**Proposed PhD Project, submitted to:**

**Department of Biology, College of Science/Salahaddin University**

**Supervisor: Prof. Dr. Abdulghany Omer Ismaeel Sarmamy**

**E-mail address:** [**Abdulghany.ismseel@su.edu.krd**](mailto:Abdulghany.ismseel@su.edu.krd)

**Proposal Title:**

**Effect of N, K and Ascorbic Acid Applied under Different Soil Moisture Content and Light Intensity on Chemical Constituents of Rosemary *Rosmarinus officinalis***

**Objectives:**

Rosemary*Rosmarinus officinalis* L. is well-known medicinal plants, which have ability to synthesize a wide variety of chemical compounds, used to perform important biological functions. Many of these [chemical](https://en.wikipedia.org/wiki/Phytochemicals) compounds have beneficial effects on human health. The beneficial chemical compounds present in plant tissues at low concentrations, but they could be increased by exposing the plants to stresses such as water stress, light deficiency or by adding of some supplements. These activities may led to some modifications or changing in genetic information of plants to produce larger amounts of chemical compounds.

**Methodology:**

1. Greenhouse (Pot) experiments could be carried out to study the effects of different treatments (application of nitrogen, potassium and ascorbic acid, different soil moisture contents and different light intensity, solely and their interactions, also) applied on Rosemary plants grown in pots through growth until maturity.
2. Studding the effects of different treatments and their interactions on chemical constituents of plants and their concentrations, especially the effects of stresses.
3. Determining the effects of different treatments and their interactions on genetic information if there is.
4. Studding the effects of Ascorbic acid and it's interactions on the plant ability to resist against stresses.

Prof. Dr. Abdulghany Omer Ismaeel Sarmamy

Department of Biology

College of Science

University of Salahaddin-Erbil.

**Effect of N, K and Ascorbic Acid Applied under Different Soil Moisture Content and Light Intensity on Chemical Constituents of Rosemary *Rosmarinus officinalis***

1. **N: 100, 200, 300 kg/hectare, = 12**
2. **K: 50, 100, 150, 200 ppm,=16**
3. **AA: 50, 100, 150 ppm, =12**
4. **Moisture: 100, 50% F.C. =8**
5. **Light: 100%, 75%, 50%, =12**
6. **O.M.: 1%, 5%, 10%.=12**

**Total =72**

**Relative Water Content (RWC) =** (Wfresh- Wdry) / (Wturgid- Wdry) x 100, where

Wfresh is the sample fresh weight, Wturgid is the sample turgid weight after saturating with distilled water for 24 h at 4 °C, and Wdry is the oven-dry (70 °C for 48 h) weight of the sample (Weatherley, 1950).

**Voltile oil Content** = The volatile oil percentages in rosemary leaves obtained from each replicate of every treatment were determined by a water distillation method described in British Pharmacopea (1963), using the following equation:

Volatile oil percentage = oil volume in the graduated tube / fresh weight of sample x 100. Then, the oil yield /plant was calculated.

**Volatile oil composition**

The obtained volatile oil from second cut was dehydrated over anhydrous sodium sulphate and stored in refrigerator until GC-MS analysis. Essential oil samples were performed using GC-HP 5890 Colum HP 130 meter Internal Diameter 0.25 millimeter equipped with flame ionization detector (FID) and a carbon wax fused silica column (50 m x 0.25 mm. i. d., film thickness 0.1 μm). Helium gas was used. The oven temperature was programmed from 60 to 240о C at 3о C per minute.

**Chlorophyll determination**

Samples of fresh leaves were taken for chlorophyll determination. Extraction in acetone was repeated until all pigments were extracted. Chlorophyll content was determined in samples of fresh leaves according to Sadasivam and Manickam

(1992). The absorbance of extracts was determined by a spectrophotometer (type Pharmacia, LKB-Novaspec II). The chlorophyll content was calculated as mg g–1 fresh weight.

**2.6.2. Carbohydrates and Nutrient Percentages**

Leaf samples were air dried at 70 °C for 24 h, then ground to fine powder and was taken for determination of the following chemical analyses:

- Total carbohydrate percentages were determined as previously described by

**H**erbert *et al.* (1971).

- Nitogen percentages were determined by micro-Kjeldahl method, phosphorus was spectrophotometrically determined and potassium was determined by flame photometer as described by A.O.A.C. (1995).

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**Journal of Medicinal Plants Studies** Vol. 1 Issue. 3 2013 www.plantsjournal.com Page | 12. **Effect of Deficit Irrigation on Growth, Yield and Volatile Oil** **Contenton *Rosmarinus officinalis* L. Plant** Hassan, F.A.S 1\*, Bazaid. S.2, Ali. E.F3

**Exp.1:** **Effect of Nitrogen and Ascorbic Acid Applied under Different Soil Moisture Content on growth and Chemical Constituents of Rosemary *Rosmarinus officinalis***

**N=3, AA=3, M=3 &Rep. =4 3x3x3x4= 108 pots(3x3x3x3= 81**

**Then, No. of Exp. Units= 3x3x3x4= 108 units**

**Anova Table**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Treatments** | **d. f.** | **Units** | **SS** | **MS** | **Ftable** | **Fcalculated** |
| A(N) | 2 | 3 |  |  |  |  |
| B(AA) | 2 | 3 |  |  |  |  |
| C(M) | 2 | 3 |  |  |  |  |
| AB | 4 |  |  |  |  |  |
| AC | 4 |  |  |  |  |  |
| BC | 4 |  |  |  |  |  |
| ABC | 8 |  |  |  |  |  |
| Error | 81 | R=4 |  |  |  |  |
| Total | 107 | 3x3x3x4  =108 |  |  |  |  |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| No. | Factors | Levels | Comb. Trea. (Intera.) | The Means | Exp Units |  |
| 1 | **N**  (effects of Nitrogen Solely) | N1(100) | N1 | N | 3 | 9 |
| 2 | N2(200) | N2 |  | 3 |
| 3 | N3(300) | N3 |  | 3 |
| 4 | **AA**  (effects of Ascorbic Acid Solely) | A1(50) | A1 | A | 3 | 9 |
| 5 | A2(100) | A2 |  | 3 |
| 6 | A3(150) | A3 |  | 3 |
| 7 | **Moisture (M)**  (effects of Soil Moisture Content Solely) | M1 (100%) | M1 | M | 3 | 9 |
| 8 | M2 (75%) | M2 |  | 3 |
| 9 | M3 (50%) | M3 |  | 3 |
| 10 | Interactions between the effects of Nitrogen and Ascorbic acid | | N1a1 | NA | 3 | 27 |
| 11 | N1a2 | NA | 3 |
| 12 |  | | N1a3 | NA | 3 |
| 13 | N2a1 | NA | 3 |
| 14 | N2a2 | NA | 3 |
| 15 | N2a3 | NA | 3 |
| 16 | N3a1 | NA | 3 |
| 17 | N3a2 | NA | 3 |
| 18 | N3a3 | NA | 3 |
| 19 | Interactions Between the Effects of Nitrogen and Soil Moisture Content | | N1m1 | NM | 3 | 27 |
| 20 | N1m2 | NM | 3 |
| 21 | N1m3 | NM | 3 |
| 22 | N2m1 | NM | 3 |
| 23 | N2m2 | NM | 3 |
| 24 | N2m3 | NM | 3 |
| 25 | N3m1 | NM | 3 |
| 26 | N3m2 | NM | 3 |
| 27 | N3m3 | NM | 3 |
| 28 | Interactions Between the Effects of Ascorbic Acid and Soil Moisture Content |  | A1m1 | AM | 3 | 27 |
| 29 | A1m2 | AM | 3 |
| 30 | A1m3 | AM | 3 |
| 31 | A2m1 | AM | 3 |
| 32 |  | A2m2 | AM | 3 |
| 33 |  | | A2m3 | AM | 3 |
| 34 | A3m1 | AM | 3 |
| 35 | A3m2 | AM | 3 |
| 36 | A3m3 | AM | 3 |
| 37 | Interactions Between the Effects of Nitrogen, Ascorbic Acid and Soil Moisture Content | | N1a1m1 | NAM | 3 | 81 |
| 38 | N1a1m2 | NAM | 3 |
| 39 | N1a1m3 | NAM | 3 |
| 40 | N1a2m1 | NAM | 3 |
| 41 | N1a2m2 | NAM | 3 |
| 42 | N1a2m3 | NAM | 3 |
| 43 | N1a3m1 | NAM | 3 |
| 44 | N1a3m2 | NAM | 3 |
| 45 | N1a3m3 | NAM | 3 |
| 46 | N2a1m1 | NAM | 3 |
| 47 | N2a1m2 | NAM | 3 |
| 48 | N2a1m3 | NAM | 3 |
| 49 | N2a2m1 | NAM | 3 |
| 50 | N2a2m2 | NAM | 3 |
| 51 | N2a2m3 | NAM | 3 |
| 52 | N2a3m1 | NAM | 3 |
| 53 | N2a3m2 | NAM | 3 |
| 54 | N2a3m3 | NAM | 3 |
| 55 | N3a1m1 | NAM | 3 |
| 56 | N3a1m2 | NAM | 3 |
| 57 | N3a1m3 | NAM | 3 |
| 58 | N3a2m1 | NAM | 3 |
| 59 | N3a2m2 | NAM | 3 |
| 60 | N3a2m3 | NAM | 3 |
| 61 | N3a3m1 | NAM | 3 |
| 62 | N3a3m2 | NAM | 3 |
| 63 | N3a3m3 | NAM | 3 |
|  |  | |  |  |  | 189 |

**Exp.2:** **Effect of *Potassium* and *Light Intensity* on growth Characteristics and Chemical Constituents of Rosemary *Rosmarinus officinalis***

**3x3x3x3=81**

**81+81=162 pots**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Treatments** | **d. f.** | **Units** | **SS** | **MS** | **Ftable** | **Fcalculated** |
| A (K) | 2 | 3 |  |  |  |  |
| B(LI) | 2 | 3 |  |  |  |  |
| AB | 2 |  |  |  |  |  |
| Error | 81 | R=4 |  |  |  |  |
| Total | 107 | 3x3x4=36 |  |  |  |  |

**108 + 36 = 144 pots**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| No. | Factors | Levels | Combined Treatments | The Means |
| 1 | K | k1 (50) | k1 | k |
| 2 |  | k2 (100) | k2 |  |
| 3 |  | k3 (150) | k3 |  |
| 4 | L | L1 (100%) | L1 | L |
| 5 |  | L2 (75%) | L2 |  |
| 6 |  | L3 (50%) | L3 |  |
| 7 |  | K1L1 |  | KL |
| 8 |  | K1L2 |  |  |
| 9 |  | K1L3 |  |  |
| 10 |  | K2L1 |  |  |
| 11 |  | K2L2 |  |  |
| 12 |  | K2L3 |  |  |
| 13 |  | K3L1 |  |  |
| 14 |  | K3L2 |  |  |
| 15 |  | K3L3 |  |  |
| 16 |  |  |  |  |
| 17 |  |  |  |  |
| 18 |  |  |  |  |
| 19 |  |  | N1L1 | NL |
| 20 |  |  | N1L2 |  |
| 21 |  |  | N1L3 |  |
| 22 |  |  | N2L1 |  |
| 23 |  |  | N2L2 |  |
| 24 |  |  | N2L3 |  |
| 25 |  |  | N3L1 |  |
| 26 |  |  | N3L2 |  |
| 27 |  |  | N3L3 |  |
| 28 |  |  | K1L1 | KL |
| 29 |  |  | K1L2 |  |
| 30 |  |  | K1L3 |  |
| 31 |  |  | K2L1 |  |
| 32 |  |  | K2L2 |  |
| 33 |  |  | K2L3 |  |
| 34 |  |  | K3L1 |  |
| 35 |  |  | K3L2 |  |
| 36 |  |  | K3L3 |  |
| 37 |  |  | N1k1L1 | NKL |
| 38 |  |  | N1k1 L2 |  |
| 39 |  |  | N1k1 L3 |  |
| 40 |  |  | N1k2L1 |  |
| 41 |  |  | N1k2L2 |  |
| 42 |  |  | N1k2L3 |  |
| 43 |  |  | N1k3L1 |  |
| 44 |  |  | N1k3L2 |  |
| 45 |  |  | N1k3L3 |  |
| 46 |  |  | N2k1L1 |  |
| 47 |  |  | N2k1L2 |  |
| 48 |  |  | N2k1L3 |  |
| 49 |  |  | N2k2L1 |  |
| 50 |  |  | N2k2L2 |  |
| 51 |  |  | N2k2L3 |  |
| 52 |  |  | N2k3L1 |  |
| 53 |  |  | N2k3L2 |  |
| 54 |  |  | N2k3L3 |  |
| 55 |  |  | N3k1L1 |  |
| 56 |  |  | N3k1L2 |  |
| 57 |  |  | N3k1L3 |  |
| 58 |  |  | N3k2L1 |  |
| 59 |  |  | N3k2L2 |  |
| 60 |  |  | N3k2L3 |  |
| 61 |  |  | N3k3L1 |  |
| 62 |  |  | N3k3L2 |  |
| 63 |  |  | N3k3L3 |  |

1. Plant height (cm),
2. Number of leaves,
3. Leaf area (cm2),
4. Stem diameter (cm),
5. No. of Branches,
6. Fresh weight of hoots**,**
7. Dry weight of **shoots,**
8. Fresh weight of roots**,**
9. Dry weight ofroots,

**Chemical analysis of shoots and Roots:**

1. Total Nitrogen (mg g-1)**,**
2. Total protein content (mg g-1)
3. Total Potassium (mg g-1),
4. Total potassium and sodium content (mg g-1)**,**
5. Mineral content (ppm)
6. Mineral nutrients included Fe,
7. Zn,
8. Mg, and
9. Ca,
10. Ash content of leaves (%),
11. Essential Oil content of shoots,
12. Proline determination,
13. Phenol content,
14. Chlorophyll a content (mg g-1 fresh weight,
15. Chlorophyll b content (mg g-1 fresh weight,

**Relative Water Content (RWC)**

**Voltile oil Content**

**Volatile oil composition**

**Carbohydrates**

Effects of Potassium, Ascorbic Acid and soil Moisture Content on some growth Characteristics and Chemical Constituents of Rosemary

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| K | A1 | | | | | | | | A2 | | | | | | A3 | | | | | |
| AA |  | B1 | | | | B2 | | B3 | | B1 | | B2 | | B3 | | B1 | | B2 | | B3 | |
| C1  (M) | R1 |  | | | |  | |  | |  | |  | |  | |  | |  | |  | |
| R2 |  | | | |  | |  | |  | |  | |  | |  | |  | |  | |
| R3 |  | | | |  | |  | |  | |  | |  | |  | |  | |  | |
| R4 |  | | | |  | |  | |  | |  | |  | |  | |  | |  | |
| Yij1 |  | | | |  | |  | |  | |  | |  | |  | |  | |  | |
| C2 | R1 | |  | |  | |  | |  | |  | |  | |  | |  | |  | |
| R2 | |  | |  | |  | |  | |  | |  | |  | |  | |  | |
| R3 | |  | |  | |  | |  | |  | |  | |  | |  | |  | |
| R4 | |  | |  | |  | |  | |  | |  | |  | |  | |  | |
| Yij2 |  | | | |  | |  | |  | |  | |  | |  | |  | |  | |
| C3 | R1 | | |  |  | |  | |  | |  | |  | |  | |  | |  | |
| R2 | | |  |  | |  | |  | |  | |  | |  | |  | |  | |
| R3 | | |  |  | |  | |  | |  | |  | |  | |  | |  | |
| Yij3 | R4 | | | |  | |  | |  | |  | |  | |  | |  | |  | |
|  |  | | | |  | |  | |  | |  | |  | |  | |  | |  | |

ABC= 3x3x3=**27** Treatments x4= **108** Experimental Units

Effects of Nitrogen and Light Intensity on some growth Characteristics and Chemical Constituents of Rosemary

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| N | N1 | | | N2 | | | N3 | | |
| LI | L1 | L2 | L3 | L1 | L2 | L3 | L1 | L2 | L3 |
| R1 |  |  |  |  |  |  |  |  |  |
| R2 |  |  |  |  |  |  |  |  |  |
| R3 |  |  |  |  |  |  |  |  |  |
| R4 |  |  |  |  |  |  |  |  |  |
| Yij. |  |  |  |  |  |  |  |  |  |

AB= 3x3= **9** Treatments x4 Replications= **36** Experimental Units