

Effect of Geographic Location, Ontogenesis on Essential Oil Composition and Spontaneously Emitted Volatile Organic Compounds of *Inula viscosa* (L.) Greuter. (Astraceae) Grown in Jordan

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Received: 15 January 2020;

Accepted: 23 June 2020;

Published online: 25 September 2020;

AJC-20069

The present study aimed at investigating the variation in the chemical composition of emitted volatile organic compounds (VOCs) and essential oils obtained from fresh aerial parts of *Inula viscosa* (Asteraceae) as affected by growth stage and geographical location zone. VOCs were extracted by solid phase microextraction (SPME) method from whole aerial parts of *I. viscosa* during the vegetative (spring-August) period, and from fresh leaves, closed pre-flowering buds and fully expanded flowers during the full flowering season (October). The essential oils were extracted by hydrodistillation from fresh organs during the flowering stage. GC/MS analysis of VOCs during the vegetative period revealed sesquiterpene hydrocarbons as main components in the samples collected from the Mediterranean zone (MID-1: 64.72-27.48%, MID-2: 32.09-76.77%). The profile of the samples from the Irano-Turanian zone (IT) was quite different. Myrcenol was the main component in the leaves, pre-flowering buds and fully expanded flowers from all locations (0.61-39.01%). E-nerolidol was the main component in the hydrodistilled oil of all organs from the different locations (73.72-88.66 %). Principle component and cluster analysis revealed that the chemical composition of the essential oils and VOCs belonging to MID-zone were similar and quite different when compared to the composition of the samples belonging to IT-zone. The results indicated the possible use of E-nerolidole as a stable chemotype marker in *I. viscosa* taxonomy.

Keywords: *Inula viscosa* L., Volatile organic compounds, Geographical variation, Principle component analysis, Cluster analysis.

INTRODUCTION

Inula viscosa (L.) Greuter. (known also as *Dittrichia viscosa*), belonging to the Asteraceae family (formerly known as Compositae) is a perennial shrubby leafy aromatic plant, reported to grow wild in different geographical regions in Jordan including the Mediterranean (MID) and Irano-Turanian (IT) zones [1]. Flowering occurs, during the period extending from late summer (August) until early winter season (December). This indigenous plant is found along the road sides and waste

places of Irbid, Ajloun, Jerash, Al-Salt, Amman, Madaba, Al-Karak, Tafila, Zarqa cities, Jordan valley and Dead sea.

I. viscosa is commonly used in the traditional medicine of different cultures [2-5] for the treatment of many ailments, especially arthritis and rheumatism. Moreover, in Jordan, a hot beverage prepared from the flowers is prescribed by traditional healers for the treatment of chronic cough and tumors [6-8]. Several studies described the phytochemical constituents and biological activities of *I. viscosa* [6-8].

| | | | | | | | | | | | | | |
|---|------|------|--------------------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|---------|
| 21 | 1202 | 1206 | <i>n</i> -Decanal | 0.66 | - | - | - | - | - | 0.38 | - | - | 1, 2 |
| 22 | 1221 | 1219 | <i>cis</i> -Sabinene hydrate acetate | - | - | - | 1.69 | - | - | - | - | - | 1, 2 |
| 23 | 1235 | 1228 | Thymol methyl ether | 2.52 | - | 2.00 | - | - | - | - | - | - | 1, 2 |
| 24 | 1244 | 1235 | 2-E-Hexenyl isovalerate | - | 0.36 | 2.80 | - | - | - | - | - | - | 1, 2 |
| 25 | 1264 | 1260 | 2-E-Decenal | - | - | 1.15 | - | - | - | - | - | - | 1, 2 |
| 26 | 1319 | 1302 | <i>cis</i> -Cycloisolongifol-5-ol | 1.89 | - | - | - | - | - | - | 0.34 | - | 1, 2 |
| 27 | 1319 | 1320 | 3-Z-Hexenyl tiglate | - | 0.72 | 3.50 | - | - | - | - | - | - | 1, 2 |
| 28 | 1351 | 1366 | α -Cubebene | 2.52 | 1.84 | 0.12 | - | 0.46 | 3.32 | - | - | - | 1, 2 |
| 29 | 1377 | 1373 | α -Copaene | 25.31 | 16.85 | 7.91 | 3.10 | 7.34 | 23.50 | 3.49 | - | - | 1, 2 |
| 30 | 1391 | 1386 | 7-Episesquithujene | - | 0.37 | - | 1.15 | 1.92 | 3.34 | 0.31 | - | - | 1, 2 |
| 31 | 1391 | 1391 | β -Elemene | - | 0.37 | 3.74 | - | - | 0.71 | 15.37 | 0.99 | - | 1, 2 |
| 32 | 1401 | 1399 | β -Longifolene | - | - | - | - | 1.06 | - | - | - | 3.18 | 1, 2 |
| 33 | 1409 | 1412 | <i>cis</i> -Caryophyllene | - | - | 1.96 | - | - | - | - | 0.44 | 1.25 | 1, 2, 3 |
| 34 | 1419 | 1418 | <i>trans</i> -Caryophyllene | 14.83 | 11.04 | 1.55 | 13.28 | 8.81 | 7.62 | - | 1.16 | 4.30 | 1, 2, 3 |
| 35 | 1435 | 1431 | <i>trans</i> - α -Bergamotene | - | - | - | - | - | 1.57 | - | - | - | 1, 2 |
| 36 | 1436 | 1446 | Neryl acetone | 0.67 | - | - | - | - | 1.13 | - | 0.45 | 1.05 | 1, 2 |
| 37 | 1457 | 1452 | β -E-Farnesene | 1.93 | - | - | - | - | - | 1.26 | - | - | 1, 2 |
| 38 | 1455 | 1450 | α -Humulene | - | 1.57 | - | 1.84 | 1.30 | 3.23 | 0.31 | 0.48 | - | 1, 2, 3 |
| 39 | 1460 | 1457 | Alloarmadendrene | 4.81 | 3.41 | 1.22 | 2.15 | 2.25 | 6.81 | 0.48 | - | - | 1, 2, 3 |
| 40 | 1482 | 1459 | γ -Irene | - | - | 1.26 | - | - | - | - | 0.07 | - | 1, 2 |
| 41 | 1477 | 1471 | γ -Gurjunene | - | 0.76 | 3.19 | 1.61 | 1.08 | - | 0.67 | 0.32 | 2.64 | 1, 2 |
| 42 | 1496 | 1474 | Valencene | - | - | - | - | - | - | - | - | 0.51 | 1, 2 |
| 43 | 1480 | 1472 | γ -Muurolene | 0.24 | - | - | - | 1.91 | - | 0.58 | 0.99 | 3.35 | 1, 2 |
| 44 | 1483 | 1476 | γ -Curcumene | - | - | - | - | 1.98 | 4.84 | - | - | - | 1, 2 |
| 45 | 1481 | 1479 | <i>ar</i> -Curcumene | - | 1.71 | - | - | 1.65 | 2.60 | - | - | - | 1, 2 |
| 46 | 1490 | 1486 | β -Selinene | 0.92 | 1.80 | 3.98 | 2.24 | 5.30 | 7.29 | 0.98 | 0.71 | 5.62 | 1, 2 |
| 47 | 1493 | 1493 | δ -Selinene | 2.91 | 5.33 | 3.81 | 6.72 | 4.81 | 5.22 | 0.97 | 2.09 | 14.96 | 1, 2 |
| 48 | 1500 | 1496 | α -Muurolene | 3.14 | 1.13 | - | - | 3.06 | 3.07 | - | - | - | 1, 2 |
| 49 | 1506 | 1502 | E,E- α -Farnesene | - | 0.80 | - | - | 0.97 | - | 0.53 | - | 0.84 | 1, 2 |
| 50 | 1506 | 1506 | β -Bisabolene | - | - | - | - | 0.91 | 0.72 | - | - | - | 1, 2 |
| 51 | 1516 | 1507 | β -Curcumene | - | - | - | - | - | 0.69 | - | - | - | 1, 2 |
| 52 | 1514 | 1515 | γ -Cadinene | 8.11 | 1.79 | - | - | 4.77 | 4.84 | 0.97 | - | - | 1, 2 |
| 53 | 1550 | 1548 | Dimethyl azelate | - | - | - | - | - | - | - | 1.21 | - | 1, 2 |
| 54 | 1563 | 1562 | E-Nerolidol | - | 8.40 | 10.71 | 2.24 | - | - | 4.66 | 5.16 | 27.94 | 1, 2, 3 |
| 55 | 1564 | 1566 | epi-Longipinanol | - | - | - | - | - | - | 0.67 | - | - | 1, 2 |
| 56 | 1569 | 1569 | Longipinanol | - | 0.63 | - | - | - | - | - | - | - | 1, 2 |
| 57 | 1583 | 1578 | Caryophyllene oxide | 0.77 | 1.34 | 1.42 | - | 0.87 | 3.28 | 1.56 | 0.45 | 2.32 | 1, 2, 3 |
| 58 | 1601 | 1589 | Cedrol | - | - | - | - | - | - | - | 5.25 | - | 1, 2 |
| 59 | 1682 | 1683 | Ishwarone | - | 0.39 | - | - | - | - | - | - | 2.48 | 1, 2 |
| 60 | 1724 | 1724 | Methyl tetradecanoate | 2.11 | - | - | - | - | - | - | - | - | 1, 2 |
| 61 | 1807 | 1799 | 2-Ethylhexyl salicylate | - | 1.41 | - | - | - | - | - | 0.26 | 2.88 | 1, 2 |
| Monoterpene hydrocarbons (1, 3, 4, 6, 9, 11, 12) | | | | 6.52 | 21.89 | 10.27 | 30.85 | 24.23 | 8.92 | 50.08 | 10.96 | 4.53 | |
| Oxygenated monoterpenes (5, 10, 14, 16, 17, 19, 22, 36) | | | | 12.22 | 11.55 | 31.93 | 20.81 | 8.78 | 1.13 | 15.17 | 44.74 | 17.92 | |
| Sesquiterpene hydrocarbons (28-35, 37-39, 41-44, 46-52) | | | | 64.72 | 47.06 | 27.48 | 32.09 | 47.93 | 76.77 | 25.92 | 7.18 | 36.65 | |
| Oxygenated sesquiterpenes (26, 40, 54-59) | | | | 2.66 | 10.76 | 12.13 | 2.24 | 0.87 | 3.28 | 6.89 | 11.25 | 32.74 | |
| Aliphatic hydrocarbons & their derivatives (2, 7, 15, 20, 21, 24, 25, 27, 53, 60) | | | | 4.73 | 2.45 | 13.59 | 3.39 | 2.37 | 3.98 | 0.89 | 5.18 | 2.71 | |
| Phenyl propanoids (8, 13, 18, 23, 45, 61) | | | | 8.55 | 4.38 | 2.00 | - | 8.03 | 2.6 | 0.56 | 20.76 | 4.11 | |
| Total identified (%) | | | | 99.4 | 98.09 | 97.4 | 89.38 | 92.21 | 96.68 | 99.51 | 99.92 | 98.66 | |

^aLinear retention index on a DB-5 column; ^bIdentification method: 1 = linear retention index; 2 = identification based on comparison of mass spectra; 3 = Co-injection with standard compounds. Serial numbers of components detected in each class are listed beside the Class name.

The main compound detected in this fraction was α -copaene (25.31%). The content of sesquiterpene hydrocarbons decreased gradually in the emitted aroma of the plant as a function of growth stage, reaching a minimum value of 27.48% prior to the flowering season. The decrease in the concentration of the main class was accompanied also by a decrease in the concentration of α -copaene, which accounted for 7.91% of the total emitted aroma content during this period.

Sesquiterpene hydrocarbons also dominated the emission profile of the aerial parts of *I. viscosa* collected from northern Jordan valley region (MID-2) and their contents increased gradually with maturation degree (32.09 to 76.77%). During

the early vegetative stage, *trans*-caryophyllene was the main component (13.28%) and its content decreased gradually to 7.62% prior to flowering period. On the contrary, the content of α -copaene increased gradually to 23.50% of the total composition during the same period.

The aerial parts of *I. viscosa* collected from IT zone had a variably interesting emission profile that was rich mainly in monoterpene hydrocarbons in the early vegetative stage (50.08%), oxygenated monoterpenes in early summer season (44.74%) and sesquiterpene hydrocarbons in late summer (36.65%). During this period of time, these three classes were dominated by limonene (32.20%), myrcenol (38.15%) and

δ -selinene (14.96%), respectively. Interestingly, the aroma of the plant collected from this location contained higher amounts of the oxygenated sesquiterpene E-nerolidol during the summer season, which amounted to 27.94% of the total content.

Volatile organic compounds (VOCs) during the flowering period: The chemical composition of the spontaneously

emitted VOCs of *I. viscosa* aerial organs including fresh leaves (L), pre-flowering buds (PF) and full inflorescences (F) were also determined during the full flowering period (October). Results are summarized in Table-2.

Sesquiterpenoids were the main class detected in the fresh leaves samples collected from the various locations, but with

TABLE-2
VARIATION OF THE SPME-VOCs CONTENT OF LEAVES (L), PRE-FLOWERING (PF)
BUDS AND FULLY EXPANDED FLOWERS (F) DURING THE FLOWERING SEASON

| No. | RI ^a | | Constituents | MID-1 | | | MID-2 | | | IT | | | b |
|-----|-----------------|------|---------------------------------------|-------|--------|-------|-------|--------|-------|-------|--------|-------|---------|
| | Lit. | Exp. | | L (%) | PF (%) | F (%) | L (%) | PF (%) | F (%) | L (%) | PF (%) | F (%) | |
| 1 | 927 | 927 | Tricyclene | - | 0.67 | 0.82 | - | - | - | - | 0.92 | - | 1, 2 |
| 2 | 930 | 930 | α -Thujene | - | - | - | - | - | - | - | - | 2.55 | 1, 2 |
| 3 | 924 | 932 | 3-Methyl-4-heptanone | - | - | - | - | - | - | - | 0.61 | - | 1, 2 |
| 4 | 939 | 933 | α -Pinene | 0.89 | 0.40 | 0.70 | 1.15 | 1.60 | 2.00 | - | 1.47 | 2.76 | 1, 2, 3 |
| 5 | 967 | 967 | <i>n</i> -Heptanol | - | 0.44 | 0.71 | - | 1.44 | 0.58 | - | - | 0.69 | 1, 2 |
| 6 | 975 | 980 | Sabinene | 1.17 | 2.34 | 2.38 | 0.56 | - | 1.13 | - | - | - | 1, 2, 3 |
| 7 | 986 | 980 | 6-Methyl-5-hepten-2-one | - | - | - | - | - | - | - | 0.63 | - | 1, 2 |
| 8 | 985 | 984 | <i>trans</i> -Isolimonene | - | - | - | - | 1.08 | 0.44 | - | - | - | 1, 2 |
| 9 | 979 | 986 | β -Pinene | 0.67 | 1.35 | 1.36 | 0.93 | 1.75 | 17.96 | 2.96 | - | 0.87 | 1, 2, 3 |
| 10 | 988 | 990 | Dehydro-1,8-cineol | 2.12 | 4.07 | 4.12 | 5.92 | 5.46 | - | - | - | 17.34 | 1, 2 |
| 11 | 999 | 1003 | <i>n</i> -Octanal | - | - | - | - | - | - | - | 0.60 | - | 1, 2 |
| 12 | 1005 | 1005 | <i>cis</i> -3-Hexenyl acetate | 1.62 | - | - | 1.53 | - | 1.73 | - | - | - | 1, 2 |
| 13 | 1017 | 1016 | α -Terpinene | 0.18 | - | - | - | - | - | - | - | - | 1, 2, 3 |
| 14 | 1025 | 1024 | <i>p</i> -Cymene | 1.94 | - | - | - | - | - | - | - | - | 1, 2, 3 |
| 15 | 1029 | 1029 | Limonene | 1.98 | 1.09 | 1.21 | 11.11 | - | 3.17 | 3.36 | - | 0.89 | 1, 2, 3 |
| 16 | 1031 | 1032 | 1,8-Cineol | 0.71 | 0.43 | 0.82 | - | 0.12 | 1.26 | - | 0.82 | 1.20 | 1, 2, 3 |
| 17 | 1037 | 1033 | <i>cis</i> -Ocimene | - | 0.80 | - | - | - | 1.21 | - | - | - | 1, 2, 3 |
| 18 | 1050 | 1045 | <i>trans</i> -Ocimene | - | 0.49 | - | - | 0.9 | 1.63 | - | 0.99 | 0.97 | 1, 2, 3 |
| 19 | 1060 | 1057 | γ -Terpinene | 0.81 | 2.96 | 2.96 | - | - | - | - | - | - | 1, 2 |
| 20 | 1068 | 1064 | <i>n</i> -Octanol | - | - | - | - | - | - | - | 1.06 | - | 1, 2 |
| 21 | 1089 | 1098 | Terpinolene | 0.98 | 1.38 | 1.46 | - | - | 0.80 | - | 2.89 | 2.91 | 1, 2, 3 |
| 22 | 1091 | 1090 | Dehydrolinalool | - | - | - | - | - | 1.09 | - | - | - | 1, 2 |
| 23 | 1097 | 1099 | Linalool | - | 0.47 | - | - | 1.22 | 0.57 | 3.40 | 0.71 | - | 1, 2, 3 |
| 24 | 1102 | 1107 | α -Thujone | 0.64 | - | - | - | - | - | - | - | - | 1, 2, 3 |
| 25 | 1101 | 1105 | <i>n</i> -Nonanal | - | - | 0.53 | 0.56 | - | - | - | 0.72 | - | 1, 2 |
| 26 | 1123 | 1113 | Myrcenol | 17.29 | 37.07 | 36.74 | 0.61 | 20.92 | 30.36 | 26.03 | 39.09 | 33.88 | 1, 2 |
| 27 | 1192 | 1193 | Methyl salicylate | - | - | - | 1.14 | - | - | - | - | - | 1, 2 |
| 28 | 1189 | 1196 | α -Terpineol | - | 0.34 | - | - | - | - | 0.60 | 0.64 | 0.97 | 1, 2, 3 |
| 29 | 1202 | 1206 | <i>n</i> -Decanal | 0.39 | 0.42 | - | - | - | - | 0.74 | 0.84 | - | 1, 2 |
| 30 | 1197 | 1215 | Butanoicacid, 2-methyl-4-methylpentyl | - | - | - | - | - | - | - | 1.00 | - | 1, 2 |
| 31 | 1229 | 1228 | 3-Z-Hexenyl 2-methyl butanoate | 18.05 | - | - | - | - | - | - | - | - | 1, 2 |
| 32 | 1235 | 1228 | Thymol methyl ether | - | - | - | - | - | - | 0.97 | - | - | 1, 2 |
| 33 | 1232 | 1235 | 3-Z-Hexenyl 3-methyl butanoate | 0.76 | - | - | - | - | - | - | - | - | 1, 2 |
| 34 | 1290 | 1293 | Thymol | 0.44 | - | - | - | - | - | - | - | - | 1, 2, 3 |
| 35 | 1319 | 1302 | <i>cis</i> -Cycloisolongifol-5-ol | - | 0.63 | 0.52 | - | - | - | - | - | - | 1, 2 |
| 36 | 1312 | 1312 | Sesamol | 0.45 | - | - | - | - | - | - | - | - | 1, 2 |
| 37 | 1333 | 1320 | Hexyl tiglate | 1.32 | - | - | - | - | - | - | - | - | 1, 2 |
| 38 | 1351 | 1366 | α -Cubebene | 1.09 | 1.76 | 1.73 | 1.67 | 1.72 | 1.20 | 0.59 | - | - | 1, 2 |
| 39 | 1377 | 1373 | α -Copaene | 7.29 | 13.04 | 12.89 | 15.44 | 11.29 | 9.13 | - | - | - | 1, 2 |
| 40 | 1391 | 1386 | 7-Episesquithujene | - | - | - | 2.94 | - | - | - | - | - | 1, 2 |
| 41 | 1388 | 1391 | β -Cubebene | - | 1.67 | 1.66 | - | - | - | - | - | - | 1, 2 |
| 42 | 1391 | 1391 | β -Elemene | 4.21 | 1.08 | 1.07 | - | 1.35 | 1.38 | 1.10 | - | - | 1, 2 |
| 43 | 1401 | 1392 | β -Longipinene | - | - | - | - | - | - | 8.94 | 1.96 | 1.92 | 1, 2 |
| 44 | 1402 | 1403 | iso-Italicene | - | - | - | 2.55 | - | - | 0.68 | - | - | 1, 2 |
| 45 | 1409 | 1412 | <i>cis</i> -Caryophyllene | 2.40 | 0.91 | 0.90 | 10.70 | 1.03 | - | 2.67 | 1.16 | 0.78 | 1, 2, 3 |
| 46 | 1419 | 1418 | <i>trans</i> -Caryophyllene | 0.71 | 2.78 | 2.76 | - | 4.93 | 9.96 | 4.88 | 11.18 | 7.15 | 1, 2, 3 |
| 47 | 1435 | 1431 | <i>trans</i> - α -Bergamotene | - | - | - | 1.62 | - | 0.41 | - | - | - | 1, 2 |
| 48 | 1436 | 1446 | Neryl acetone | - | 0.63 | 0.63 | 0.42 | 1.29 | - | 2.22 | - | - | 1, 2 |
| 49 | 1441 | 1440 | Aromadendrene | 0.70 | 0.83 | 0.82 | - | - | - | - | - | - | 1, 2 |
| 50 | 1454 | 1445 | α -Neoclovene | - | - | - | - | - | - | - | - | 0.81 | 1, 2 |
| 51 | 1457 | 1452 | β -E-Farnesene | - | - | - | - | - | - | 1.43 | 1.60 | - | 1, 2 |
| 52 | 1455 | 1453 | α -Humulene | 1.04 | - | - | 1.11 | 3.06 | 0.90 | - | - | 0.97 | 1, 2, 3 |
| 53 | 1460 | 1457 | Alloarmadendrene | 1.61 | 2.70 | 2.68 | 2.08 | 4.48 | 1.93 | - | - | - | 1, 2, 3 |

| | | | | | | | | | | | | | |
|--|------|------|---|-------|-------|-------|-------|-------|-------|-------|-------|-------|---------|
| 54 | 1463 | 1461 | Dehydroaromadendrane | - | 0.80 | 0.80 | 0.40 | 1.04 | - | - | - | - | 1, 2 |
| 55 | 1482 | 1462 | γ -Irene | 0.97 | - | - | - | - | - | 7.45 | 2.65 | - | 1, 2 |
| 56 | 1477 | 1471 | γ -Gurjunene | 1.27 | 1.40 | 1.39 | - | 1.48 | - | 1.89 | 2.77 | 1.72 | 1, 2 |
| 57 | 1480 | 1472 | γ -Muurolene | 2.34 | - | 2.03 | 0.63 | - | - | 0.89 | - | 1.71 | 1, 2 |
| 58 | 1496 | 1471 | Valencene | - | 2.05 | - | - | 2.20 | 1.33 | 7.87 | 3.22 | 2.67 | 1, 2 |
| 59 | 1483 | 1476 | γ -Curcumene | - | - | - | 13.55 | - | - | - | - | - | 1, 2 |
| 60 | 1481 | 1479 | α -Curcumene | - | - | - | 8.05 | - | - | - | - | - | 1, 2 |
| 61 | 1490 | 1486 | β -Selinene | 3.20 | 2.87 | 2.84 | 2.36 | 11.32 | 3.54 | 3.89 | 3.70 | 3.46 | 1, 2 |
| 62 | 1493 | 1493 | δ -Selinene | 5.12 | 4.22 | 4.18 | 1.31 | 1.89 | 0.84 | 5.85 | 10.10 | 7.71 | 1, 2 |
| 63 | 1500 | 1496 | α -Muurolene | 0.71 | 0.92 | 0.91 | 1.28 | 3.39 | 2.05 | 0.55 | - | - | 1, 2 |
| 64 | 1516 | 1507 | β -Curcumene | - | - | - | 2.31 | - | - | - | - | - | 1, 2 |
| 65 | 1514 | 1516 | γ -Cadinene | 0.67 | 1.11 | 1.10 | 3.00 | 2.00 | 1.00 | - | - | - | 1, 2 |
| 66 | 1535 | 1536 | 10-epi-Cubebol | - | 0.67 | 0.67 | - | - | - | - | - | - | 1, 2 |
| 67 | 1563 | 1562 | E-Nerolidol | 9.82 | 2.85 | 2.82 | - | 12.09 | 1.38 | 5.55 | 7.45 | 3.67 | 1, 2, 3 |
| 68 | 1564 | 1566 | epi-Longipinanol | 0.50 | 0.53 | - | - | - | - | 0.37 | - | - | 1, 2 |
| 69 | 1583 | 1578 | Caryophyllene oxide | 1.35 | - | 0.53 | - | 0.87 | 0.56 | 2.38 | 1.20 | 0.78 | 1, 2, 3 |
| 70 | 1641 | 1631 | Caryophylla-4(14),8(15)-dien-5- β -ol | - | - | - | 0.49 | - | - | - | - | - | 1, 2 |
| 71 | 1648 | 1652 | Agarspirol | 1.0 | - | - | - | - | - | 1.02 | - | - | - |
| 72 | 1667 | 1662 | Intermedeol | - | - | - | - | - | - | 0.45 | - | - | 1, 2 |
| 73 | 1682 | 1678 | Ishwarone | 0.51 | - | - | - | - | - | 0.38 | - | - | 1, 2 |
| Monoterpene hydrocarbons (1, 2, 4, 6, 8, 9, 13, 15, 17, 18, 19, 21) | | | | 6.68 | 11.48 | 10.89 | 13.75 | 5.33 | 28.34 | 6.32 | 6.27 | 10.95 | |
| Oxygenated monoterpenes (10, 16, 22, 23, 24, 26, 28, 48) | | | | 20.76 | 43.01 | 42.31 | 6.95 | 29.01 | 33.28 | 32.25 | 41.26 | 53.39 | |
| Sesquiterpene hydrocarbon (38-47, 49-54, 56-65) | | | | 32.36 | 39.47 | 39.10 | 62.95 | 51.18 | 33.67 | 41.23 | 35.69 | 28.90 | |
| Oxygenated sesquiterpenes (55, 66-73) | | | | 14.15 | 4.68 | 4.54 | 0.49 | 12.96 | 1.94 | 17.6 | 11.3 | 4.45 | |
| Aliphatic hydrocarbons & their derivatives (3, 5, 7, 11, 12, 20, 25, 29, 30, 31, 33, 37) | | | | 22.14 | 0.86 | 1.24 | 2.09 | 1.44 | 2.31 | 0.74 | 5.46 | 0.69 | |
| Phenyl propanoids (14, 32, 34, 36) | | | | 2.83 | - | - | 9.19 | - | - | 0.97 | - | - | |
| Total identified | | | | 98.92 | 99.50 | 98.08 | 95.42 | 99.92 | 99.54 | 99.11 | 99.98 | 98.38 | |

^aLinear retention index on a DB-5 column; ^bIdentification method: 1 = linear retention index; 2 = identification based on comparison of mass spectra; 3 = Co-injection with standard compounds. Serial numbers of components detected in each class are listed beside the Class name.

some qualitative and quantitative differences regarding the types of sesquiterpenoids (hydrocarbons or oxygenated).

While sesquiterpene hydrocarbons dominated the emission profile of the fresh leaves collected from waste lands of MID-1 (32.36%), IT (41.23%) and MID-2 (62.95%), other classes afforded the most abundant compounds detected in the emission profiles of *I. viscosa* leaves. These included the aliphatic hydrocarbon 3-Z-hexenyl-2-methyl butanoate (18.05%, MID-1) and the oxygenated monoterpene myrcenol (26.03%, IT zone). α -Copaene (15.44%) was the main sesquiterpene hydrocarbon detected in the emitted aroma of leaves samples collected from MID-2 region.

The emission profiles of the flowers at the pre-flowering stage revealed a total of 39, 26 and 26 constituents detected in the spontaneously emitted VOCs from the three locations, and accounting for 98.92% (MID-1), 99.92% (MID-2), 99.98% (IT) of the total emission content. Oxygenated monoterpenes dominated the emission profiles of the PF buds of MID-1 and IT samples (43.01% and 41.26%, respectively) mostly represented by myrcenol (37.07% and 39.09%, respectively). On the other hand, sesquiterpene hydrocarbons dominated the aroma of the PF buds collected from MID-2 region (51.18%) followed by oxygenated monoterpenoids (29.01%), with myrcenol (20.92%) as the main component. The main sesquiterpene hydrocarbons detected included β -selinene (11.32%) and α -copaene (11.29%).

Oxygenated monoterpenes dominated the emission profile of the inflorescences during the full flowering stage collected in IT (53.39%) and MID-2 (33.28%) samples. Samples from MID-1 area were rich in sesquiterpene hydrocarbons (39.10%).

Despite this variation, myrcenol remained the main component in the emissions of all samples from the three different locations (MID-1: 36.74%; MID-2: 30.36%, and IT: 33.88%).

Chemical composition of the hydrodistilled oils obtained from *I. viscosa* fresh leaves and flowers at the pre- and full flowering stages: Analysis of the hydrodistilled oils obtained from the leaves and the inflorescences during the pre-flowering and full flowering stages resulted in the identification and characterization of a total of 48 compounds (Table-3). All samples from the two geographical zones were characterized by high content of oxygenated sesquiterpenoids amounting to more than 67% of the total content. In most cases, E-nerolidol had the highest contribution to the distilled oils obtained from leaves, pre-flowering buds and fully flowering blooms of MID-1 (50.2%, 70.4% and 54.7%, respectively), MID-2 (88.7%, 40.1 and 41.2%, respectively) and IT (37.7%, 44.4% and 47.1%). In addition, other compounds belonging to this class were detected in appreciable amounts. Table-3 lists the main constituents detected in the different hydrodistilled organs.

Principal component analysis (PCA) and cluster analysis (CA): In the current work, PCA has been applied to the data obtained from the GC/MS analysis (Tables 1-3) and was then used to investigate similarities or differences in the chemical composition of emission profiles of the aerial parts and organs as affected by growth stage and origin (MID-1, MID-2 and IT). Therefore, only the first few PCs were studied, best result was obtained upon using a two-dimensional PCAs score models that for about 40% (Table-1), 42% (Table-2) and 21% (Table-3) of the total variation in the data sets using the first two PCs.

TABLE-3
 CHEMICAL COMPOSITION OF THE HYDRO-DISTILLED OILS OBTAINED FROM THE LEAVES (L),
 PRE-FLOWERING BUDS (PF) AND FULLY EXPANDED FLOWERS (F) OF *I. viscosa* GRWOING WILD IN JORDAN

| No. | RI ^a | | Constituents | MID-1 | | | MID-2 | | | IT | | | b |
|---|-----------------|------|-------------------------------------|-------|--------|-------|-------|--------|-------|-------|--------|-------|---------|
| | Lit. | Exp. | | L (%) | PF (%) | F (%) | L (%) | PF (%) | F (%) | L (%) | PF (%) | F (%) | |
| 1 | 979 | 979 | β-Pinene | - | - | - | - | - | - | 0.63 | - | - | 1, 2 |
| 2 | 991 | 990 | Dehydro1,8-cineol | - | - | - | - | - | 0.50 | - | - | 1.30 | 1, 2 |
| 3 | 1189 | 1196 | α-Terpineol | - | - | - | - | - | 1.01 | - | - | 0.69 | 1, 2, 3 |
| 4 | 1290 | 1293 | Thymol | 0.54 | - | - | - | - | - | 0.63 | - | - | 1, 2, 3 |
| 5 | 1377 | 1373 | α-Copaene | 2.23 | 0.66 | 2.09 | 0.49 | 2.40 | 2.42 | - | - | - | 1, 2 |
| 6 | 1391 | 1392 | β-Elementene | 2.69 | - | - | - | - | - | 2.67 | 0.87 | 1.67 | 1, 2 |
| 7 | 1409 | 1412 | cis-Caryophyllene | 2.53 | - | - | - | - | - | 2.67 | 0.74 | 1.43 | 1, 2, 3 |
| 8 | 1419 | 1418 | trans-Caryophyllene | 0.50 | - | - | 0.44 | 1.91 | 1.98 | 1.05 | 0.63 | 1.48 | 1, 2, 3 |
| 9 | 1436 | 1446 | Neryl acetone | 0.59 | - | - | - | - | - | 3.48 | - | 2.68 | 1, 2 |
| 10 | 1454 | 1445 | Neoclovene | - | - | - | - | - | - | - | - | 1.83 | 1, 2 |
| 11 | 1455 | 1454 | α-Humulene | 1.83 | - | - | 0.13 | 2.14 | - | - | - | - | 1, 2, 3 |
| 12 | 1460 | 1457 | Alloarmadendrene | 1.22 | - | - | 0.20 | - | 1.12 | - | - | - | 1, 2 |
| 13 | 1482 | 1468 | γ-Irone | 4.57 | 1.43 | 3.34 | 0.43 | 2.60 | 1.38 | - | 3.66 | - | 1, 2 |
| 14 | 1477 | 1471 | γ-Gurjunene | - | - | - | - | - | - | 5.03 | 2.16 | 1.05 | 1, 2 |
| 15 | 1480 | 1472 | γ-Murolene | 5.30 | 0.92 | - | 0.61 | - | - | 5.03 | 1.50 | 3.98 | 1, 2 |
| 16 | 1481 | 1479 | ar-Curcumene | 0.59 | - | - | 0.61 | - | - | - | - | - | 1, 2 |
| 17 | 1490 | 1486 | β-Selinene | 2.46 | 1.15 | 1.38 | 0.19 | 3.63 | 3.85 | 1.40 | 0.76 | 5.70 | 1, 2 |
| 18 | 1493 | 1493 | δ-Selinene | 1.72 | - | 2.07 | 0.28 | 1.87 | 1.90 | - | 1.86 | - | 1, 2 |
| 19 | 1496 | 1489 | Valencene | - | - | - | - | - | - | 1.22 | 3.82 | 2.19 | 1, 2 |
| 20 | 1500 | 1496 | α-Murolene | 0.53 | - | 0.54 | 0.19 | - | 1.11 | - | - | - | 1, 2 |
| 21 | 1514 | 1515 | γ-Cadinene | 1.23 | 0.62 | 0.87 | 0.53 | 1.52 | 1.56 | - | - | - | 1, 2 |
| 22 | 1539 | 1536 | α-Copaen-11-ol | 0.48 | - | 1.77 | 0.29 | 1.51 | 1.55 | - | - | - | 1, 2 |
| 23 | 1563 | 1562 | E-Nerolidol | 50.16 | 70.42 | 54.71 | 88.66 | 40.14 | 41.19 | 37.72 | 44.38 | 47.07 | 1, 2, 3 |
| 24 | 1569 | 1569 | Longipinanol | - | - | - | - | - | - | 1.24 | 0.73 | 1.48 | 1, 2 |
| 25 | 1564 | 1566 | epi-Longipinanol | 1.32 | - | 0.93 | - | - | - | 0.07 | - | 0.54 | 1, 2 |
| 26 | 1583 | 1579 | Caryophyllene oxide | 3.56 | 2.24 | 4.11 | 2.20 | 6.65 | 6.81 | 5.13 | 3.32 | 7.10 | 1, 2, 3 |
| 27 | 1582 | 1585 | Globulol | - | - | - | - | - | - | 0.94 | - | - | 1, 2 |
| 28 | 1596 | 1592 | ar-Dihydroturmerone | - | 3.65 | - | - | - | - | - | - | - | 1, 2 |
| 29 | 1596 | 1593 | Fokineol | 2.30 | - | - | 0.71 | 5.77 | - | 2.94 | 3.39 | 2.32 | 1, 2 |
| 30 | 1601 | 1603 | Guaiol | - | - | 1.02 | - | - | - | 0.67 | - | - | 1, 2 |
| 31 | 1608 | 1606 | Humulene epoxide II | - | - | - | 0.09 | 1.40 | - | - | - | - | 1, 2 |
| 32 | 1608 | 1613 | cis-Isolongifolanone | - | - | - | - | - | - | 0.45 | 0.89 | - | 1, 2 |
| 33 | 1619 | 1614 | Isolongifolan-7a-ol | - | - | - | 0.12 | 1.70 | 1.76 | - | - | - | 1, 2 |
| 34 | 1637 | 1621 | Gossonol | - | - | - | - | - | - | 6.54 | - | - | 1, 2 |
| 35 | 1613 | 1623 | trans-Isolongifolanone | 0.91 | 1.01 | 1.92 | - | - | - | 0.84 | 3.11 | 1.39 | 1, 2 |
| 36 | 1641 | 1631 | Alloarmadendrene epoxide | 1.64 | 1.06 | 1.73 | 0.62 | 1.62 | - | - | - | - | 1, 2 |
| 37 | 1641 | 1632 | Caryophylla-4-(14)8(15)-dien-5-β-ol | 1.14 | 0.88 | 1.21 | - | 1.67 | 1.11 | 2.98 | 1.40 | 0.59 | 1, 2 |
| 38 | 1651 | 1635 | β-Eudesmol | 3.04 | 1.48 | 7.59 | - | - | - | 1.34 | 1.30 | 1.04 | 1, 2 |
| 39 | 1654 | 1649 | α-Eudesmol | - | - | - | - | 2.17 | 2.20 | - | - | - | 1, 2 |
| 40 | 1648 | 1652 | Agarospinol | 3.30 | 5.24 | 5.21 | 1.11 | 4.91 | 5.09 | 10.66 | 11.79 | 7.11 | 1, 2 |
| 41 | 1667 | 1662 | Intermedeol | - | - | - | 0.17 | - | - | - | - | 0.84 | 1, 2 |
| 42 | 1661 | 1664 | E-Amyl-cinnamic alcohol | - | - | 0.51 | - | - | - | - | - | - | 1, 2 |
| 43 | 1682 | 1683 | Ishwarone | 0.96 | 1.76 | 2.32 | 0.48 | 2.41 | 3.55 | 1.28 | 2.75 | 5.04 | 1, 2 |
| 44 | 1691 | 1698 | z-α-trans-Bergamotol | - | 0.61 | - | 0.49 | - | - | - | 2.09 | - | 1, 2 |
| 45 | 1745 | 1740 | γ-Costol | - | 1.55 | - | 0.41 | 2.86 | 2.81 | 0.66 | 2.08 | - | 1, 2 |
| 46 | 1760 | 1761 | Benzyl benzoate | - | - | - | 0.13 | - | - | - | - | - | 1, 2 |
| 47 | 1766 | 1762 | β-Costol | 0.76 | - | 1.64 | - | 6.19 | 6.15 | 1.24 | - | - | 1, 2 |
| 48 | 1773 | 1765 | α-Costol | 1.02 | 4.58 | 4.22 | - | 4.91 | 4.87 | - | 4.78 | 1.44 | 1, 2 |
| Monoterpene hydrocarbons (1) | | | | - | - | - | - | - | - | 0.63 | - | - | |
| Oxygenated monoterpenes (2, 3, 9) | | | | 0.59 | - | - | - | - | 1.51 | 3.48 | - | 4.67 | |
| Sesquiterpene hydrocarbons (5-8, 10-12, 14, 15, 17-21) | | | | 22.24 | 3.35 | 6.95 | 3.06 | 13.47 | 13.94 | 19.07 | 12.34 | 19.33 | |
| Oxygenated sesquiterpenes (22-27, 29-33, 35-41, 43-45, 47-48) | | | | 75.16 | 92.26 | 91.72 | 95.78 | 86.51 | 78.47 | 68.16 | 85.67 | 75.96 | |
| Phenyl propanoids (4, 16, 28, 34, 42, 46) | | | | 1.13 | 3.65 | 0.51 | 0.74 | - | - | 7.17 | - | - | |

^aLinear retention index on a DB-5 column; ^bIdentification method: 1 = linear retention index; 2 = identification based on comparison of mass spectra; 3 = Co-injection with standard compounds. Serial numbers of components detected in each class are listed beside the Class name.

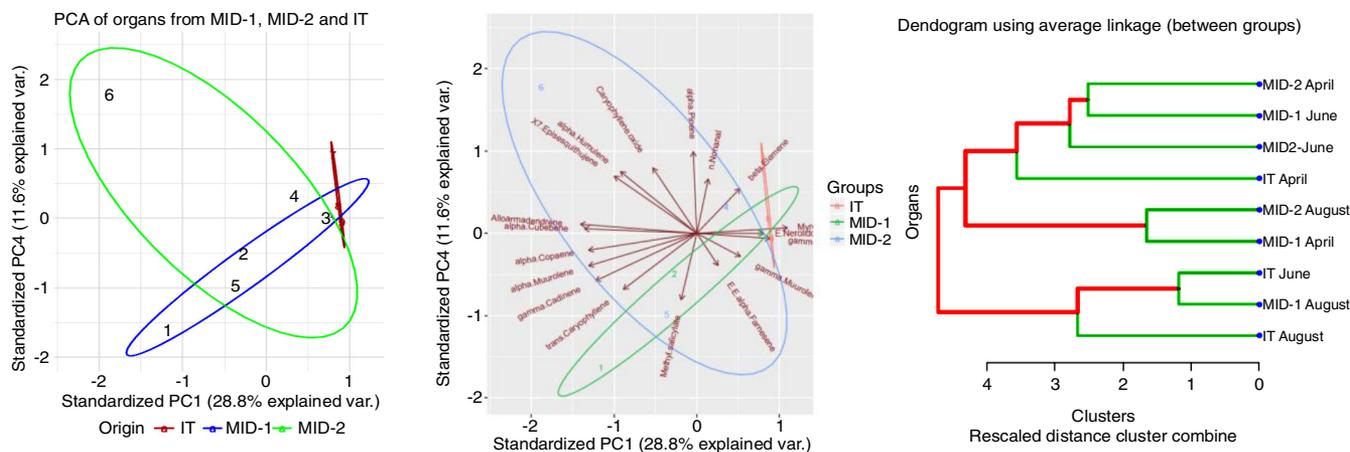


Fig. 1. PCA scores plot, PCA bi-plots and dendrogram based on Table-1 data

Fig. 1 represents the resulted PCA scores plot, PCA bi-plots and dendrogram corresponding to the VOCs emissions obtained from the whole aerial parts of the plant during the vegetative state (Table-1).

Two crossed clusters were obtained, one corresponding to the plant data collected from MID-1 and the other corresponding to the plant samples collected from MID-2, indicating similarities in the chemical composition of the emission profiles from the two locations, the third location which belongs to the collections from a different bio-geographical zone (IT) was quite different. Every point in Fig. 1 of the three clusters represents the data obtained from the collection of the plant in particular month from the three different locations and the shown clusters in this figure clearly reveals no significant differences in the chemical compositions extracted from different months in MID-1 and MID-2. Further investigation of the data has been carried out in an attempt to identify the major chemical constituents responsible for the observed behaviour during the vegetative state of the plants' life cycle. Hence, bi-plots (scores and loadings) PCA were created. As can be concluded, γ -muurolene, E,E- α -farnesene, methyl salicylate, γ -gurjunene, E-nerolidol and myrceneol had the greatest impact on the similarity in the first two PCs. For more analysis, CA was applied to the same data matrix used for the PCA. The resulted dendrogram of this test is shown in Fig. 1 using complete link-

age distances between the samples (organs). The obtained results were very consistent to those obtained from the PCA application revealing strong relation between the emitted VOCs belonging to MID collection during the vegetative period.

PCA scores plot, PCA bi-plots and dendrogram were generated for the data given in Table-2 (Fig. 2). The results during the vegetative period were in agreement with the two crossed clusters correspond to obtained MID-1 and MID-2 collections, revealing good similarities in the composition of their emission profiles while the third location showed a different emission profile.

The main constituents responsible for this behaviour during the flowering period that had the greatest impact on the similarity included β -selinene, *n*-heptanol, dehydroaromadendrane, γ -muurolene, alloarmadendrene, α -cubebene and γ -cadinene and to a lower impact each of *cis*-3-hexenyl acetate and *cis*-caryophyllene. Also, it was noticed that there is a strong relations between all IT organs emissions supporting its separation in a different cluster.

The variation in the chemical composition of the hydro-distilled essential oils was also statistically investigated, the results of the PCA, bi-plots (scores and loadings) PCA and dendrogram are shown in Fig. 3. In agreement with present results obtained from the emission profiles during vegetative and flowering stages, the two crossed clusters corresponding

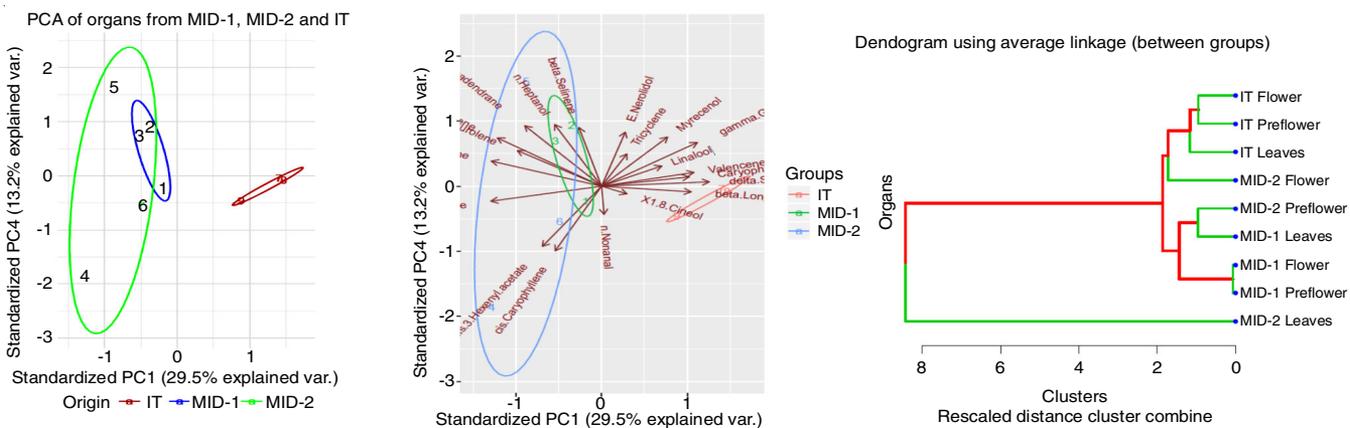


Fig. 2. PCA scores plot based on Table-2 data

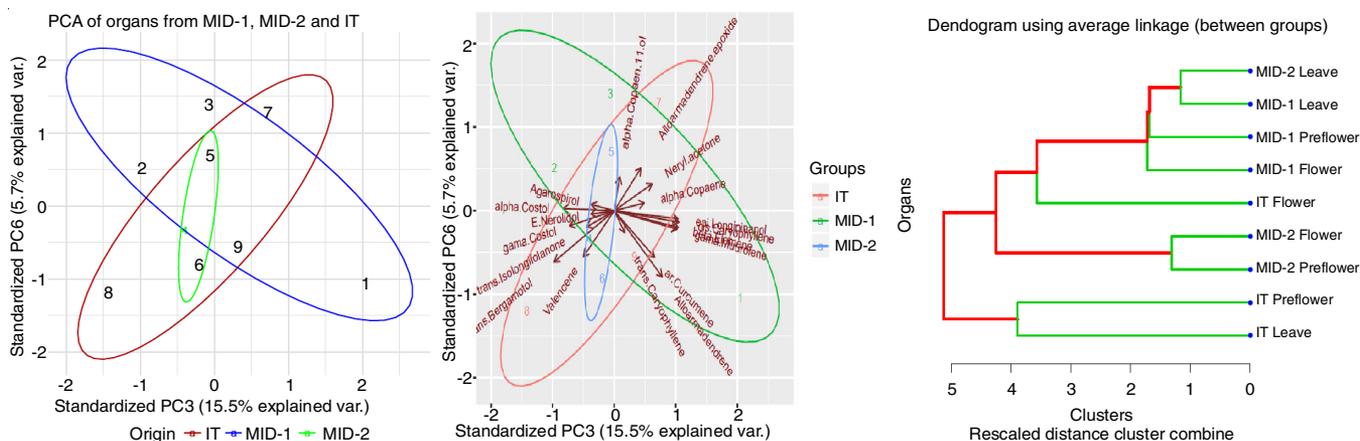


Fig. 3. PCA scores plot for the applied to the data in Table-3

to MID-1 and MID-2 collections revealed quite similar chemical composition. Despite the observed intersection of IT with both MID-1 and MID-2, it can be noticed easily that most of the points belonging to the IT collections (PF buds (7) and F (8)) are out of the intersection area of MID-1 and MID-2. Components having the high impact on the observed similarity of the first two principal components included α -costol, γ -costol, α -copaen-11-ol, alloarma-dendrene, ar-curcumene, epi-longipinanol, *cis*-caryophyllene, β -elemene and γ -muurolene, while agarospirol, E-nerolidol and *trans*-isolongifolanone despite their less impact still had important role in the observed similarity.

Conclusion

The current investigation is the first report presenting the geographical and seasonal variation effects on the chemical composition of *I. viscosa* using two different extraction methods SPME and hydrodistillation. The current results, revealed that plants collected from the Mediterranean (MID-1 & 2) and Irano-Turanian (IT) bio-geographical zones, exhibited quite different qualitative and quantitative variations in their content (emitted scent and essential oil), as confirmed from the statistical analysis results. Such variations could be mainly attributed to exogenous factors (environmental) like soil nature, light, water and other related climatic conditions. It was noticed that E-nerolidol was the main component detected in the hydrodistilled oils of the different organs. Careful inspection of the literature indicates that there are several chemotypes of *I. viscosa*. Regarding the current findings, E-nerolidol is the main chemotype of *I. viscosa* growing wild in different bio-geographical zones of Jordan.

ACKNOWLEDGEMENTS

The authors wish to thank the Deanship of Scientific Research at Al-Balqa Applied University for their financial support (Project Number: 46/2014/2015).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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