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# Essential oils and Heavy Metals accumulation in *Salvia officinal*is cultivated at different Inter-raw Spaces in Ash-Shoubak South Region of Jordan

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## Introduction

Salvia is a well-known medicinal and culinary herb that has been used for many centuries in the Mediterranean region. In Jordan, twenty species of salvia are grown widely throughout the territories of country (Al-Eisawi, 1982). Amr and Đorđević (2000) reported that the sage originating from Jordan can be used as high quality raw materials for the production of phytopreparations. The leaves of *S. officinalis* are used to relive Headache, flatulence, toothache, abdominal pain, and common cold (Abu-Rmaileh and Fatma Afifi, 2003). Essential oil extracted from *S. officinalis* exhibited antioxidant and antimicrobial properties (Dragan et al., 2003). Heavy metals are presented as contents of many medicinal plants (El- Rjoob et al., 2008; Abu–Darwish et al., 2009). They can affect the production and yield of certain biological compounds (Aziz and Gad, 2007) having positive and negative roles in living tissues (Malencic et al., 2003). Therefore, this study was designed to Pb, Cd, Cr, Co, Ni, Zn, Fe, Cu, Mn, and essential oil contents in *Salvia officinalis* cultivated at 15, 30, and 45cm intra-raw spaces in Ash-shoubak south region of Jordan.

## Materials and Method

This study was performed in an experimental area of Ash-shoubak University Collage in Ash-Shoubak region (30° 32N, 35° 33 E), 1365 m above the sea level. Ambient temperature and seasonal means of rainfall are (4.11 – 19.9C°) and 294.2 mm/year, respectively. *S.officinalis* seedlings that have a well-developed root system were transplanted and cultivated in the experimental area in May of 2007 in 50 cm-rows with intra-row spacing of 15, 30 and 45 cm apart. Five plants from each intra-row spacing were harvested at the following phenological stages, vegetation (VEG), beginning of blooming (BB), full-blooming (FB) and fruit Maturation (FM) stages on middle of June , July, august, September, 2007, respectively. All the plant samples were dried in draughty place at about 20˚C for heavy metals and essential oil treatments.

## Essential Oil Extraction

20gr of the dried mild and sieved collected specimens including leaves, stems, flowers, and fruits (depending on the phonological stage) of *S.officinalis were* steam distilled for 2 h at a rate of 2-3ml/ min using a Clevenger-type system. The values reported are the mean of at least 3 distillations.

**Determination of Heavy Metals**

Heavy metals content were analyzed in selected samples with highest and lowest essential oil contents at 15, 30, and 45cm intra-raw spacing by using Atomic Absorption Flame Emission Spectrophotometer Model (Shimadzu Japan) AA-6200. The plant samples were oven dried at 70 ˚C for 24 hours until the dry weight was constant. The dried samples were then ground and passed through a 0.2 mm plastic sieve. 0.5gm of plant sample was wet digested with ultra-pure nitric acid ($HNO_3$ (10-15 ml) in a polyethylene test tube using a heating blocks digestion unit at 120 $^{o}$C. The final solution was filtered into a 25 ml or 50 ml volumetric flask through a 45-µm filter paper and diluted to the mark with ultra–pure water.

## Results and Discussion

**Essential Oils**

The results of essential oil yield are shown in table (1). The oil content extracted during the VEG and BB phonological stages of *S.officinalis* planted in 15, 30, and 45cm intra-raw spaces were found to satisfy the requirements of European Pharmacopoeia (EP), while samples collected at FB and FM stages from all studied intra-raw spaces were lower than EP, that requires an oil yield of 1.0% v/w or above (European Pharmacopoeia, 2005). The results showed a clear effect of both intra–raw planting spacing and phenological stage on the oil yields. The maximum yields of oil extracts were 2.00, 1.80, and 1.73% during the VEG stage of *S.officinalis* planted in 15, 30, and 45cm intra-raw spaces, respectively, then decreased to 0.80, 0.90,and 0.87% during the FM and BB stage, respectively; the minimum range of oil extracted from *S.officinalis* in the south Shoubak region was ranged from 0.80 to 0.90% during the FB and FM stages, respectively. These results were on the contrary with the results obtained from the middle region of the country (Amr and.Đorđević, 2000S). They found that the maximum oil extracted from *S.officinalis* was 2.13% during the blooming phase of a four year plant age, then decreased to 1.83% during fruition phase, to reach its minimum of 1.28% before blooming phase. The results of essential oils extracted during the life cycle stages of all studied intra-raw planting spaces of *S.officinalis* were decreased in the order: VEG>BB>FB>FM. The same trend was observed by Mirjalili et al., (2006). These differences could be explained by the effect of geographical and climate variations among regions (Perry et al., 1999), seasonal variations and harvesting time (Qiu et al., 2005).

Table (1): The % of oil content in *saliva officinalis* cultivated at 15, 30, and 45 cm planting spaces in Ash-shoubak region.

| Inter- Raw Planting Space | Phenological Stage | | | |
|---|---|---|---|---|
| | VEG | BB | FB | FM |
| 15 cm | 2.00±0.1155 | 1.8±0.2517 | 0.84±0.0058 | 0.80±0.00 |
| 30 cm | 1.80±0.0058 | 1.70±0.1155 | 0.93±0.0058 | 0.90±0.001 |
| 45 cm | 1.73±0.1155 | 1.50±0.1155 | 0.93±0.002 | 0.87±0.001 |

**Heavy metals in Plant samples**

The results of trace element contents are shown in Table (2). Lead (Pb), was not detectable during VEG and FM pheneological stages of *S.officinalis* planted at 15, 30 and 45cm intra-raw spaces. On the contrary, *S.officinalis* cultivated in two other locations in the middle region of Jordan has been contaminated with Pb in samples collected during various phonological stages in Hfashiet Al Dbajbe near Amman-Sahab highway, ranged from 17 to 32mg/kg (Amr and Đorđević, 2000). The absence of Pb in our studied samples *S.officinalis* is mainly due to the location of cultivation area that is away from the road and motor vehicles; the leading factors of plant contamination with Pb (El- Rjoob et al., 2008). Cadmium (Cd) values were not detectable in all specimens of *S.officinalis* collected from Ash-shoubak region. On the other hand, Cd was detected below the toxic level (3-30ppm) in *S.officinalis* cultivated in other regions near the main road exposed to a high traffic density than other locations (El- Rjoob et al., 2008). Chromium (Cr) and Cobalt (Co) were not detectable. The same results were found in the middle region of Jordan (Amr and and.Đorđević, 2000). The average content of Ni in plant is 0.10 – 5.0 ppm, the toxic level ranges from 10 to 100 ppm. The lowest content of Ni recorded 0.42 ppm at 15 cm intra-raw planting space and harvested during the VEG stage, while the highest was 4.94 ppm, detected in samples harvested during the FM stage in the same intra-raw planting space. On the other hand, Ni content during the VEG stage at 30 and 45cm intra-raw planting spaces were 2.78 and 4.17 ppm, respectively, these results showed that the content of Ni was increased by increasing intra-raw planting space, indicating to the activity of the root, Ni absorbing system and/or the metabolic activity of the tissue-metal accumulating (Sengar et al., 2008). Zinc (Zn) concentration in plant may vary between 30-150 ppm, but usually it is 20-50 ppm (Malencic et al., 2003). The lowest concentration of Zn (95.81ppm) was found in *S.officinalis* during the VEG stage within 30cm intra-raw planting space, the highest was 125.71ppm and detected in plants collected during FM stage in 15 cm intra-raw planting space. On the other hand, Zn content was increased during the life cycle of *S.officinalis* cultivated at 15 cm intra-raw space from 116.91ppm in VEG to reach a maximum of 125.71 ppm during FM stage indicating to the Zn accumulation in the leaf tissue of *S.officinalis* (Angelova et al., 2005).

Table (2) Concentration of heavy metals (ppm) in *Saliva Officials* cultivated in Ash-shoubak region depending on phenological stages and intra-raw spacing.

| Stage / cm | Pb | Cd | Cr | Co | Ni | Zn | Fe | Cu | Mn |
|---|---|---|---|---|---|---|---|---|---|
| VEG (15cm) | nd | nd | nd | nd* | 0.421 ±0.25 | 16.91 ±0.0.36 | 736.17 ±6.94 | 7.32 ±0.81 | 45.0 ±0.46 |
| VEG (30cm) | nd | nd | nd | nd | 2.78 ±1.08 | 95.87 ±1.191 | 68.97 ±5.41 | 7.02 ±0.50 | 51.35 ±0.42 |
| VEG (45 cm) | nd | nd | nd | nd | 4.17 ±1.38 | 108.85 ±0.52 | 524.67 ±3.35 | 3.07 ±0.70 | 44.63 ±0.72 |
| FM (15cm) | nd | nd | nd | nd | 4.94 ±1.09 | 125.71 ±0.29 | 935.40 ±8.84 | 12.14 ±0.93 | 45.00 ±0.64 |

*nd: not detected

In our studied samples the concentration of Fe reached its maximum of 935.40ppm in 15cm intra-raw planting space and harvested during FM stage, while the minimum was 524.67ppm in the samples cultivated within 45 cm intra-raw planting space and harvested during VEG stage. The content of Fe was found to be increased proportionally with a progressive growth stage of plant life cycle planted in 15 cm intra-raw space. However, *S.officinalis* cultivated in Ash-shoubak environment is more rich in Fe, than that cultivated in the middle region. (Amr and.Đorđević, 2000). These results seem to indicate that both soils and plants in Ash-shoubak south region are well supplied with this essential microelement (Fe) compared to the middle region of Jordan (Amr and.Đorđević 2000) and other various geographical locations in the world (Malencic et al., 2003).The average content of copper (Cu) in dry plant material is 2.0-20 ppm (Malencic et al., 2003). However the concentrations of Cu in all samples of *S.officinalis* grown in Ash-shoubak south environment were within the normal range and lower than those recorded in the middle region of Jordan, (Amr and.Đorđević, 2000). The contents of Mn in all samples of *S.officinalis* were found to be very closely to each other, the highest content was 51.35ppm in samples cultivated in 30cm intra-raw spacing and harvested during the VEG stage, while the lowest was 44.63 ppm in 45cm intra-raw spacing. In conclusion, the yields of essential oil and heavy metals content in *S. officinalis* are affected by phenological stages and intra-raw planting spaces. *Salvia officinalis* L., cultivated in Ash-shoubak south region of Jordan is rich in essential oil content and free from hazard heavy metals.

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