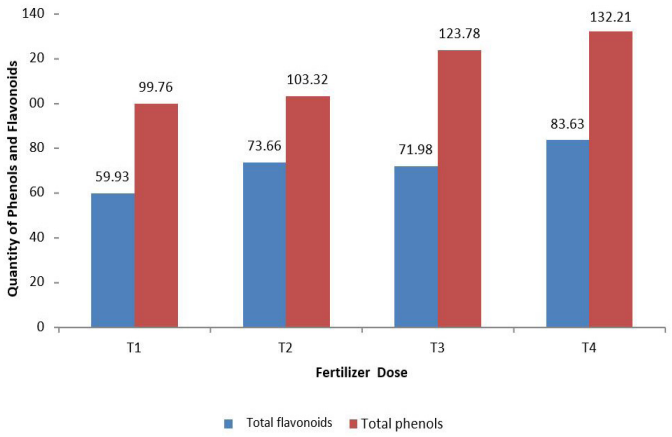

Figure 4: *Effect of fertilizer dose on total phenols and flavonoids in Origanum vulgare.*

The biosynthesis of biologically active compounds is influenced by nitrogenous fertilizers. It was observed that the amount of total phenolics and flavonoids was improved as the dose of NPK ascended (Figure 4). In four different treatments of fertilizer, NPK:Urea in 2:2 treatment showed highest amount of total polyphenols and flavonoids which was 132.21 mg GAE/g DW and 83.63 mg CE/g DW, respectively. Lowest amount was recorded at T_1 1:1 which was 99.76 mg GAE/g DW and 59.93 mg CE/g DW. Correspondingly, Nguyen and Niemeyer (2008) reported higher levels of polyphenols, falvonoids and anthocyanins in basil when nitrogenous fertilizer dose was increased. Zhao *et al.* (2021) attributed higher polyphenol and flavonoid contents in *Allium fistulosum* to higher doses of nitrogen fertilizer ultimately contributing in higher anticancer activities. Ma *et al.* (2023) studies the effect of nitrogen fertilizer on phenols, falvonoids and antioxidant activity of brown rice. They observed an increase in phenols and flavonoids with increasing the level of nitrogen but at 260 kg N/100 m^2 polyphenols experienced a decline which may be due to specific response of each phenol to nitrogen fertilizer.

The results showed that salinity stress significantly increased the yield of essential oil in oregano. An increase in essential oil yield (1.4%) was observed up to a salt concentration of 17.5ds/m^{-1} but it decreased at 25ds/m^{-1} (Figure 5). Oregano did not tolerate to increased level of $NaCl_2$ 25ds/m concentration. Salinity reduced essential oil yield as salt concentration increased up to 25ds/m.

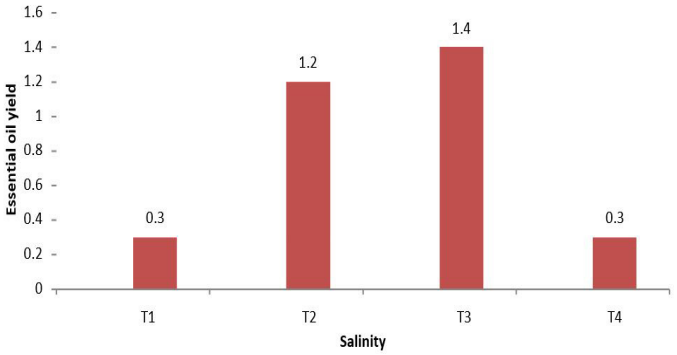

Figure 5: *Effect of salinity on essential oil yield.*

Our results are strongly supported by Olfa *et al.* (2009), they also noticed decrease essential oil contents in higher saline conditions in *Origamum majorana*. Aziz *et al.* (2008) also reported decrease in vigour in growth parameter but increased essential oil percentage in peppermint cultivated in salinity condition. Safikhani *et al.* (2007) and Khammari *et al.* (2007) stated decline in germination rate, less initiation of flowers and suppression of oil contents in *Nigella sativa* which may be due to unavailability of water for plant growth and toxicity of NaCl.

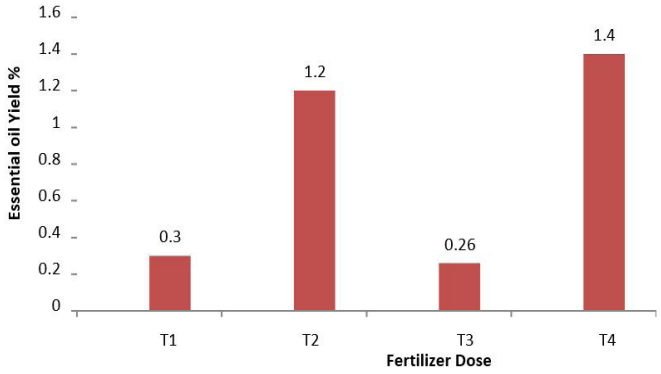

Figure 6: *Effect of fertilizer dose on essential oil yield.*

Application of nitrogen doses increased essential oil yield. Various doses of NPK:Urea with different ratios were applied to asses optimal essential oil yield. Provision of nitrogen enhances vegetative growth and proper functioning of different metabolic pathways. Moreover, nitrogen is integral part of many organic compounds of the plant metabolic system and activation of photosynthesis. Nitrogen fertilization might enhance the biosynthesis of essential oil through its direct or indirect role in plant metabolism resulting in more plant metabolites. During present studies, highest essential oil yield was observed at 2:2 (NPK:Urea), 1.4% and lowest essential oil yield 0.28% (Figure 6). A decline in essential oil

yield was recorded at 2:1 (NPK:Urea). Mauyo *et al.* (2008) attributed higher essential oil yield with increasing fertilizer to increased fresh herbage yield. These findings correlate with those of Omer *et al.* (2008), who said that nitrogen fertilizer was helpful in increasing essential oil and *de novo* meristem cell building and metabolism in *Origanum syriacum* and *Ocimum americanum*.

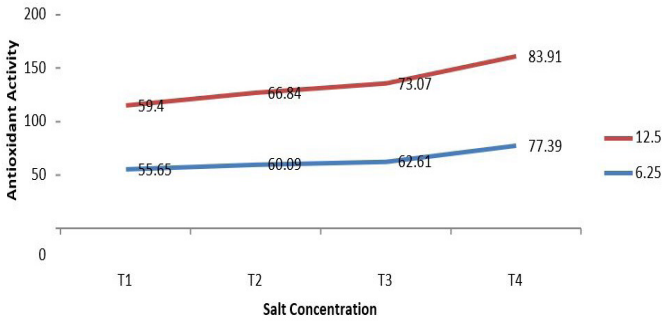

Figure 7: *Effect of salinity on antioxidant activity in Origanum vulgare.*

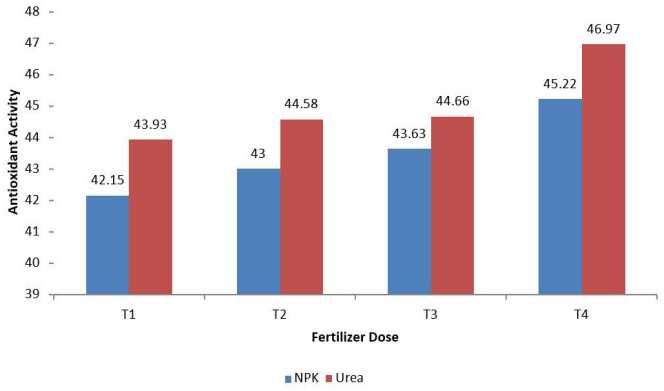

Figure 8: *Effect of fertilizer dose on antioxidant activity in Origanum vulgare.*

Antioxidant activity of essential oil of *Oreganum vulgare* was evaluated through DPPH free radical scavenging assay at all four treatments of salinity and fertilizer application. Investigations revealed that with increase of salinity and fertilizer dose, higher antioxidant activities were observed. Zhu (2000) reported that Salt stress can induce oxidative stress. Younessi-Hazekhanlu *et al.* (2021) reported higher antioxidant activities induced by mild salinity levels in *Ballota nigra* which may be due to shift in metabolite expression. Zhou *et al.* (2018) also depicted higher antioxidant activities at low and mild salinity levels in *Schizonepeta tenuifolia* but lower antioxidant activities at 75 and 100mM NaCl concentration. It was found that at low and moderate salinity level, glandular trichome density is increased in leaves which enhances secondary metabolite production. In accordance to present studies, Hess *et al.* (2022) documented higher antioxidant activities in basil cultivars with higher doses of nitrogenous fertilizers. Nguyen and Niemeyer (2008) indicated that manipulation of nitrogen fertilization levels may be an effective method to increase the expression of polyphenolic compounds in basil and ultimately antioxidant activities.

It could be inferred that concentration of bioactive compounds; essential oil yield and antioxidant activity could be manipulated in oregano herb. Further agronomic trials should be conducted in field with physiological attributes for potential exploration of the herb under local conditions.

Novelty Statement

Oregnao is an exotic culinary herb introduced in Pakistan. No studies are available on agronomic practices for high yield of the herb having higher medicinal potential.

Author's Contribution

Riffat Tahira: Conceived the idea, supervised the experiments and manuscript writing.
Ashtar Khan: Conducted the Nitrogen and salinity application trials.
Tariq Rafique: Provided lab facilities, supervised biochemical tests.
Zoha Abeer: Conducted biochemical test.
Umer Iqbal: Conducted essential oil extraction experiments and methodology write up.
M. Muneer: Literature review and proof reading of manuscript.
Vania Areej Cheema: Conducted biochemical tests and results write up.
Nadar Khan: Conducted statistical analysis and result write up.

Conflict of interest

The authors have declared no conflict of interest.

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