The effect of N, P and micronutrients on yield and essential oil of \textit{Satureja hortensis} L.

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\textbf{ABSTRACT}

This study was conducted to investigate the effect of different amounts of nitrogen, phosphorus and micronutrient on yield and essential oil of \textit{satureja hortensis} L. The experiment was conducted as factorial experiments in randomized complete block design with three replications. This experiment included three levels of nitrogen from ammonium nitrate 0,150,300 kg/ha, two levels of phosphorus from triple super phosphate 50,100 kg/ha and two levels of Fe, Zn and Mn from the iron sulfate, zinc sulfate and manganese sulfate in 50 kg/ha. The analysis of variance results showed that it was significant effect nitrogen in: height, dry weight, number of derivative branches, length of inflorescence, essential oil, content of chlorophyll a in the level of 1% and the effect of phosphorus fertilizer on the length of florescent, essential oil, chlorophyll b in the level of 5% and in the dry weight and the total chlorophyll in the level of 1%. The interaction effect of nitrogen and phosphorus fertilizers were significant in dry weight, phosphorous amount, chlorophyll a and total chlorophyll in the level of 1% and chlorophyll b in the level of 5%. Comparison of mean results showed that the consumption of nitrogen source about 300 kg/ha and phosphorous with the amount of consumption 100 kg/ha have the most effect on the most studied traits, also micronutrients caused to increase the studied traits, however, this increase was not statistically significant. According to the result of these investigation can be concluded that \textit{satureja hortensis} L. optimum yield with the ammonium nitrate fertilizer in 300 kg/ha and supper phosphate triple fertilizer 100 kg/ha obtaind. Since the highest nitrogen application in this experiment demonstrated a better plant function, further investigations using greater amounts of nitrogen is recommended.

\textbf{INTRODUCTION}

Origny (\textit{Satureja hortensis} L.) is an aromatic plant, belonging to \textit{Lamiaceae} species; it is an annual medicinal plant. As origany, it has been known as native to south Europe and parts of North America [1]. Among Lamiaceae species, there are medicine type and therapeutic species which are utilized in treatments for different illnesses. Some of them contain essential oils and great number of these species is used in nutrition, cooked or raw and they are grown because of their beautiful and fragrant flowers [2, 3]. Even though their secondary metabolites is under control of genes, the amount of their production is considerably influenced by environmental conditions among which one can consider the physical and chemical properties of soil and highly consumed and little consumed nutrients.

Nitrogen is one of the important elements for agricultural plants and influences both factors of quality and quantity related to the plant secondary metabolites. In this respect, Niakan et al., [4] analyzing the effect of three fertilizers NPK on dry and fresh weights, leaf area and the amount of essential oil in peppermint (\textit{Menta piperita} L.), could find out that treatment with 200-100-200 kg/ha NPK, the leaf area and the amount of essential oil increased. Babalar et al., [5] studied the influences of nitrogen and calcium carbonate on the growth, the amount of Rosemarinic acid and the yield of origany and demonstrated that increasing nitrogen fertilizer up to 100 kg/ha, plants height, and its dry and fresh weight increased considerably and with the fertilizer level of 150 kg/ha was set into a statistical group.

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Since the most important issue in medicinal plants is whether the material being extracted from them is natural, one should be careful in applying the chemical fertilizers so it is critical to determine the right amount of fertilizer [6]. Abbaszadeh et al., [7] Investigating the effect of different amounts of nitrogen fertilizer and various methods of using it on the amount of essential oils in Melissa Officinalis noticed the importance of nitrogen fertilizer in proliferating the seeds of these plants. Ademar et al., [8] found that phosphorus fertilizer caused the biological yield, flowering crop yield and essential oil of coriander (Coriandrum sativum L.) medicinal plant to increase. Rahmani et al., [9] reported in relation to the influence of nitrogen on Melissa Officinalis that in different growth levels led to the increase in the number of lateral branches, early flowering, and elongation of flowering crops and seed germination percentage and there was a significant difference relative to the evidence.

The concentration of trace elements in soil is one of the basic criteria in producing the available medical compounds that are freshly cultivated. It indicates the fact that the amount of absorption and their entrance is proportional to the concentration which has great influence on the medicinal compounds biosynthesis [10].

Following the rapid growth of demand for medicinal plants and herbal medicines worldwide, their cultivation has specifically increased during recent years and using chemical fertilizers the level of food products has increased, however, the influence of different amounts of chemical fertilizers on the types and amounts of secondary metabolites in aromatic and medicinal plants have been paid little attention [11]. So, considering the influence of environmental factors such as nutritional elements on the quality and quantity of origny, in this paper, the nutritional effect of nitrogen, phosphorus and trace elements on the quality and quantity of origny (Satureja hortensis L.) as an aromatic and important medicinal plant has been evaluated.

2-Research Methods:

This investigation was conducted in Marand area in the longitude of 45°, 45', 4'' east, with the latitude of north 38°, 25', 52''. The place is at the height 1320 meter above sea level. To determine the physicochemical properties of the soil of the place being investigated, a sample of compound soil was provided from a depth of 0-30 centimeter from soil and the relevant results have been included in table 1.

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>EC</th>
<th>pH</th>
<th>T.N.V</th>
<th>OC</th>
<th>sand</th>
<th>clay</th>
<th>Silt</th>
<th>Pava</th>
<th>Kava</th>
<th>Fe</th>
<th>Zn</th>
<th>Mn</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-30</td>
<td>1.3</td>
<td>8.2</td>
<td>13.2</td>
<td>0.66</td>
<td>24</td>
<td>42</td>
<td>4.51</td>
<td>4.51</td>
<td>780</td>
<td>3</td>
<td>0.22</td>
<td>4.9</td>
</tr>
</tbody>
</table>

The experiment was conducted in the form of three element factorial with randomized complete blocks designs and three replications. The characteristics of experimental factors are: first factor: nitrogen in three levels (N1: with no nitrogen application, N2: 150 kg/ha, and N3: 300 kg/ha from Ammonium nitrate source), second factor: phosphorus in two levels (P1: 50 kg/ha, P2: 100 kg/ha from triple supper phosphate), Third factor: trace elements in two levels (M0: no application and M1: 50 kg/ha iron sulphate, zinc sulphate, manganese sulphate). In the preparation stage, nitrogen, phosphorus, zinc, iron and manganese fertilizers were determined and consumed according to experimental issues.

After the preparation stage and applying the plan for an experimental farmland, irrigation was conducted. The size of each part was considered as 3.5 *2.5 m2. After the land was ploughed, cultivation of origany (Satureja hortensis L.) was cultivated. Subsequent to complete flowering of origany, the cultivated parts were taken. To determine the yield of dry matter, samples were dried in 75°C. The number of lateral branches was measured via counting and the length of inflorescence was scaled using a ruler. To extract the essential oil, Klevenger machine was used. Phosphorus was measured by colorimetric methods of Molybdite Vanadate, the concentration of chlorophyll was measured using Sairam method [12] method and chlorophyll (a, b and total) was accounted for using formulation (Chlorophyll a=12.25(A663.2)-2.79(A646.8), Chlorophyll b= 21.5(A646.8)-5.1(A663)) and by having the fresh weight of leaf and the concentrations of chlorophyll a, b and total in mg/gr. In these relations, (A663), (A646) were the absorption in wavelength 663 and 646 nanometer, respectively. The obtained data and the relevant variance were analyzed through SPSS software. The comparison of averages was done using the multi-amplitude test of Dunken with the probability level of 5 percent.

RESULTS AND DISCUSSION

Yield of dry matter:

The results of variance analysis related to the yield of dry matter indicated that factors including nitrogen, phosphorus, trace elements and interaction effect of nitrogen with phosphorus and nitrogen with trace element had a substantial effect on this quality. However, the interaction between phosphorus and trace elements and the threefold interaction among nitrogen, phosphorus and trace elements were not meaningful (Table 2). Comparison of the average interaction effect between nitrogen and phosphorus indicated that the most amount
The number of lateral branches:

In flowering stage, levels of nitrogen and phosphorus had a substantial influence on the number of lateral branches in the bush and the interaction of applications didn’t have substantial influence for this quality (table 3). The most number of lateral branches in 300 kg/ha of ammonium nitrate was 22.0 which had a substantial increase compared to non-consumption of nitrogen. The least number of lateral branches was related to the non-consumption of nitrogen being 18.9 (table 3). In application of 100 kg/ha phosphorus, the highest number of lateral branches was observed to be 21.29 which was substantially different from 50 kg/ha phosphorus (Table 2).

The number of lateral branches in the plant is one of the most important features for evaluating the increased weight of origany bush which widens the plant and because of that, photosynthesis level increases. This leads to the increased productive organs and increased yield. Nitrogen fertilizer leads to the increased branch and leaf production in spearmint plants [4] that conforms to the results of the present research. In studies carried out by [17] on Lavandula stoechas plants, they found that the influence of various amounts of nitrogen on the number of subordinate branches is meaningfully substantial.

Inflorescence length:

The obtained results indicated that from the perspective of Inflorescence length, only nitrogen had a meaningful effect in the probability level of %1. Using phosphorus, trace elements and also the interaction effect among different factors didn’t have a meaningful effect on Inflorescence length (table 2). The results of comparing means indicated that in the 300 kg/ha of ammonium nitrate in, the highest Inflorescence length was 20.4 centimeter that was meaningful and remarkable compared to control. The least Inflorescence length was observed at control case to be 18.4 centimeter (table3). The increase in nitrogen causes increase in plant height and it also causes increase in the uptake of nutrients from the soil so it will have a positive influence for the plant growth. In the medicinal plant Dracocephalum moldavica, consumption of nitrogen fertilizer in different levels of growth led to an increase in the length of flowering top branches which was meaningful compared to the control [18].

Phosphorous content:

The results of variance analysis indicate that the consumption phosphorous and nitrogen doesn’t have a meaningful effect on the plant’s phosphorous content (table 2). Yet, trace elements and the interaction effect between nitrogen and phosphorous were meaningful in their influence on the level of phosphorous. The comparison of means interaction effect between phosphorous and nitrogen on the plant’s phosphorous content (table 5) indicated that the highest amount of phosphorous 186.7 milligram on dry weight was obtained from the application of 100 and 300 kg/ha ammonium nitrate and triple supper phosphate which had a meaningful statistical difference with other levels of phosphorous and nitrogen being consumed. In a study by David and
Rothstein [19], it was stated that in *Abies fraseri* plant, the uptake of phosphorous decreased as nitrogen increased in nutritional solutions which was not in conformity with the findings of the present study. It seems that in their experiment, the presence of antagonistic effects between two dissolved nutritional elements led to such conclusion.

**Essential oil content:**

The results of variance analysis indicated that nitrogen and phosphorous had a meaningful effect on the amount of essential oil. The interaction effect among applications and consuming trace elements didn’t have a meaningful effect on the amount of essential oil (table 2). Comparison of means also indicated that in 300 kg/ha ammonium nitrate with an average essential oil 1.93 milliliter the highest amount of essential oil was obtained which had a meaningful difference compared to the levels before that (table 3). Among factors influential on the amount of secondary metabolite production in plants are nutritional and environmental conditions. So, by supplying enough nutritional elements, the amount of essential oil will increase. Studies carried out by Alizadeh et al. [1] also indicate increase in the amounts of essential oil due to the fertilizers containing highly consumed elements like nitrogen, potassium, phosphorous and trace elements including iron and zinc.

The consumed phosphorous was also effective on the amount of extracted essential oil so that the highest amount of essential oil was obtained by consuming 100 kg/ha triple supper phosphate (table 4). So an increase in nitrogen and phosphorous led to an increased amount of essential oil production. The results of these studies conform to Fernander [20] and Arabis and Bayram [21] in the case of sweet basil (*Ocimum basilicum* L.), who asserted that by consuming nitrogen, the amount of essential oil increases. A number of researchers reported that high function of essential oil in spearmint is achieved through more nitrogen application [22, 23]. In a research carried out about the medicinal plant Melissa officinalis it was demonstrated that appropriate nitrogen application can enhance the efficiency of essential oils in this plant noticeably [7].

**Chlorophyll a, b and total:**

The results obtained from variance analysis of chlorophyll a, b and total indicated that the influence of nitrogen and phosphorous on chlorophyll a, b and chlorophyll total was meaningful. The interaction effect of nitrogen and phosphorous also indicated a meaningful difference from the perspectives of chlorophyll a, b and total (table 2). Nitrogen has an important role in chlorophyll structure and increase in nitrogen leads to germination development of plant like increased leaf area and an increase in this feature results into increased amount of chlorophyll and photosynthetic materials [24]. Jampeetong and Brix [25] studying the amounts of chlorophyll a, b and total in garden sage (*Sativa officinalis* L.) demonstrated that the amounts of chlorophyll a, b and total were meaningfully influenced by *NH₄NO₃*, applications. Plants grown in *NH₄NO₃* had greater chlorophyll compared to other applications.

<table>
<thead>
<tr>
<th>Source</th>
<th>Degree of freedom</th>
<th>Yield dry matter (%)</th>
<th>lateral brach (%)</th>
<th>Inflorrence length (cm)</th>
<th>Phosphor (mg/g wt)</th>
<th>Essential oil (mg/g wt)</th>
<th>Chlorophyl a (mg/g wt)</th>
<th>Chlorophyl b (mg/g wt)</th>
<th>Total Chlorophyl (mg/g wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replication 2</td>
<td>3888.38**</td>
<td>4.316**</td>
<td>6.282**</td>
<td>149.612**</td>
<td>0.003**</td>
<td>0.023**</td>
<td>0.003**</td>
<td>0.002**</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>2</td>
<td>540596.75**</td>
<td>30.663</td>
<td>13.196**</td>
<td>1195.042**</td>
<td>0.326</td>
<td>0.324</td>
<td>0.335**</td>
<td>0.534**</td>
</tr>
<tr>
<td>P</td>
<td>2</td>
<td>111630.38</td>
<td>16.134</td>
<td>1.381**</td>
<td>15544778**</td>
<td>0.041</td>
<td>0.291</td>
<td>0.068</td>
<td>0.592**</td>
</tr>
<tr>
<td>N*P</td>
<td>2</td>
<td>517465.02**</td>
<td>1.054**</td>
<td>0.281**</td>
<td>73.387**</td>
<td>0.020**</td>
<td>0.101**</td>
<td>0.055**</td>
<td>0.300**</td>
</tr>
<tr>
<td>Micr.</td>
<td>1</td>
<td>26674.70**</td>
<td>7.200**</td>
<td>2.377**</td>
<td>107.468**</td>
<td>0.020**</td>
<td>0.012**</td>
<td>0.013**</td>
<td>0.049**</td>
</tr>
<tr>
<td>N*Micr.</td>
<td>2</td>
<td>104616.19**</td>
<td>0.168**</td>
<td>0.592**</td>
<td>21.717**</td>
<td>0.009**</td>
<td>0.004**</td>
<td>0.007**</td>
<td>0.018**</td>
</tr>
<tr>
<td>P*Micr.</td>
<td>1</td>
<td>32442.03**</td>
<td>0.147**</td>
<td>0.433**</td>
<td>32.111**</td>
<td>0.001**</td>
<td>0.016**</td>
<td>0.006**</td>
<td>0.041**</td>
</tr>
<tr>
<td>N<em>P</em>Micr.</td>
<td>2</td>
<td>4656.30**</td>
<td>1.551**</td>
<td>1.358**</td>
<td>22.007**</td>
<td>0.002**</td>
<td>0.001**</td>
<td>0.003**</td>
<td>0.001**</td>
</tr>
<tr>
<td>Error</td>
<td>22</td>
<td>33671.82</td>
<td>3.216</td>
<td>1.184</td>
<td>0.001</td>
<td>0.007</td>
<td>0.017</td>
<td>0.013</td>
<td>0.001</td>
</tr>
<tr>
<td>CV(%)</td>
<td>6.85</td>
<td>8.70</td>
<td>25.5389</td>
<td>2.89</td>
<td>1.88</td>
<td>2.25</td>
<td>8.06</td>
<td>11.12</td>
<td></td>
</tr>
</tbody>
</table>

Note: **, * ns, are significant difference in 1 and 5 percent and non significant, respectively

<table>
<thead>
<tr>
<th>Nitrogen rate</th>
<th>lateral brach (%)</th>
<th>Inflorrence length (cm)</th>
<th>Essential oil (mg/g wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N₀</td>
<td>18.8b</td>
<td>18.4b</td>
<td>0.53c</td>
</tr>
<tr>
<td>N₁</td>
<td>18.9a</td>
<td>20.1a</td>
<td>1.52b</td>
</tr>
<tr>
<td>N₂</td>
<td>22.0a</td>
<td>20.4a</td>
<td>1.93a</td>
</tr>
</tbody>
</table>

*N₀, N₁ and N₂: Control, 150 and 300 kg/ha Amonium Nitrate, respectively

*Means with the same letter(s) at each column were not significant differences at 0.05 probability level.
Table 4: Effect of phosphorus on experimental traits

<table>
<thead>
<tr>
<th>Phosphorus rate</th>
<th>lateral branch</th>
<th>Inflorescence length (cm)</th>
<th>Essential oil (mg/g wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_1$</td>
<td>19.95 b</td>
<td>19.26 b</td>
<td>0.67 b</td>
</tr>
<tr>
<td>$P_2$</td>
<td>21.29 a</td>
<td>19.85 a</td>
<td>0.75 a</td>
</tr>
</tbody>
</table>

*P$_1$ and P$_2$: 50 and 100 kg/ha triple super phosphate, respectively.
*Means with the same letter(s) at each column were not significant differences at 0.05 probability level.

Table 5: Effect of Nitrogen and Phosphorus on experimental traits

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Yield (kg/ha)</th>
<th>Chlorophyl a</th>
<th>Chlorophyl b</th>
<th>Total Chlorophyl</th>
<th>Phosphorus</th>
</tr>
</thead>
<tbody>
<tr>
<td>N$_1$P$_1$</td>
<td>1987.6d</td>
<td>0.98b</td>
<td>0.63b</td>
<td>1.60b</td>
<td>123.6f</td>
</tr>
<tr>
<td>N$_1$P$_2$</td>
<td>2055.2d</td>
<td>1.03b</td>
<td>0.66ab</td>
<td>1.69b</td>
<td>163.2c</td>
</tr>
<tr>
<td>N$_1$P$_3$</td>
<td>2565.9c</td>
<td>0.92b</td>
<td>0.57b</td>
<td>1.49b</td>
<td>131.6e</td>
</tr>
<tr>
<td>N$_2$P$_1$</td>
<td>2726.1c</td>
<td>0.99b</td>
<td>0.56b</td>
<td>1.55b</td>
<td>169.5b</td>
</tr>
<tr>
<td>N$_2$P$_2$</td>
<td>2953.8b</td>
<td>1.07b</td>
<td>0.55b</td>
<td>1.62b</td>
<td>136.5d</td>
</tr>
<tr>
<td>N$_2$P$_3$</td>
<td>3782.5a</td>
<td>1.45a</td>
<td>0.79a</td>
<td>2.24a</td>
<td>196.7a</td>
</tr>
</tbody>
</table>

*Means with the same letter(s) at each column were not significant differences at 0.05 probability level.

Conclusion:

Origany (*Satureja hortensis* L.) grown in the garden as a medicinal plant is easily cultivated in Marand area and has an appropriate potential for production. The best nitrogen level was 300 kg/ha from ammonium nitrate source with dry weight yield of 3368.1 kg/ha. Applying this level of ammonium nitrate resulted into the most efficient inflorescence length, number of lateral branches, amounts of chlorophyll a,b and total of the plant, amount of essential oil and the amount of phosphorus. Also, consumption of 100 kg/ha triple super phosphate had a positive effect on each one of the above mentioned features. Considering the obtained results, we can conclude that the optimized function was obtained by applying 300 kg/ha ammonium nitrate. Since the highest nitrogen application in this experiment demonstrated a better plant function, further investigations using greater amounts of nitrogen is recommended.

REFERENCES