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Ecological and Ethnobotanical Study of *Myrtus communis* L. (Myrtaceae) in Al-Jabal Al-Akhdar, Libya

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A B S T R A C T

The Myrtle (*Myrtus communis*) is a common shrub widespread in the Mediterranean. Its fruit and leaves have antioxidant, antibacterial and antifungal properties, and are used for their content of essential oils; however, most commonly used is as an ingredient in locally made juice. The uncontrolled exploitation of Myrtle has reduced both the species geographical coverage and the size of individual populations. This study was selected to investigate the ethnobotanical of *M. communis* and to identify the main reasons for their folk uses, methods of uses and their geographical distribution in the Al Jabal Al Akhdar area, Libya. Also, an experiment was carried out to determine the allelopathic, as an ecological process in regulating plant population in ecosystems, the potential of plant parts by which may other plants be affected. Such volatile substances released from Myrtle leaves for controlled cultivation requires a characterization present both within and between populations. The use of Myrtle as a flavouring agent and stomachic is 90% and 10.9%, respectively. The plant use was also recorded for Diabetes (60%), Anti-septic, Blood purification and Constipation (6.6%). Our results of allelopathic on germination and growth bioassay experiment demonstrated that germination percentage of *Hordeum vulgare* and *Triticum aestivum* (Recipient Species) was significant ($P \leq 0.05$). The germination percentage decreased with the increase in *Myrtus communis* Leaves Aqueous Extract (MCLAE) concentration. Shoot Length, Root Length also decreased with the increase in the extract concentration, whereas the reverse was true for Seedling Dry Weight increased with the increase of MCLAE concentration.

دراسة بيئية ومعرفية عن نبات المرسين (الفصيلة الأسية) في الجبل الأخضر– ليبيا.

خديجة محمد الحداد[1] نجاة محمد المسماري [2]

نبات الميرسين (*Myrtus communis*) شجيرة دائمة الخضرة ومنتشرة في البحر الأبيض المتوسط. تحتوي ثمارها وأوراقها على خصائص مضادة للأكسدة ومضادة للبكتيريا ومضادة للفطريات، و تستخدم اساساً لمحتواها من الزيوت العطرية، أما الأكثر استخداماً محلياً هو كعصير المصنوع من الثمار. مما أدى إلى الاستغلال المفرط لنبات الميرسين و تقليل التغطية الجغرافية النباتية و حجم الأفراد للنوع .أجريت هذه الدراسة للتحري عن أصل النبات و معرفة الأسباب الرئيسية لاستخداماته الشعبية ethnobotanical وطريقة استخدامه، و توزيعه الجغرافي في منطقة الجبل الأخضر بليبيا. كما تم إجراء تجربة التضاد الكيميائي allelopathic كعملية بيئية في تنظيم تجمع النباتات في النظم البيئية. فكانت استخدامات النبات في الطب الشعبي كعامل منكه وللعلاج المعدي والتقليل من الحركة المعوية هو 90٪ و 10.9٪ على التوالي. مستخلص أوراق الميرسين يُساعد على خفض مستويات السكر في الدم لدى المصابين بفرط مستويات السكر في الدم كما تم تسجيل استخدام النبات لمرض السكر (60٪)، تنقية الدم والإمساك (6.6٪). وأظهرت نتائج اختبار allelopathic على الإنبات والمقاييس الحيوية للنمو أن نسبة الإنبات (GP) لنبات الشعير *Hordeum vulgare* و لنبات القمح *Triticum aestivum* (الأنواع المتلقية) كانت معنوية ($P \leq 0.05$). انخفت نسبة الانبات مع زيادة تركيز خلاصة أوراق نبات الميرسين كما انخفض طول النبات وطول الجذور مع زيادة تركيز المستخلص الورقي لنبات الميرسين، بينما كان العكس صحيحاً بالنسبة للوزن الجاف للشتلات التي زادت مع زيادة تركيز المستخلص.

INTRODUCTION

Myrtaceae is a family of woody flowering plants that encompasses around 5500 species, classified in 144 genera, and 17 tribes. Within Myrtaceae, the tribe Myrteae represents half of the family's biodiversity with 51 genera and about 2500 species mostly restricted to the Neotropics, though 15 genera and about 450 species are found in other continents, such as Southeast Asia, Northeast Australia, and the Pacific islands, including New Caledonia and New Zealand. The genus *Myrtus* is the sole found in European/Northern African, Asia, particularly in the Mediterranean region of southern Europe as far west as Macaronesia (Madeira and the Azores), the Saharan mountains, and as far east as western Asia (Iran and Afghanistan) (**Migliore *et al*., 2012; Thornhill *et al*., 2015 and Vasconcelos *et al*., 2017).**

Myrtus communis L. is a species of genus *Myrtus* L. belongs to the family Myrtaceae, which contain about 140 genera and 3,400 species growing in tropical, sub-tropical, and temperate regions of the world **(Treveset *et al*., 2001).** It grows spontaneously as an evergreen shrub or a small tree. The plant can reach a height of 2.5 m **(Giampieri *et al*., 2020)**, stem is branched having dark evergreen leaves which are glabrous, opposite, glossy, whorled, or paired, coriaceous, with stiff structure, lanceolate to ovate, margined entire, aromatic, acuminate and 2.5-3.8cm long, and the lamina glands is absent **(Babaee *et al*., 2010).** flowers are starry, scented, and can be white or pink, axillary in position having slender peduncle, of 2cm in diameter, stamen possess yellow anthers **(Serce *et al*., 2010).** The flowers bloom from June-September having five sepals, five petals and many stamens **(Sumbul *et al*., 2011).** Whereas berry fruits are edible, small, with a round shape and many seeds inside, generally blue-black, even if some varieties have white-yellow fruits, and ripen in autumn, between October and February **(Giampieri *et al*., 2020).** *Myrtus communis* L. is used as folk medicine, different parts of the plants are used such as leaves, fruits, flowers, roots **(Ertug *et al*., 2004)** and volatile oils for several purposes **(Tuzlasi *et al*., 2006; Bahadırlı *et al*., 2020).**

Myrtus communis L. a common and widespread shrub of the Mediterranean, Asia, and northern sub-tropical Africa, urbanisation and agricultural expansion may have caused loss and fragmentation of the habitat. The IUCN red list identifies the plant as endangered **(Al-Zani, 1986; El-Barasi & Saaed, 2013; Kishwar *et al*., 2017).** Myrtle fruit and leaf material often collected from wild subpopulations; increasing urbanisation, natural fires, grazing by livestock and other wild herbivores, and the cutting of wood are all putting pressure **on wild subpopulations (Melito *et al*.,2013). On the other hand, the species has a high ecological tolerance as it produces a large number of seeds with a high germination** rate making it an efficient successional species in abandoned fields **(Santos, 2000).**

Ethnobotany is broadly defined as the study of relationships between people and plants. Some prefer to define it as the scientific study of the interactions between human cultures and plants. The scope of ethnobotany includes the study of plants used for a variety of economic and non-economic societal purposes (e.g., as tools and construction materials, as food, in ritual, for divination, as cosmetics, for ornamentation, as textiles or clothing, as currency, and in social life) **(Soejarto *et al*., 2011).**

Reigosa *et al*. (2006) postulated that allelopathy is a biological phenomenon that an organism produces and excretes one or more chemical substances by which the growth, survival, and reproduction of other organisms influenced. The magnitude of the effect of allelopathy depends on the extent of habitat characteristics and any other stresses, such as environmental conditions, soil salinity and type **(El-Darier and Youssef, 2017)**, moisture, less than optimal nutrients or biological factors (insect or disease pressure) that occur during the growing season or different growth forms **(Labbafi *et al*., 2010).** Allelopathic potential considers a differential chemical marker among some growth forms and species **(El-Darier *et al*., 2018).** The differential allelopathic potential of different growth forms was positively correlated with the contents of total phenolic acids **(Bertholdsson, 2012).** Allelopathy indirectly reflects the information coded in DNA respecting the chemical print and is thus considered amenable technique to provide a chemical basis for molecular phylogeny **(Junaedi *et al*., 2010).** The present work aims to investigate the ethnobotanical survey of *Myrtus communis* L., the main reasons for their eradication and evaluate allelopathic effects as an ecological process in regulating plant population in ecosystems of extracts of the Myrtle with different concentrations on germination of *Hordeum vulgare* and *Triticum aestivum*.

MATERIAL AND METHODS

Data collection

Myrtus communis is an important medicinal plant; the survey was carried out during January 2020. A total of 30 local people was interviewed and were classified into three groups (**Table 1**). The participants were asked about the folk uses, recipes, and its community, reason of extinction and survival of the myrtle.

Table 1. Classification of participant into groups

S. No	Group	Age Ranges	Participant
1	I	20-30	8
2	II	31-40	13
3	III	41-50	9

Quantitative ethno-medicinal data analysis

Conversion of the qualitative data into quantitative is essential for hypothesis-testing, statistical validation, and comparative analysis.

Relative frequency of citation (RFC)

The collected ethno-medicinal data were quantitatively analysed using the Relative Frequency Citation **(RFC)** index. This indicator shows the local importance of the species and is calculated from the frequency of citation (**FC**, the number of informants mentioning the usage of the specie) divided by the total number of informants in the survey **(N),** without considering the use categories **(Khan *et al*., 2016).**

RFC can be defined by formula, RFC=FC/N value ranges from zero (none of the informant cites the plant as useful) to one (every informant reports the plant to be useful) **.**

Use value (UV)

The use value **(UV)** demonstrates the relative importance of locally known plants. It is calculated using formula **UV=∑Ui/N**

Where **Ui** is the number of uses mentioned by each information for a given species and **N** is the total number of informants.

Geographical distribution in Al Jabal Al Akhdar region

Literatures and studies that done on natural vegetation in Libya were reviewed, in order to collect the location of the *Myrtus communis* L.; the locations of the sites then were plotted as points on google earth (GE). The points were then converted from KML format, which were created in GE, to a GIS vector (shapefile) using QGIS software in order to visualise the distribution of the *Myrtus communis* L. in the study area. The sites were georeferenced according to the Universal Transverse Mercator (UTM) projection, with reference to World Geodetic System datum (WGS84) and Zone 34N and converted to GIS format using QGIS.

Soil database/vector format for the eastern zone of Libya that modified after Selkhozprom export 1980 was used to identify the type of soil on which the *Myrtus communis* L. is grown.

Germination bioassay

Petri-dish experiment was applied to investigate the potential allelopathic effects of *Myrtus communis* leaves Aqueous Extract (MCLAE) on germination percentage **(GP).**

To accomplish this experiment, 10 seeds of crop plants *Hordeum vulgare* and *Triticum aestivum* (recipient species) were arranged in 9-cm diameter petri-dishes on two discs of whatman No.1 filter paper under normal laboratory conditions with day temperature ranging 25-30°C and night temperature ranging 20-25°C. Ten ml of MCLAE were added daily to three replicates. **GP** were recorded daily for successive seven days.

Calculations of germination parameters

Germination percentage (GP) was calculated according to the general equations:

$$\textbf{Number of germinated seeds} / \textbf{total number of seeds} \times \textbf{100}$$

Shoot length ratio was calculated according to the general equations:

$\mathbf{SHLR = SHL/L}$ Where: SHL is the length of shoot per individual and L is the total length of the individual.

Root length ratio was calculated according to the general equations:

$\mathbf{RTLR = RTL/L}$ Where: RTL is the length of root per individual and L is the total length of the individual.

Seedling dry weight (mg/seedling) (SDW)

Five homologous seedlings of *Myrtus communis* L. were taken and each individual was separated into shoot and root then the oven dry weight was determined after drying in an oven at 60 °C till constant weight.

RESULTS AND DISCUSSION

Ethnobotanical description of *Myrtus communis* L. had been done using an open-ended questioner in order to identify the plant by local inhabitants, knowing about the growing season, associated crops, beneficial and harmful effects, and also medicinal uses in treating different diseases. Most of the locals living in the study area were familiar with the study plant, however, its medicinal values were not well known.

Internationally, the market value of myrtle is not well known due to its wild nature but in some region of the world, its fruit and leaves are sold in the local markets such as, in turkey, fruits of myrtle are sold in the south-west provinces such as Milas, Mulga and Bodrum, in the Mediterranean town and Aegean markets **(Ali *et al*., 2013**). The present research was carried out to study the different aspects of Myrtle the Folk uses of Myrtle shows that it is used most abundantly as flavouring agent (90%) Extract the essential oil and Food (86.6 %) followed by use as a Diabetic (60%). The lowest used value (6.6 %) for Anti-septic, Blood purification and Constipation **(Table2**).

Table 2. Medicinal folk uses of Myrtle in Al Jabal Al Akhdar area, Libya

Folk uses	FC			∑Ui	%UV
	(20-30)	(31-40)	(41-50)		
Flavouring agent	6	13	8	27	90
Extract the essential oil	5	13	8	26	86.6
Food	5	13	8	26	86.6
Diabetic	4	6	8	18	60
Anti-septic	0	0	2	2	6.6
Blood purification	0	1	1	2	6.6
Constipation	0	0	2	2	6.6

Note: FC=Frequency of Citation, ∑Ui=the total number of uses mentioned by each informant and %UV is total percentage of use value.

Kishwar *et al*. (2017) observed that 46% people use it as a flavouring agent, 31% people used its dry leaves with tea; **Montoro *et al*., (2006)** also state the same result. The Myrtle is used for stomach treatment (10.90%), food (9.54%), blood clotting (4.09%) which is supported by **(Hosseinzadeh *et al*., 2011; Serio *et al*., 2014)** also reported the anti-diabetic (3.18%) and anti-septic (2.72%) uses of the specie.

Geo-referenced locations of *Myrtus communis* L. in the present research, the current sites of Myrtle were recorded and summarized in **Figure 1** based on (**Keith, 1965; Qaiser & Siddiqi, 1986; OMU, 2005**).

The soil data show that the *Myrtus communis* L. is grown on Mollisols (Rendolls), Alfisols, Vertisols, and Inceptisols, the soil classification is shown in **Table 3**.

Myrtus communis L. is endangered species we investigate their occurrence in Al Jabal Al Akhdar, Libya over time, some factors affect their habitat destruction made it vulnerable during the survey it was noted that deforestation. Myrtle fruit and leaf material are often collected from wild subpopulations thought to significantly affect viability. The accessibility to reach the sites of Myrtle trees due to the close distance of their location to populated areas (**Figure 1**), led to make the plant subject to clear cut and /or damage. Beside plant leaves and fruits collection, Al Jabal Al Akhdar area subject to human activities such as urbanisation **(Ahwaidi, 2017; Almesmari, 2019)**, overgrazing or grazing and cutting of wood **(El-Barasi and Saaed, 2013)**, agriculture expansion **(El-Barasi and Saaed, 2013; Almesmari, 2019),** and fires (wild and/or resulting in human activity) **(El-Barasi and Saaed, 2013; Zatout and Soliman, 2014).** All those anthropogenic factors are putting pressure on wild subpopulations which may lead to degradation or extinction of the plant species.

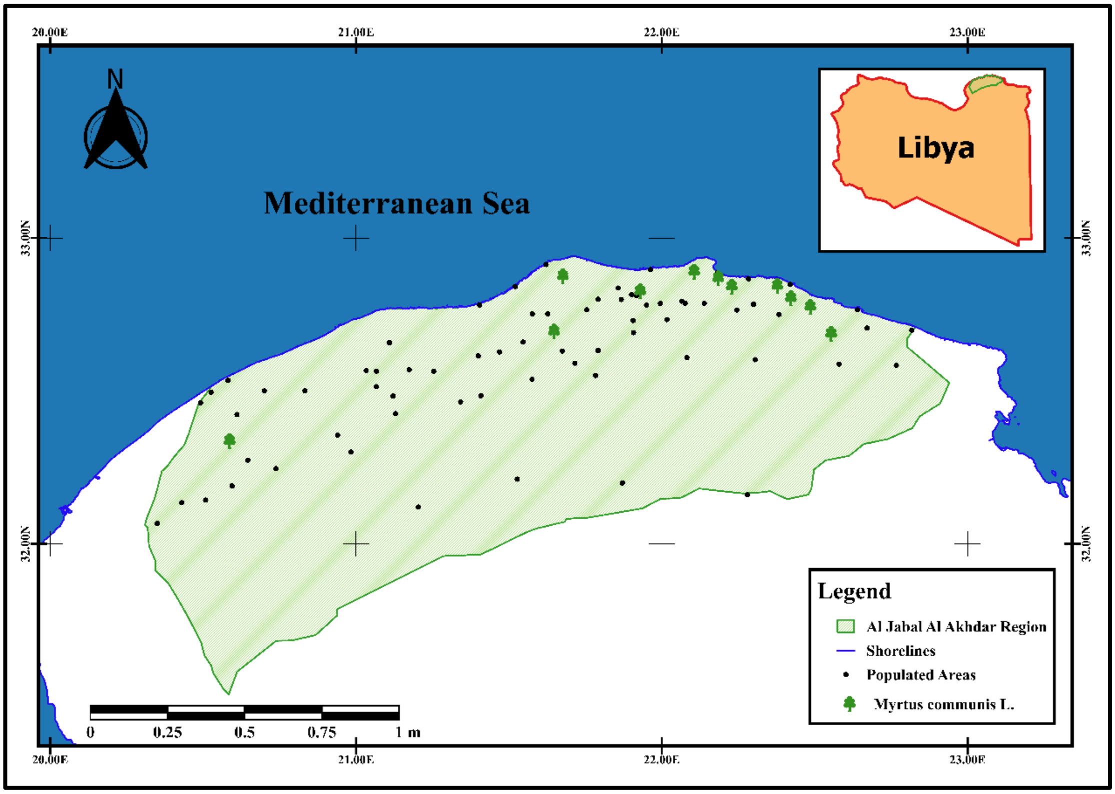

Figure 1. The distribution of *Myrtus communis* L. in Al Jabal Al Akhdar region- Libya (source: Author)

Table 3. The soil classification of the Myrtus's site

ID	Site name	Soil order	Soil suborder	Soil great group
1	15 Km west of Derna to Ras'Elhelal	Alfisols	Xeralfs	Rhodoxeralfs
2	24 km from Derna along costal road to Susa	Mollisols	Xerolls	Calcixerolls / Argixerolls
3	25 km E. of Ras'Elhelal	Mollisols	Rendolls	Haprendolls
4	Alhamama to Al Baydah	Mollisols	Rendolls	Haprendolls
5	Habun (Ain Al-Shallala)	Vertisols	Xererts	Haploxererts
6	Ras El Helal &Wadi Ras El Helal	Mollisols	Rendolls	Haprendolls
7	Wadi El Kuf	Mollisols/ Inceptisols	Xerolls/ Xerepts	Calcixerolls / Argixerolls Calcixerepts
8	Wadi El Mahbool	Mollisols	Rendolls	Haprendolls
9	Wadi El Tier	Mollisols	Rendolls	Haprendolls
10	Wadi Qalah	Mollisols	Xerolls	Calcixerolls / Argixerolls
11	Wadi Zaza	Mollisols	Rendolls	Haprendolls

Germination Bioassay Experiment

Seed germination parameters

Data concerning the germination percentage (GP) of recipient species are illustrated and statistically represented in **Table 4**. Petri-dish experiment demonstrated that GP significantly ($p \leq 0.05$) affected upon applying different concentration levels of *M. communis* Leaves aqueous extract (MCLAE). GP decreased with the increase in MCLAE concentration. At the end of the experiment (after fourteen days), GP showed value of 100% at the control level for the recipient species. At 0.25% MClAE concentrations, GP reduced to 80%, 90% for *Hordeum vulgare* and *Triticum aestivum* respectively. Additionally, at 0.75% MCLAE concentration, the percentage was reduced to values of 70%, 60% for *H. vulgare* and *T. aestivum* respectively. Finally, GP recorded for *H. vulgare* and *T. aestivum* reached values of 60%, 50% at 1.25% MCLAE concentration, respectively.

Table 4. Variation in germination percentage (GP) of recipient species seeds as affected by *Myrtus communis* L. Leaves aqueous extracts (MCLAE). Data are means of three replicates

Treatment (%)	Recipient Species	
	Hordeum vulgare	***Triticum aestivum***
Control	100	100
0.25	80	90
0.75	70	60
1.25	60	50

Different letters indicate significance at p≤0.05 as evaluated by two-way ANOVA test.

Shoot (SHL) and root (RTL) lengths

The allelopathic effect of MCLAE concentration on the shoot (SHL) and root (RTL) lengths of Recipient Species are illustrated in .خطأ! لم يتم العثور على مصدر المرجع. Petri-dish experiment demonstrated that the shoot (SHL) and root (RTL) lengths of Recipient Species was significantly affected upon different concentration levels of *M. communis* L. leaves aqueous extracts (MCLAE).

MCLAE extract concentration levels have statistically reduced SHL. The applied concentrations are significant at $p \leq 0.05$. Generally, SHL decreased with the increase of MCLAE concentration. SHL attained a value of 5.5 and 12cm for *Hordeum vulgare* and *Triticum aestivum* (Recipient species), respectively at control level, while it increased to 9 and 12cm at 0.25% MCLAE concentration for *H. vulgare* and *T. aestivum*, respectively. Constantly, at 0.75% MCLAE concentration, the values reduced to 5.5 and 12cm for *H. vulgare* and *T. aestivum* respectively.

Table 5. Variation in shoot length (SHL) and root length (RTL) (cm) of recipient species seeds as affected by *Myrtus communis* L. Leaves aqueous extracts (MCLAE). Data are means of three replicates

Treatment (%)	Recipient Species			
	Hordeum vulgare		***Triticum aestivum***	
	SHL (cm)	RTL (cm)	SHL (cm)	RTL (cm)
Control	5.5	6	12	11
0.25	9	6	16	14
0.75	5.5	2.5	12	3
1.25	4.5	0.2	6	1

Different letters indicate significance at p≤0.05 as evaluated by two-way ANOVA test

Finally, SHL reached the minimum value 4.5 and 6cm at 1.25% MCLAE concentration, respectively. These results are consistent with a study (**Saeed, 2004**), where the decrease was evident in the high concentrations of extracts, especially when using Nicotiana tabacum where the length of *H. vulgare* and *T. aestivum*, at high concentrations, is due to the fact that these extracts contain compounds that act in their high concentrations as Gibberellin substances, which increase the effectiveness of the nutrient-dissolving enzymes present in the seed and thus reduce their access to the active tissues in the seed. Compared with control, a gradual decrease in RTL was observed along gradual MCLAE concentration (.خطأ! لم يتم العثور على مصدر المرجع). RTL implication was significantly affected at ($p \leq 0.05$). At the end of the experiment, RTL showed a value of 6 and 11cm at the control level for *H. vulgare* and *T. aestivum* respectively, while it increased only for *T. aestivum* to 14cm at 0.25% MCLAE concentration. On contrary, at 0.75% MCLAE concentration, the values decrease of 2.5 and 3cm in that order has occurred. Finally, constantly at 1.25% MCLAE concentration the values decrease of 0.2 and 1cm for *H. vulgare* and *T. aestivum*, respectively. These results are in agreement with the study (**Saeed, 2004),** where the decrease was evident in the high concentrations of extracts, especially when using the *Nicotiana* sp.. The reasoning is these extracts that work with high concentrations as anti-Gibberellin substances, which increases the effectiveness of the enzymes analysed for the nutrients found in the seed, and thus reduces their access to the effective tissues in the seed such as roots and seedlings.

Table 6 demonstrates that the seedling dry weight (SDW) and wet weight (SWW) of *H. vulgare* and *T. aestivum* were significantly affected by applying different concentration levels of *M. communis* leaves aqueous extracts (MCLAE).

Table 6. Variation in seedling dry and wet weight of recipient species seeds as affected by *Myrtus communis* Leaves aqueous extracts (MCLAE). Data are means of three replicates

Treatment (%)	Recipient Species			
	Hordeum vulgare		*Triticum aestivum*	
	Seedling dry weight (g)	Seedling wet weight (g)	Seedling dry weight (g)	Seedling wet weight (g)
Control	0.21	1.07	0.17	0.34
0.25	0.20	1.49	0.16	0.55
0.75	0.25	1.32	0.16	0.60
1.25	0.22	0.94	0.20	0.83

Different letters indicate significance at p≤0.05 as evaluated by two-way ANOVA test.

MCLAE extract concentration levels have statistically increase seedling dry weight (SDW) and wet weight (SWW). The applied concentrations are significant at $p \leq 0.05$. Generally, SDW increases with the increase of MCLAE concentration. At the end of the experiment, SDW has a value of 0.21 and 0.17g at control level, while it reduced to 0.20 and 0.16g at 0.25% MCLAE concentration for *H. vulgare* and *T. aestivum*, respectively. Constantly, at 0.25, 0.75% SDW has the same values of 0.16g for *T. aestivum* and the maximum value of 0.25g for *H. vulgare* at 0.75%. While, at 1.25% SDW reached the maximum values of 0.22 and 0.20g for *H. vulgare* and *T. aestivum*, respectively. Finally, SWW attained value of 1.07 and 0.34g for *H. vulgare* and *T. aestivum*, respectively; at the control level; where the values of SWW continuously increased to 0.83cm for *T. aestivum* at 1.25% while, *H. vulgare* decreased to 0.94cm at 1.25%. As an allelopathic response of *Triticum aestivum* towards Cassia angustifolia; shoot weight of *T. aestivum* was significantly affected while root length of was non-significantly affected **(Hussain *et al*., 2007)**. **Tehmina and Bajwa (2005)** tested efficacy of *Helianthus annuus* for suppressing wheat weeds like *Phalaris minor*, *Chenopodium album*, *Coronopus didymus*, *Rumex dentatus* and *Medicago polymorpha* and concluded that stem and root extracts of H. *annuus* caused 30-90% reduction in dry weight of the weeds relative to the control.

CONCLUSION

Myrtle grows under Quercus faginea Lamk. Forests in humid and subhumid bioclimatic stages. However, myrtle is found in association with *Pinus halepensis* Mill, *Juniperus phoenicea* L., *Ceratonia siliqua* L. and with shrubs such as *Pistacia lentiscus* L. Most of the myrtle biomass has been harvested from wild plants without consideration of the reduction of natural biodiversity subsequently, the natural populations are progressively decreasing in number and size. Additionally, phytochemical production depends upon internal and external factors affecting the plant as genetic structures and environmental conditions. In fact, the investigation of the chemical and morphological diversity of myrtle populations represented an essential step to verify biomass production and to constitute an important resource for agro-industrial purposes.

Currently, the wild myrtle tree is a source of raw material for oil extraction. The continuous use of resource and the increase in demand for its several uses has led to a decrease in its natural areas. For these reasons, steps must be taken to avoid the extinction of this species from its natural ecology, to reduce genetic diversity and, in the worst case, to disappear the species.

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