The changes of yield and essential oil components of German Chamomile (*Matricaria recutita* L.) under application of phosphorus and zinc fertilizers and drought stress conditions

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**KEYWORDS**

German chamomile; Drought stress; Phosphorus; Zinc; Essential oil

**Abstract** In order to investigate the effect of drought stress and phosphorus and zinc fertilizers on physiological traits, yield and essence components of German chamomile (Gural cultivar), a split plot factorial experiment was conducted based on randomized complete block design with three replications at Research farm of University of Zabol in 2013. Drought stress consisted of three levels 75% (control), 50% (mild stress) and 25% of field capacity (severe stress) as main plots, and factorial combinations of three triple superphosphate fertilizer (CaH$_4$P$_2$O$_8$) levels (0, 150, and 300 kg ha$^{-1}$) and two zinc sulfate fertilizer (ZnSO$_4$H$_2$O) levels (0 and 30 kg ha$^{-1}$) as subplots. Studied traits were included carbohydrate, prolin, carotenoid, essential oil percentage, essential oil yield, chamanzulene content, β-farenzn, bisabolo oxide A, and bisabolo oxide B. The results showed significant effect of drought stress on all studied traits, whereas application of phosphorus fertilizer was significant on essential oil percentage, essential oil yield, chamanzulene content, β-farenzn, bisabolo oxide A, and bisabolo oxide B, and zinc fertilizer was significant on essential oil percentage, essential oil yield and chamanzulene content. Also can note that high application of phosphorus fertilizer (300 kg ha$^{-1}$) had negative effects on yield of chamomile. In general, the results suggest that irrigation based on 50% of field capacity with application of 150 kg phosphorus

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1. Introduction

The German chamomile (*Matricaria recutita* L.) is mainly cultivated for essential oil. Chamomile is a well-known medicinal plant species from the Asteraceae family often referred to as the “star among medicinal species”. Nowadays it is a highly favored and much used medicinal plant in folk and traditional medicine. Its multitherapeutic, cosmetic, and nutritional values have been established through years of traditional and scientific use and research (Singh et al., 2011). About 40 different compounds were identified in chamomile essential oil that the most important ones included sesquiterpenes, chamazulene, β-farnesn, bisabolo oxide A, and bisabolo oxide B (Jaymand and Rezaee, 2001). Water deficit is one of the most important limiting factors on crop production in arid and semi-arid regions (Sharafi et al., 2002). Drought stress by reducing water content of tissues limits the growth of plants and caused some metabolic and physiological changes (French and Turner, 1991). On the other hand the availability of nutrients in the soil is affected by drought stress (Munns, 1993). Thus, nutritional management of plants under drought stress conditions is one of the most important factors in crop production. When plants receive a sufficient quantity of nutrients will show better resistance to drought (Lal et al., 1993). The better understand of nutrient roles in plant resistance to drought, is associated with improvement of fertilizer management in arid and semi-arid regions (Solinas et al., 1996). Water is one of the most important environmental factors that affect the growth and development of chamomile (Wagner, 1993). Have been reported that in Thymus vulgaris the highest essential oil percentage obtained from irrigation based on 70% of field capacity and with the intensification of water stress essential oil yield decreased (Letchamo et al., 1994). The results of Khakshu Moghaddam et al. (2011) have showed that drought stress has a significant effect on germination and morphological traits such as leaf area, fresh and dry weight of shoots and roots. They also have found that drought stress increased the prolin and soluble carbohydrate of shoots and roots. Drought stress in mint increased the content of menthol and total sesquiterpenes while decreased content of Cineol and Pulegone monofuran (Charles et al., 1990). The results of different studies have showed that application of sufficient amount of phosphorus fertilizer has a direct effect on flowering, dry weight of flower and essential oil yield of German chamomile (Omidbéygi, 1995). The role of phosphorus especially during reproductive phase is remarkable and it is one of the most important elements in chamomile production (Lopes, 1996). In many cases it has been reported that zinc deficiency caused limitation of plant response to phosphorus and even decreased yield (Ronaghi et al., 2002). The Zn has an especial role in plant protection against drought stress (Cakmak, 2009). Omidbéygi (2006) has reported that application of Zn fertilizers increased essential oil of mint. It has been found that zinc increased uptake efficiency of nitrogen and phosphorus (Sharafi et al., 2002). Our objectives were to investigate the effects of drought stress and phosphorus and zinc fertilizers on physiological traits, yield and essential oil components of German chamomile.

2. Materials and methods

The experiment was conducted in split plot factorial based on randomized complete block design with three replications at Research farm of University of Zabol in 2013. The results of chemical analysis of the field soil is given in Table 1.

Drought stress consisted of three levels 75% (control), 50% (mild stress) and 25% of field capacity (severe stress) as main plots, and factorial combinations of three triple superphosphate fertilizer (CaH4P2O8) levels (0, 150, and 300 kg ha⁻¹) and two zinc sulfate fertilizer (ZnSO4H2O) levels (0 and 30 kg ha⁻¹) as subplots (the fertilizers were applied before planting time). Generally 18 treatments were used with three replications. In order to increasing in germination percentage, the seeds were mixed with soft sawdust in ratio of 1 (seed) to 2 (sawdust) (the ratio is based on weight). The seeds were sown at 20 cm apart in rows 40 cm wide, on first half of March 2013. Drought stress levels were determined by the Time Domain Reflectometry (TDR) (Imko, Germany). Water suitable for irrigation was obtained from a deep well in the area. The normal cultural practices of growing chamomile plants were followed until harvest. The success of chamomile cultivation as a commercial venture lies in how efficiently and effectively one can collect the flowers at the right stage during the peak flowering season extending over a period of 3–6 weeks. Flower plucking is a selective process as flowers in all stages, namely, buds, semi-opened buds, flowers in all stages of bloom appear on the plants. Flowering will be observed on plants here and there all over the field and these flowers are plucked at the appropriate stage (Singh et al., 2011). So flowers were selectively collected on 27 April, 30 April, 4 May, 8 May, and 12 May 2013. Fresh flowers weighted immediately after each collection and after the final collection, total weight of the harvested flowers were considered as a fresh flowers yield. A sample of the harvested fresh flowers were used for the assessment of carbohydrate (0.2 g of fresh leaf tissue), prolin (0.2 g of fresh leaf tissue) and carotenoid (0.1 g of fresh leaf tissue).

<table>
<thead>
<tr>
<th>EC (ds/cm)</th>
<th>pH</th>
<th>N%</th>
<th>C</th>
<th>P (mg/kg)</th>
<th>K (mg/kg)</th>
<th>Na (mg/kg)</th>
<th>Z (mg/kg)</th>
<th>Soil texture</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.46</td>
<td>8.4</td>
<td>0.05</td>
<td>0.47</td>
<td>9.2</td>
<td>115</td>
<td>38.7</td>
<td>4.8</td>
<td>Loamy sandy</td>
</tr>
</tbody>
</table>

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German chamomile essential oil extracted from the dried flowers and using Clevenger system. The carbohydrate contents according to Kerepsi et al. (1996) method (using ethanol 95% and based on sulfuric acid), prolin contents according to Bates et al. (1973) method, carotenoid using spectrophotometer, (Uni Germany), essential oil percentage using Clevenger system, chamanzulene content, β-farenzn, bisabolo oxide A, and bisabolo oxide B using Chromatography, (GMl, United states) were measured. Essential oil yield was determined through calculating the weight percentage of the flower essential oil. Statistical analysis was carried out using SAS version 9.1 software. Significant difference was set at \( P \leq 0.05 \) and determined using the Duncan’s multiple range test.

3. Results

The analysis of variance of studied traits is given in Table 2. The results showed a significant effect of irrigation levels on all traits at 1% probability level, a significant effect of phosphorus levels on essential oil percentage, chamanzulene, β-farenzn, bisabolo oxide A and bisabolo oxide B contents and essential oil yield at 1% probability level and a significant effect of zinc levels on essential oil percentage, chamanzulene content and essential oil yield at 5% probability level. Interaction between drought stress and phosphorus had a significant effect on prolin and bisabolo oxide A contents at 5% and 1% probability level, respectively. Interaction between drought stress and zinc had a significant effect on bisabolo oxide A content at 1% probability level and on chamanzulene content and essential oil yield at 5% probability level.

The results of interaction between phosphorus and zinc showed a significant effect on chamanzulene, β-farenzn, bisabolo oxide A and bisabolo oxide B contents at 5% probability level and on essential oil yield at 1% probability level. The interaction between drought stress, phosphorus and zinc also had a significant effect on bisabolo oxide A content at 1% probability level and on essential oil yield at 5% probability level. The highest content of β-farenzn obtained from irrigation based on 75% of field capacity and the highest content of carbohydrate, prolin and bisabolo oxide A obtained from irrigation based on 25% of field capacity (Table 3). It seems that with increasing in stress levels, the amount of these traits increases.

The results of interaction between drought stress and phosphorus showed that the highest prolin and bisabolo oxide A were belonged to irrigation based on 25% of field capacity

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Effect of drought stress on physiological traits of chamomile.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irrigation levels (field capacity) (%)</td>
<td>Carbohydrate (mg/g)</td>
</tr>
<tr>
<td>---------</td>
<td>----------------------</td>
</tr>
<tr>
<td>75</td>
<td>8.44c</td>
</tr>
<tr>
<td>50</td>
<td>10.26b</td>
</tr>
<tr>
<td>25</td>
<td>13.35a</td>
</tr>
</tbody>
</table>

Figures followed by the same letter in each column are not significantly different at 5 probability level by Duncan’s multiple range test.

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Interaction of drought and phosphorus levels on prolin and bisabolo oxide A of chamomile.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irrigation levels (field capacity) (%)</td>
<td>Phosphorus levels (kg)</td>
</tr>
<tr>
<td>---------</td>
<td>------------------------------------------</td>
</tr>
<tr>
<td>75</td>
<td>0</td>
</tr>
<tr>
<td>150</td>
<td>3.30bc</td>
</tr>
<tr>
<td>300</td>
<td>3.49abc</td>
</tr>
<tr>
<td>50</td>
<td>0</td>
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<td>150</td>
<td>3.53abc</td>
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<td>300</td>
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<td>150</td>
<td>4.30a</td>
</tr>
<tr>
<td>300</td>
<td>3.93abc</td>
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</table>

Figures followed by the same letter in each column are not significantly different at 5 probability level by Duncan’s multiple range test.

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along with application of 150 kg phosphorus fertilizer ha$^{-1}$ (Table 4).

The results of interaction between drought stress and zinc showed that the highest content of chamanzulene obtained from irrigation based on 50% of field capacity along with application of 30 kg zinc fertilizer ha$^{-1}$ and the highest content of bisabolo oxide A obtained from irrigation based on 25% of field capacity with application of 30 kg zinc fertilizer ha$^{-1}$ that with irrigation based on 25% of field capacity without application of zinc and also with irrigation based on 50% of field capacity with application of 30 kg zinc were listed statistically into one group. But the highest essential oil yield obtained from irrigation based on 50% of field capacity with application of 30 kg zinc fertilizer ha$^{-1}$ and the lowest essential oil yield obtained from irrigation based on 25% of field capacity with application of 30 kg zinc fertilizer ha$^{-1}$ (Table 5).

<table>
<thead>
<tr>
<th>Table 5</th>
<th>Interaction of drought and zinc levels on chamanzulene, essential oil yield and bisabolo oxide A of chamomile.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irrigation levels (field capacity) (%)</td>
<td>Zinc levels (kg)</td>
</tr>
<tr>
<td>75</td>
<td>0</td>
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<tr>
<td></td>
<td>30</td>
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<tr>
<td>50</td>
<td>0</td>
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<tr>
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<td>30</td>
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<tr>
<td>25</td>
<td>0</td>
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<td></td>
<td>30</td>
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Figures followed by the same letter in each column are not significantly different at 5 probability level by Duncan’s multiple range test.

<table>
<thead>
<tr>
<th>Table 6</th>
<th>Interaction of phosphorus and zinc levels on essential oil percentage and essential oil compositions and bisabolo oxide A of chamomile.</th>
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<tbody>
<tr>
<td>Phosphorus levels (kg)</td>
<td>Zinc levels (kg)</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>30</td>
</tr>
<tr>
<td>150</td>
<td>0</td>
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<td>0</td>
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Figures followed by the same letter in each column are not significantly different at 5 probability level by Duncan’s multiple range test.

<table>
<thead>
<tr>
<th>Table 7</th>
<th>Interaction of drought, phosphorus and zinc levels on bisabolo oxide A and essential oil yield of chamomile.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irrigation levels (field capacity) (%)</td>
<td>Phosphorus levels (kg)</td>
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<tr>
<td>75</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>30</td>
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<tr>
<td>150</td>
<td>0</td>
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<td>30</td>
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</tbody>
</table>

Figures followed by the same letter in each column are not significantly different at 5 probability level by Duncan’s multiple range test.
The results of interaction between phosphorus and zinc showed that the highest content of essential oil percentage, essential oil components and essential oil yield obtained from treatment with application of 150 kg phosphorus fertilizer ha\(^{-1}\) and 30 kg zinc fertilizer ha\(^{-1}\) (Table 6).

The interaction between drought stress, phosphorus and zinc showed that the highest content of bisabolene oxide A was belonged to irrigation based on 25\% of field capacity with application of 150 kg phosphorus fertilizer ha\(^{-1}\) and 30 kg zinc fertilizer ha\(^{-1}\). But the highest essential oil yield was belonged to irrigation based on 50\% of field capacity along with application of 150 kg phosphorus fertilizer ha\(^{-1}\) and 30 kg zinc fertilizer ha\(^{-1}\) (Table 7).

4. Discussion

The results showed that exposing chamomile plants to soil moisture stress during its life cycle might lead to a significant effects on physiological traits, essential oil yield and essential oil components. As the roots of the chamomile are shallow, the plant is unable to draw moisture from the lower moist horizon of the soil and therefore needs frequent irrigation to maintain an optimum moisture level (Singh et al., 2011). It seems that moisture stress reduces chamomile productivity and this effect is influenced by the severity of the stress. Water stress adversely impacts many aspects of the physiology of plants. If the stress is prolonged, plant growth and productivity are severely diminished. Plants have evolved complex physiological and biochemical adaptations to adjust and adapt to a variety of environmental stresses (Osakabe et al., 2014). We found that with increasing in water stress levels the contents of prolin and carbohydrate increased significantly. When plants experience environmental stresses, such as drought, they activate various metabolic and defense systems to survive. Many genes and products commonly appear in response to drought, salinity, and low-temperature stresses. For example, osmoprotectants, such as proline (Pro), glycine betaine, mannitol, and sugars confer stress tolerance (Yamada et al., 2005). Proline, an amino acid, is a compatible solute involved in cell osmotic adjustment (OA) and protection of cell components during dehydration (Zhang et al., 2009). Osmotic adjustment helps to maintain cell turgor, which can allow cell enlargement and plant growth during water stress; and it can allow stomata to remain at least partially open and CO\(_2\) assimilation to continue at water potentials that would be otherwise inhibitory (Alves and Setter, 2004). Proline also functions as a free radical scavenger and suppresses free radical-mediated damage during drought stress. Several studies have indicated that proline content increases during drought stress, and proline accumulation is associated with improvement in drought tolerance (Seki et al., 2007; Zhang et al., 2009). According to the results of this experiment it seems that application of zinc under drought stress condition can decrease damage of drought stress. The microelements play critical roles in plant nutrition and production. The Zn plays a key role in the synthesis of proteins, DNA, and RNA (Welch, 2001) and plays very important role in plant metabolism by influencing the activities of hydrogenase and carbonic anhydrase, stabilization of ribosomal fractions and synthesis of cytochrome (Tisdale et al., 1984). Plant enzymes activated by Zn are involved in carbohydrate metabolism, maintenance of the integrity of cellular membranes, protein synthesis, regulation of auxin synthesis and pollen formation (Marschner, 1995). The regulation and maintenance of the gene expression required for the tolerance of environmental stresses in plants are Zn dependent (Cakmak, 2000). Zinc seems to affect the capacity for water uptake and transport in plants and also reduce the adverse effects of short periods of heat and salt stress (Kasim, 2007; Disante et al., 2010; Peck and McDonald, 2010; Tavallali et al., 2010). The results of researchers have showed that application of zinc and manganese increases plants resistance to the environmental stress such as drought and reduces harmful effects of drought stress (Movahhedy-Dehnay et al., 2009). Also we observed that high application of phosphorus fertilizer (300 kg ha\(^{-1}\)) had negative effects on yield of chamomile. This plant growth disorder is maybe because of interaction between P and Zn that is usually termed ‘P-induced-Zn deficiency’. This disorder in plant growth is associated with high levels of available P or with application of P to soil. The Zn deficiency symptoms can be prevented by the application of Zn fertilizers. The actual causal relationship and mechanisms are still not fully understood. In general, four possible causes have been considered responsible for P induced-Zn deficiency. These include (i) a P–Zn interaction in soil; (ii) a slower rate of translocation of Zn from the roots to shoots; (iii) a simple dilution effect on Zn concentration in plant tops due to growth responses to P; (iv) a metabolic disorder within plant cells related to an imbalance between P and Zn (Olsen, 1972). Many researchers have reported that applied P accentuated Zn deficiency symptoms in plants (Lonergan et al., 1979; Sharma et al., 1968). The higher P levels in soil reduced the Zn concentrations in the plant tops and also reduced total Zn contents (Singh et al., 1986; Clark, 1978). These scientists suggested that P–Zn antagonism existed in the roots of the plants. Other studies suggested that although P decreased the Zn concentrations in the tops, the total Zn contents either increased or remained the same (Boawn and Brown, 1968; Boawn and Leggett, 1968). The cause of this P-induced-Zn deficiency has been suggested to be due to interference by P with the uptake, translocation, or utilization of Zn (Adriano et al., 1971). Thus, it is important that the application of nutrients to be in balance. Having a good nutrient balance is therefore an important factor to improve plant growth by indicating the actual amount and combination of nutrients that the production needs. Also this is a good way to save money (Kulmala, 2012). The results of this experiment are in agreement with the results of Roshanzameir (2010) in basil, and Ardekani et al. (2007) in Melissa officinalis L.

5. Conclusion

In summary, the results of this study indicate that drought stress caused significant effects on physiological traits, essential oil yield and essential oil components. Proline, carotenoid, carbohydrate, and essential oil components increased, whereas essential oil yield decreased in response to severe drought stress. The results showed that optimum amount of Zn and P can improve the studied traits of chamomile. According to the results of this experiment it seems that application of Zn under drought stress condition can decrease damage of drought stress that this is maybe because of critical roles of that in plant nutrition and production. Also we observed that high application of phosphorus fertilizer (300 kg ha\(^{-1}\)) had negative effects on yield of chamomile which this plant growth...
disorder is maybe because of interaction between P and Zn. In general, the results suggest that irrigation based on 50% of field capacity with application of 150 kg phosphorus fertilizer ha⁻¹ and 30 kg zinc fertilizer ha⁻¹ can improve essential oil yield and medicine components of German chamomile essential oil.

References


