Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 4, No. 3, p. 296-302, 2014
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

# The study of relationship between two cytotypes and chemotypes of *Achillea millefolium* L. (Asteraceae) from Iran

**Ali Mazooji[1*], Fahimeh Salimpour[2], Talayeh Poorfarzaneh[3]**

[1]*Department of Biology, Roudehen Branch, Islamic Azad University, Roudehen, Iran*

[2]*Department of Biology, North Tehran Branch, Islamic Azad University, Tehran, Iran*

[3]*Department of Biology, Basic Science Faculty, , Islamic Azad University, Science and Research Branch, Tehran, Iran*

Article published on March 22, 2014

**Key words:** Asteraceae, chromosome number, essential oil composition, Medicinal.

**Abstract**

Cra Achillea L. (Asteraceae) comprises 115 species, which are mainly distributed in Europe, Asia and North Africa. 19 species of this genus are described in the Flora Iranica, of which Achillea milllefolium L. is a very important medicinal plant and has been used in traditional medicine. To determine the relation between cytotypes and chemotypes of this species, two accessions were chosen. Cytological analyses and the composition of the essential oils from aerial parts of these two acssessions were performed. The results indicated two ploidy levels of 2n=6x=54 and 2n=8x=72 in this species. Also the main composition of the essential oils of them was different especially in contents. Our results suggesting that the octaploidy level of this species is the best chemotype for pharmacological usage.

***Corresponding Author:** Ali Mazooji ✉ amazooji@yahoo.com

## Introduction

The genus *Achillea* grows in temperate climate in dry or semi-dry habitats. It belong to family Asteraceae and comparises of about 115 species ( Chopra *et al*, 1956; Nemeth, 2005 ). The most important among them having pharmacological significance is *Achillea millefolium* L. ( Khaniki, 1995), commonly known as yarrow or milfoil (Pino and Fuents, 1998 ). It is a very important medicinal plant and has been used in traditional medicine. It uses as herbal tea for headaches, hepato-biliary disorders, gastrointestinal complaints and as an appetite enhancing drug and as lotions against skin inflammations, wounds, cuts and abrasions (Nadim *et al*., 2011; Safiuddin,2002; Si *et al*., 2006; Mockute and Judzentiene, 2003). The most medicinally active parts of the plant are the flowering tops containing essential oil. On the other hand, this taxon is one of the youngest evolutionary genera of the Asteraceae family, and X=9 is often reported as the basic chromosome number in it. But the successive cycle of polyplodization, hybridization and differentiation were reported specially in *A. millefolium* (Hofmann *et al*., 2002; Kiran *et al*., 2008; Mabberley, 1997). By attention to correlation between environmental factors and qualitative contents of components of essential oil, and different ploidy level of this plant, the main objective of this study is to determine the relation between ploidy level and essential oil composition in the two population of *A.millefolium*, that growing in the Northern parts of Iran.

## Materials and Methods

1- Plant material

2- Two acsessions were collected during the flowering period from natural habitats of Iran (Tabel 1).

2- Essential oil extraction

**Tabel 1.** Accessions and their localities.

| label | Acsession | Locality |
|---|---|---|
| Am8 | *A. millefolium* | Alborz: Karaj to Chalous road, Gachsar to Dizine, 1983 m, Mazooji, IRAN-61052 |
| Am3 | *A. millefolium* | Mazandaran: Haraz road, 2331 m, Mazooji, IRAN-63071 |

For isolation of the volatile oils, the aerial parts of the plants were dried at room temperature and hydrodistilled for 4 h using a clevanger-type apparatus. The oil was dried over anhydrous sodium thiosulfate and kept at 4°C in sealed brown vials until required. Analytical gas chromatography was capillary column DB-5 (30 m. 0.25 mmid, 0.25 µm film thickness); carrier gas, He; split ratio, 1: 25, and using a flame ionization detector. The column temperature was programmed at 50°C for 1 min, and then heated to 265°C for 20 min. Gas chromatography- mass spectrum (GC-MS) was performed on a thermoquest 2000 with quadruple detector, on capillary column DB-5 (GC), carried gas, He; flow rate, 1.5 ml/min. The column was held at 50°C for 1 min, and programmed up to 265°C for 20 min. Quantitative data were obtained from the electronic integration of the FID peak areas. The components of the oils were identified by comparison of their mass spectra and retention indicates with Wiley library.

3- Chromosome counting

For chromosome counting, seeds were germinated on wet filter paper in petri dishes and left at 22°C temperature for two days. Root tip meristems obtained from seedling were pretreated in 8-hydroxyl- quinalin (2Mm) at 4°C for five h, fixed in 1:1 (v/v) solution of formalin 10% and chromic acid 1% for 24 h at 4°C, then root tips NaOH 1N for 10 min at 60°C and stained with aceto-iron-hematoxilin solution for 4 h at 30°C. After each step, root tips were washed briefly in distilled water. Meristematic region with 1 mm of length excised and macerated in cytase enzyme at room temperature for 1 h. Squash preparations on slides were made in 45% acetic acid

(Aghayev, 1998). Chromosome measurements including long arm (LA), short arm (SA), total length of chromosomeset (TL). Karyotype formula was determined by chromosome morphology based on centromere position according to classification of Levan (Levan *et al*., 1964).

**Results**

Gas-choromatographic analysis of the composition of essential oil and determine the chromosome numbers and the karyotypes of two examined accessions of *A. millefolium*, showed interesting results as follows:

1. *A. millefolium* from Alborz (Am8):

The oils isolated by hydrodistillation from the aerial parts were obtained in yields 3.26% (w/w). 63 constituents representing 84.12% of the total components in the oil of this accession, characterized by 1,8-cineol (9.48%); cis-cadin-4-en-7-ol (9.38%); ß-Eudesmol (5.86%); Thymol (4.64%); Carvacrol (4.36%); Caryophyllene oxide (4.13%) and Terpinene-4-ol (3.65%) were the main constituents. The results of chromosome number showed that (2n=8x=72) in Am8, so this accession was octaploid (Figure 1). Karyotype analysis revealed a symmetrical patern (Table 2). In terms of the Stebbins system, the karyotype of this taxon, mostly sizes 2A class.

2. *A. millefolium* from Mazandaran (Am3):

The hydrodistillation of aerial parts yielded 1.13% (w/w). Based on our results, among the 73 characterized comprising 85.59% of the total components, containing 1,8-cineol (9.16%); cis-chrysanthenyl acetate (4.98%); trans-nerolidol (4.10%); α-terpinol (3.51%); camphor (3.27%); Caryophyllene oxide (3.41%) and Chromosome studied of this accession, (2n=6x=54) were showed. So this accession was hexaploide (Figure 2). Base on Stebbins classification, karyotype is symmetric and mostly sizes 2A class (Table 2).

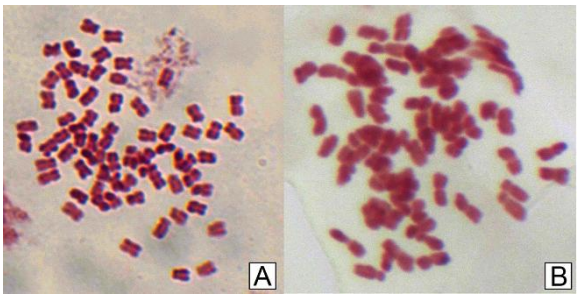


**Fig. 1.** Mitotic metaphase chromosomes of two *A.millefolium* accessions, (A) Am8 (2n=8x=72); (B) Am3 (2n=6x=54).

**Table 2.** Karyotype features of two accessions of *A. millefolium*.

| Accession | 2n | Ploidy level | TF% | S% | Karyotype formula | SC |
|---|---|---|---|---|---|---|
| Am8 | 72 | 8x | 40.27 | 62 | 1M+28m+14sm | 2A |
| Am3 | 54 | 6x | 40.69 | 58.8 | 1M+21m+5sm | 2A |

TF% = Total form percentage (sum of short arm lengths of individual/total haploid length of the complement chromosomes), S% = Symmetry index, Karyotype formula (m= medium region; sm= sub medium region; M= medium point), and SC= Stebbin's classification.

**Table 3.** Comparison between the main components of essential oil between two accession of *A. millefolium*.

| No | Components | Area% (Am8) | RT | RI |
|---|---|---|---|---|
| 1 | 1,8-Cineol | 9.48 | 12.1 | 1036.79 |
| 2 | Terpinene-4-ol | 3.65 | 16.22 | 1182.17 |
| 3 | α-Terpineol | 2.61 | 16.57 | 1194.41 |
| 4 | Thymol | 4.64 | 19.12 | 1285.97 |
| 5 | Carvacrol | 4.39 | 19.41 | 1296.4 |
| 6 | Spathulenol | 2.83 | 26.95 | 1591.29 |
| 7 | Caryophyllene oxide | 4.13 | 27.14 | 1599.17 |
| 8 | cis-Cadin-4-en-7-ol | 9.38 | 28.2 | 1645.22 |
| 9 | β-Eudesmol | 5.86 | 28.65 | 1664.78 |
| 10 | Selin-11-en-4α-ol | 2.52 | 28.8 | 1671.3 |
| No | Components | Area% (Am3) | RT | RI |
| 1 | α-Pinene | 2.37 | 9.47 | 937.5 |
| 2 | β-Pinene | 2.14 | 10.6 | 981.641 |
| 3 | 1,8-Cineol | 9.61 | 12.1 | 1036.79 |
| 4 | β-Thujone | 3.18 | 14.37 | 1117.48 |
| 5 | Camphor | 3.27 | 15.26 | 1148.6 |
| 6 | cis-Chrysanthenol | 5.78 | 15.72 | 1164.69 |
| 7 | Terpinene-4-ol | 3.28 | 16.22 | 1182.17 |
| 8 | α-Terpineol | 3.51 | 16.57 | 1194.41 |
| 9 | cis-Chrysanthenyl acetate | 4.98 | 18.47 | 1262.59 |
| 10 | Lavandulyl acetate | 3.06 | 19.07 | 1284.17 |
| 11 | trans-Nerolidol | 4.10 | 26.26 | 1562.66 |
| 12 | Caryophyllene oxide | 3.41 | 27.14 | 1599.17 |

**Table 4.** Comparison absence or present composition in two accession of *A. millefolium*.

| Components | Area% (Am3) | Area% (Am8) |
|---|---|---|
| Santolina triene | 0.09 | 0 |
| α-Thujene | 0.40 | 0.21 |
| *α*-Pinene | 2.37 | 1.85 |
| Camphene | 0.22 | 0.09 |
| Thuja-2,4(10)-dien | 0.27 | 0 |
| Sabinene | 1.24 | 0.68 |
| *β*-Pinene | 2.14 | 1.81 |
| *β*-Myrcene | 0.11 | 0.11 |
| *α*-Terpinene | 0.69 | 0.5 |
| *p*-Cymene | 1.58 | 0.9 |
| 1,8-Cineol | 9.61 | 9.48 |
| γ-Terpinene | 1.20 | 0.93 |
| *cis*-Sabinene hydrate | 0.28 | 0 |
| *α*-Terpinolene | 0.46 | 0.21 |
| Linalool | 0.98 | 0 |
| Filifolone | 0.35 | 0.41 |
| *α*-Thujone | 0.78 | 0.24 |
| *β*-Thujone | 3.18 | 1.01 |
| Chrysanthenone | 0.65 | 0.84 |
| trans-Verbenol | 1.27 | 0.74 |
| Camphor | 3.27 | 0.38 |
| *cis*-Chrysanthenol | 5.78 | 1.55 |
| Pinocarvone | 1.00 | 0.93 |
| Borneol | 1.40 | 0 |
| *p*-Mentha-1,5-dien-8-ol | 1.15 | 0.71 |
| Terpinene-4-ol | 3.28 | 3.65 |
| α-Terpineol | 3.51 | 2.61 |
| Myrtenal | 0.64 | 0.57 |
| Verbenone | 0.10 | 0 |
| *trans*-Chrysanthenyl acetate | 0.17 | 0.15 |
| Cumin aldehyde | 0.09 | 0 |
| *cis*-Chrysanthenyl acetate | 4.98 | 1.91 |
| Lavandulyl acetate | 3.06 | 0.38 |
| Thymol | 0.45 | 4.64 |
| Carvacrol | 0.00 | 4.39 |
| *Trans*-Carvyl acetate | 0.09 | 0 |
| Eugenol | 0.45 | 0 |
| Neryl acetate | 0.00 | 0.33 |
| *α*-Copaene | 0.19 | 0.12 |
| *β*-Elemene | 0.20 | 0 |
| *trans*- Caryophyllene | 1.67 | 1.01 |
| *α*-Humulene | 0.43 | 0.59 |
| γ-Curcumene | 0.15 | 0 |
| ar-Curcumene | 0.55 | 0.17 |
| Germacrene D | 0.82 | 0.2 |
| Indipone | 0.23 | 1.01 |
| β-Bisabolene | 0.32 | 0.24 |
| δ-Cadinene | 0.57 | 0.09 |
| *trans*-Nerolidol | 4.10 | 0.7 |
| Spathulenol | 1.44 | 2.83 |
| Caryophyllene oxide | 3.41 | 4.13 |
| Salvial-4(14)-en-1-one | 0.38 | 0.4 |
| Humulene epoxide II | 0.51 | 0.38 |
| Ledene oxide-(II) | 0.92 | 0.63 |
| cis-Cadin-4-en-7-ol | 1.71 | 9.38 |
| allo- Aromadendrene epoxide | 1.02 | 0.69 |
| β-Eudesmol | 1.01 | 5.86 |
| Selin-11-en-4α-ol | 0.87 | 2.52 |
| *α*-Bisabolol | 0.25 | 0 |
| Eudesma-4(15),7-dien-1β-ol | 1.31 | 1.71 |
| 6S,7R-Bisabolone | 1.27 | 0.18 |
| Hexahydrofarnesyl acetone | 0.16 | 0.18 |
| Tricosane | 0.45 | 1.01 |
| Tetracosane | 0.31 | 0.46 |

**Discussion**

*A. millefolium* is named (Hazanbel) in traditional Iranian medicine and sighted in Iranian Plant Pharmacopae. There are some chemotypes of this species in the world but it is unknown in Iran and only essential oil compositions is found (Nadim *et al*., 2011; Newal *et al*., 1996; Sheidaie *et al*., 2009). This species has some ploidy levels (Kiran *et al*., 2008; Si *et al*., 2006 ). Manzaneda *et al.* said that, there is relationship between climate and cytotype distribution. Our results showed two ploidy levels in this species. One accession (Am3: 2n=6x=54) collected from humidity area with 2300 m height but Am8 (2n=8x=72) is from timberline area with lower heigh and semi desert climate. So, our results indicate that changing the environmental factors are effective to change the ploidy level and occurrence cytotypes. On the other hand, it has been found that the essential oil of *A. millefolium* grown under different environmental conditions is rich in 1,8-cineol, Terpinene-4-ol, α-Pinene, ß-Pinene, Caryophyllene oxide, Spathulenol and Pinocarvone. But the content of these components showed different in these two taxa (Table 3). Some components were absent between two accessions (Table 4). For example, in Am8 (2n=8x=72), Santolina triene, Borneol, Cumin aldehyde, Linalool, Eugenol, ß-Elemene were not found. Also, among the major common components isolated in Am3 (2n=6x=54), Carvacrol, Neryl acetate were not found. On the other hand , Therefore, it seems that, with change the ploidy level, the biosynthesis of volatile oil and their contents can be change. So there is correlation between environmental factors, cytotypes and chemotypes and essential oil contents, yield and chemical profile were slightly affected under climate conditions.

**References**

**Chopra RN, Nayar SL and Chopra IC.** 1956. Glossary of Indian Medicinal Plants. National Institute of Science Communication, New Delhi, 3-4.

**Ebrahim F, Pakniyat H, Arzani A and Rahimmalek M.** 2012. Karyotype analysis and new chromosome number reports in *Achillea* species. Biologia. **67**(2), 284-288.

**Hofmann L, Fritz D, Nitz S and Kollmannsberger Drawert H.** 1992. Essential oil composition of three polyploids in the *Achillea millefolium* complex. Phytochemistry. **31,** 537-542.

**Khaniki GB.** 1995. Chromosome numbers and morphometry in *Achillea* (Anthemideae, Compositae). Nucleus **38,** 104- 111.

**Kiran Y, Arabaci T, Sahin A and Turkoglu I.** 2008. Karyological notes on another eight species of *Achillea* (Asteraceae) from Turkey. Biologia **63,** 343-348.

**Mabberley Dj.** 1997. The Plant-Book, 2th ed. Cambridge University Press, Cambridge.

**Mockute D and Judzentiene A.** 2003. Chemotypes of essential oils of *Achillea millefolium* L. subsp.*millefolium* growing wild in eastern Lithuania, Journal of Esential Oil Research **17**(6), 664-667.

**Nadim MM, Malik AA, Ahmad J and Bakshi SK.** 2011. The Essential Oil Composition of *Achillea millefolium* L. Cultivated Under Tropical Condition in India. World Journal of Agriculture Science. **7**(5), 561-565.

**Nemeth E.** 2005. Essential oil composition of species in the genus *Achillea*. Journal of Essential Oil Research **17,** 501-512. Healthcare Professionals. Pharmaceutical Press, London.

**Pino JA and Fuents V.** 1998. Chemical composision of the leaf oil of *Achillea millefolium* grow in Cuba. Journal of Essential Oil Research **10,** 427-428.

**Safiuddin A.** 2002. Unani Adviyat Mufarrada, 9th ed. National Council for Propagation of Urdu Language, New Delhi, India.

**Sheidai M, Azani N and Attar F.** 2009. New chromosome number and unreduced pollen formation in *Achillea* Species (Asteraceae). Acta Biologica Szegediensis **53(1),** 39-43.

**Six T, Zhang ML, Shi QW and Kiyota H.** 2006. Chemical constituents of the plants in genus *Achillea*. Chemistry and Biodiversity **3,** 1163-1180.