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Eco-geographical Influence on β-Carboline Alkaloid Accumulation in Peganum Harmala L. Seeds: A Comparative Study Between Naâma (Algeria) and Selected Regions Using HPLC and GC-MS

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SUMMARY:
Background: Peganum harmala L. (Zygophyllaceae) is a perennial herbaceous plant widely distributed across arid and semi-arid ecosystems of the Mediterranean basin and Central Asia. Its seeds are exceptionally rich in β-carboline alkaloids, particularly harmine and harmaline, which are of considerable pharmacological interest. Environmental stress conditions typical of steppe ecosystems including drought, extreme temperatures, and poor soils are known to influence secondary metabolite biosynthesis in plants, yet the quantitative impact of eco-geographical factors on alkaloid accumulation in this species remains incompletely understood.
Objectives: This study aimed to quantify the major β-carboline alkaloids (harmine and harmaline) in seeds of Peganum harmala collected from the El Kasdir region (Province of Naâma, Algeria), and to assess how eco-geographical and climatic factors influence alkaloid accumulation through a comparative multi-regional analysis. This interdisciplinary approach combining plant ecology and analytical chemistry constitutes the original contribution of this work.
Methods: Seeds were harvested in July 2018 from the El Kasdir region, which is characterized by a steppe climate with cold, arid winters and sandy soils of basic pH (8.4). Alkaloid extraction was performed following the protocol of Herraiz and al. (2010)[1], using a perchloric acid/methanol solution (1:1). The resulting extracts were analyzed by High-Performance Liquid Chromatography with Diode Array Detection (HPLC-DAD) and Gas Chromatography coupled with Mass Spectrometry (GC/MS). The quantitative results obtained from five samples were then compared with six published studies covering six geographical regions: Setif and Djelfa (Algeria), Toledo (Spain), Agadir (Morocco), Tehran (Iran), and Xinjiang (China).
Results: HPLC-DAD and GC/MS analyses identified harmaline (retention time: 19.29 min; 44.39%) and harmine (retention time: 19.71 min; 46.05%) as the two dominant alkaloids, together representing more than 90% of the total alkaloid fraction. Mean concentrations were 32.7 ± 1.7 mg/g for harmaline and 20.96 ± 1.18 mg/g for harmine. A minor alkaloid, tetrahydroharmine (THH), was also detected at 0.5%. The comparative analysis revealed marked interregional variability: the highest concentrations were recorded in Xinjiang, China (58.46 mg/g harmaline; 40.77 mg/g harmine) and Toledo, Spain (56.0 mg/g; 43.2 mg/g), Agadir, Morocco (0.874 mg/g; 8.514 mg/g). Tehran, Iran (0.25%; 1, 84%), Setif, Algeria (3,8 % ; 2,9 %) and Djelfa, Algeria (48.009%; 38.44 %)
Conclusion: This study demonstrates that β-carboline alkaloid biosynthesis in Peganum harmala is strongly modulated by eco-geographical factors, including climate (aridity, precipitation, temperature), edaphic conditions (soil type, organic matter content), and altitude. Arid continental climates appear to favor higher alkaloid accumulation, while milder, ocean-influenced conditions are associated with lower concentrations. These findings highlight the importance of considering geographical origin and environmental context in the standardization of medicinal plant products derived from Peganum harmala.
KEYWORDS *Peganum harmala; eco-geographical variation; arid ecosystems; secondary metabolites; β-carboline alkaloids; environmental stress.*

INTRODUCTION:

Peganum harmala L., commonly known as "Harmal," is a perennial drought-resistant herbaceous plant belonging to the Zygophyllaceae family. It is a spermatophyte angiosperm of the dicotyledon class [2].
The plant is widely distributed across arid and semi-arid regions, saline lands, and desert pastures [3];[4] .Its stems are typically sparsely branched, reaching 30 to 90 cm in height, with fairly short internodes and dense foliage.[5]
This plant was selected for this study due to its exceptional richness in indole alkaloids. Our research focuses on the extraction of the alkaloids harmine and harmaline from the seeds of Peganum harmala collected in the El Kasdir region (provence of Naâma, Algeria). The primary objective is to conduct a quantitative comparative analysis with previous literature to determine whether environmental factors and the specific location of the study site influence the production and composition of the extracted alkaloids, namely harmine and harmaline.

Consequently, the presentwork aims to establish an interdisciplinary approach combining ecology and chemistry, which constitutes the originality of this work.
Numerous researchers have investigated the harmine and harmaline alkaloids in Peganum harmala, notably the study of Herraiz and al. (2010) [1] .Other significant studies include those by Bensalem and al. (2014)[6],Sassoui and al. (2015) [7], among many others.
In conclusion, our findings indicate a difference in the quantitative aspect. The variation observed in our results compared to other studies could therefore be attributed to environmental factors, plant-specific characteristics, or differences in geographical origin.

2. MATERIALS AND METHODS:

2-1.Description of *Peganum harmala* Fruit and Seeds:

The fruit is a small spherical capsule of 6 to 10 millimeters in diameter, comprising three locules **[8].** These locules contain more than 50 small, triangularseeds **[9].**The seeds are angular, dark brown in color, and have a bitter taste**[10].**

Fig.1. Fruits and seeds of *Peganum harmal*(Picture taken by: Yassine SEBAA, El-Kasdir, provence of Naama July 2018)

2-2. Seed Sampling Site:

Seeds of *Peganum harmala* were harvested in July 2018 in the El Kasdir provence (Naâma, Algeria) (Fig. 2).
The geographical coordinates of the sampling site are:
Latitude (N): 33°37'34.20"
Longitude (W): 1°29'70.60"**[11].**

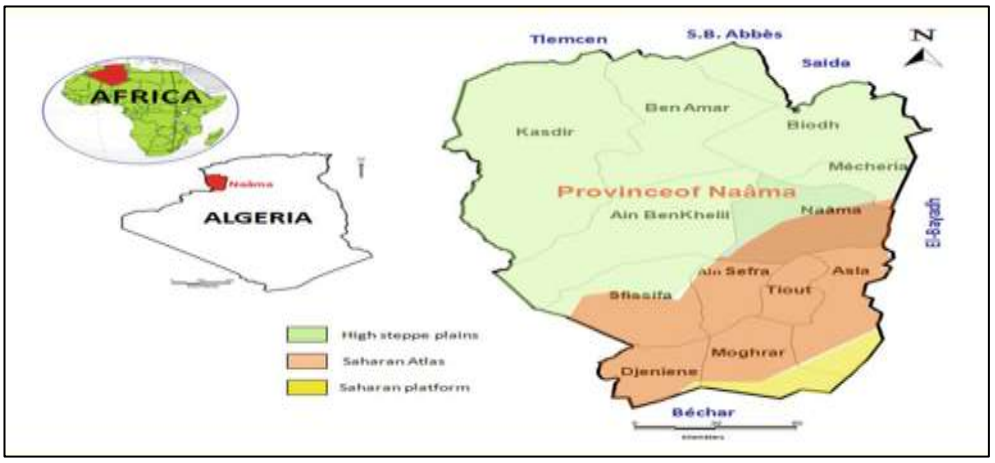

Fig.2. Location of the seed collection area **(Abdelkrim Benaradj and al., 2021).**

2-3. Climatic Study of the Sampling Site:

To characterize the climate of the seed collection region, we referred to rainfall and temperature data from the nearest meteorological station, located in Mecheria (provence of Naama). The monthly average precipitation and temperature data, corresponding to the period from 1990 to 2014, are presented in Table 1(Source: https://www.intechopen.com/chapters/77198#).

Table.1.Climatic data from the Mecheria meteorological station (1990-2014),**(Abdelkrim Benaradj and al., 2021).[12].**

Month	January	February	March	April	May	June
T (°C)	6.95	8.06	11.31	14.12	22.91	23.87
P (mm)	18.49	17.88	27.95	26.24	21.05	10.6
Month	July	August	September	October	November	December
T (°C)	27.9	27.12	22.03	17.01	11.01	7.54
P (mm)	5.28	10.14	26.34	35.52	26.98	16.62

P (mm): Precipitation in millimeters
T°C: Temperature in degrees Celsius

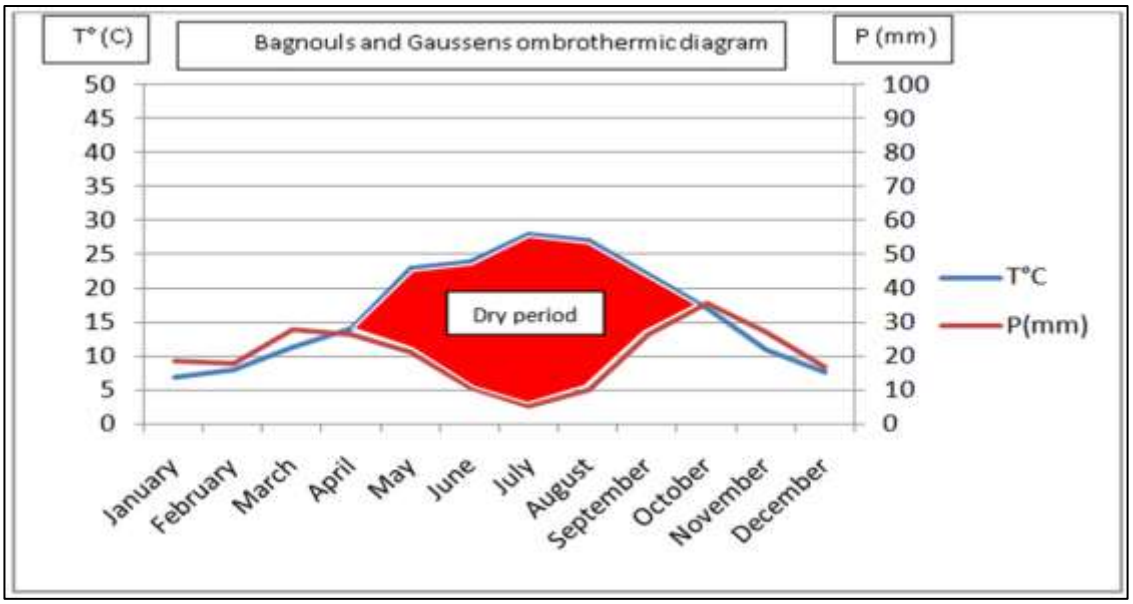

Fig.3. Ombrothermic diagram of Bagnouls and Gaussen for the Mecheria Station (P=2T).

The dry period extends from April to October, lasting for seven months (Fig. 3). July is the driest month with 5.28 mm of precipitation. In contrast, October and November are the wettest months, with 35.52 mm and 26.98 mm of precipitation, respectively.
According to **Abdelkrim Benaradj and al. (2021),** the climate of the Mecheria station is a steppe climate with a cold and arid winter.

2-4.Climate study of the selected regions:

2-4-1- Setif region:

Table 2: Climatic data from the Setif meteorogical station (21-year period 2000 to 2020) **(Marrouche hind, 2023) [13]**

Month	January	February	March	April	May	June
T (°C)	5.52	6.41	9.53	12.64	16.91	20.95
P (mm)	47.8	40.1	44.5	45.1	41.6	21

Month	July	August	September	October	November	December
T (°C)	26.52	25.74	20.99	16.36	9.92	6.45
P (mm)	9.86	19.9	38.3	30.7	40	37

P (mm): Precipitation in millimeters
T°C: Temperature in degrees Celsius

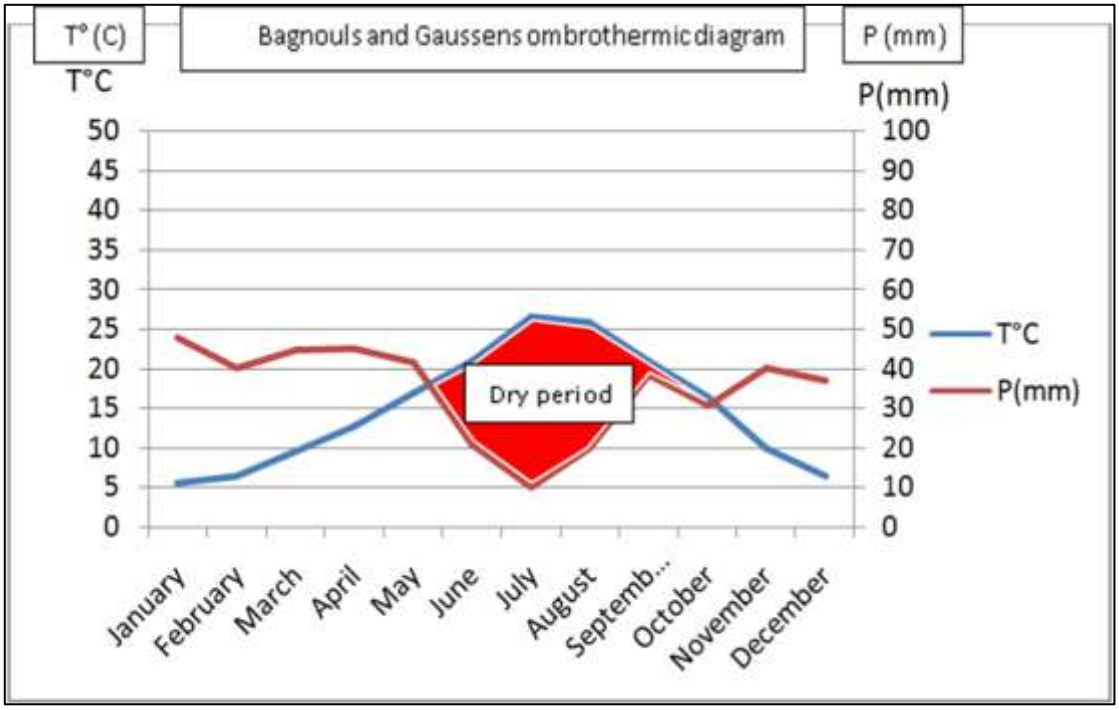

Fig.3. Ombrothermic diagram of Bagnouls and Gaussen for the Setif Station (P=2T).

2-4-2- Djelfa region:

Table.3. Climatic data from the Djelfa meteorogical station**(Mecheri Hadjer, 2018) [14]**

Month	January	February	March	April	May	June
T (°C)	4.8	6.3	9.1	12.5	17	22.5
P (mm)	33.9	29.7	29.5	30.2	34	19.7
Month	July	August	September	October	November	December
T (°C)	26.6	26.5	20.9	15.5	9.4	6
P (mm)	11.1	20.6	30.9	26.6	29.7	27

P (mm): Precipitation in millimeters
T°C: Temperature in degrees Celsius

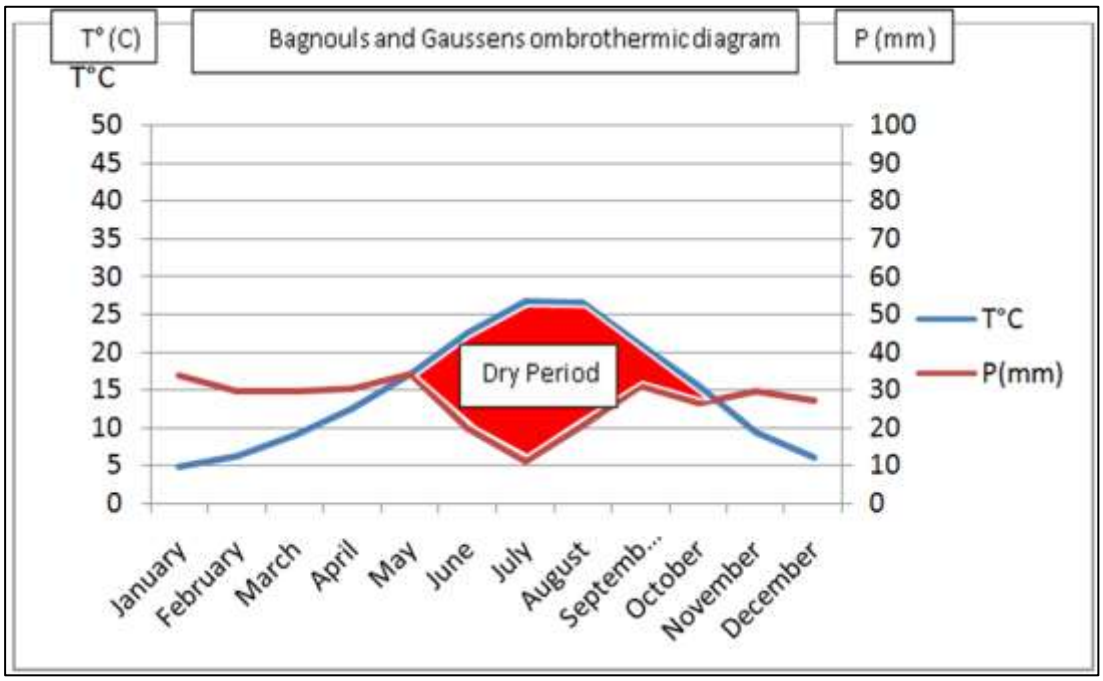

Fig.4. Ombrothermic diagram of Bagnouls and Gaussen for the Djelfa Station (P=2T)

2-4-3- Tolede region:

Table.4. Climatic data from the Tolede meteorological station: AEMET 2020 agencia estatal de meteorologia (1991-2020)

Month	January	February	March	April	May	June
T (°C)	6.8	8.4	11.7	14.1	18.4	23.7
P (mm)	25	25	33	40	39	19
Month	July	August	September	October	November	December
T (°C)	27.2	26.8	22.1	16.6	10.7	7.3
P (mm)	6	9	21	49	38	36

P (mm): Precipitation in millimeters
T°C: Temperature in degrees Celsius

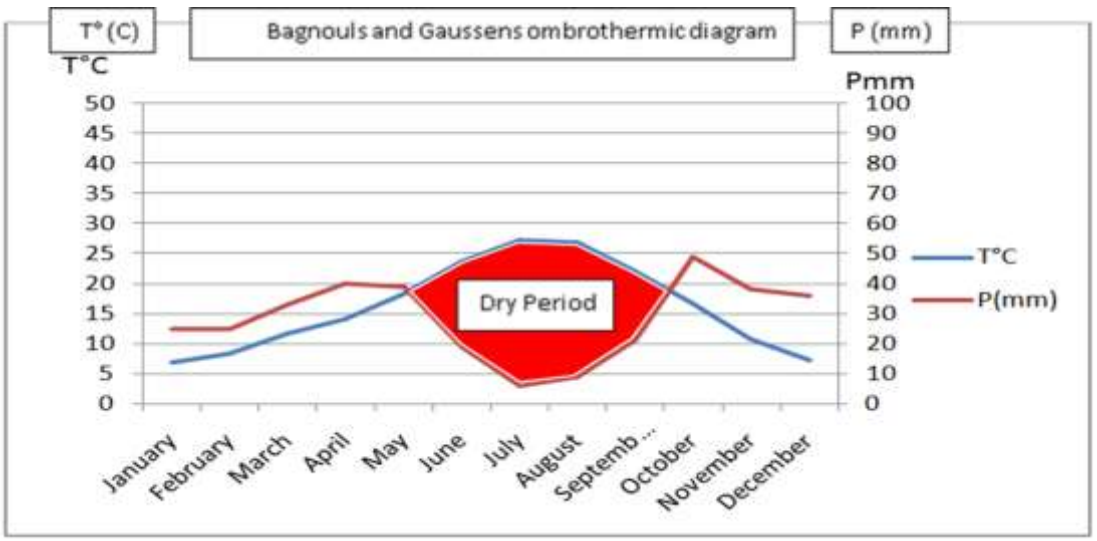

Fig.5.Ombrothermic diagram of Bagnouls and Gaussen for the Tolede Station (P=2T)

2-4-4- Agadir region:

Table.5.Climatic data from the Agadir meteorological station, Ibn Zohr University, Faculty of Sciences, Department of Geology, Agadir 2018

Month	January	February	March	April	May	June
T (°C)	14	15	16.5	17	18.5	20
P (mm)	25.5	22	18	14.5	3	1
Month	July	August	September	October	November	December
T (°C)	22	22	21.5	20	18	15
P (mm)	0	0	2	15	28.5	33.5

P (mm): Precipitation in millimeters
T°C: Temperature in degrees Celsius

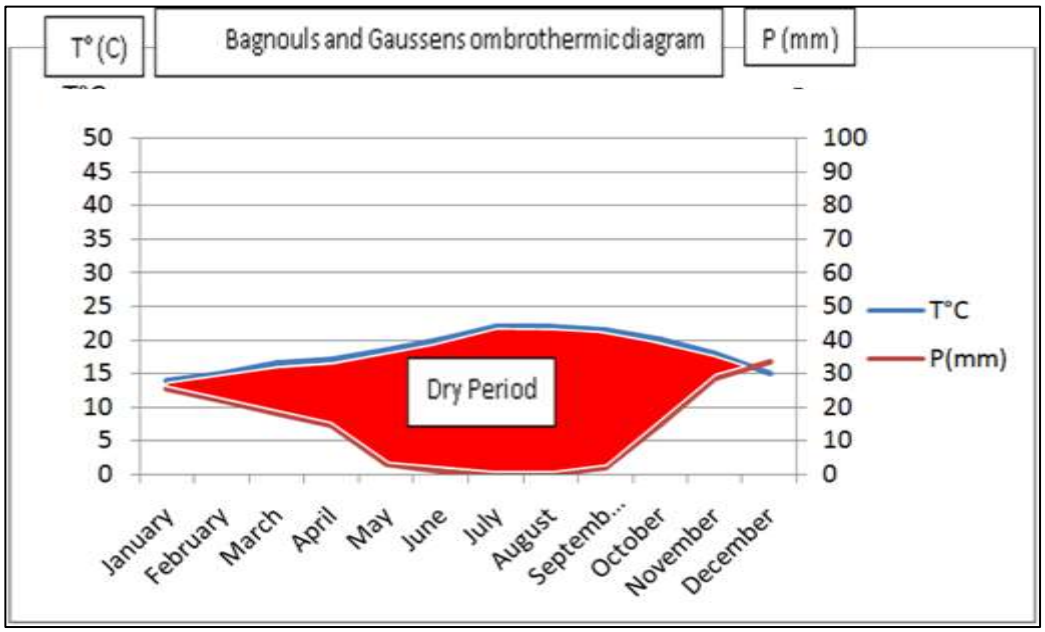

Fig .6.Ombrothermic diagram of Bagnouls and Gaussen for the Agadir station (P=2T)

2-4-5- Tehran region:

Table.6.Climatic data from the Tehran meteorological station: National metrology office (Iran)

Month	January	February	March	April	May	June
T (°C)	-2	-1	3	7	10	14
P (mm)	70	70	65	130	65	25
Month	July	August	September	October	November	December
T (°C)	17	16	13	8	3	0
P (mm)	10	5	5	55	75	55

P (mm): Precipitation in millimeters

T°C: Temperature in degrees Celsius

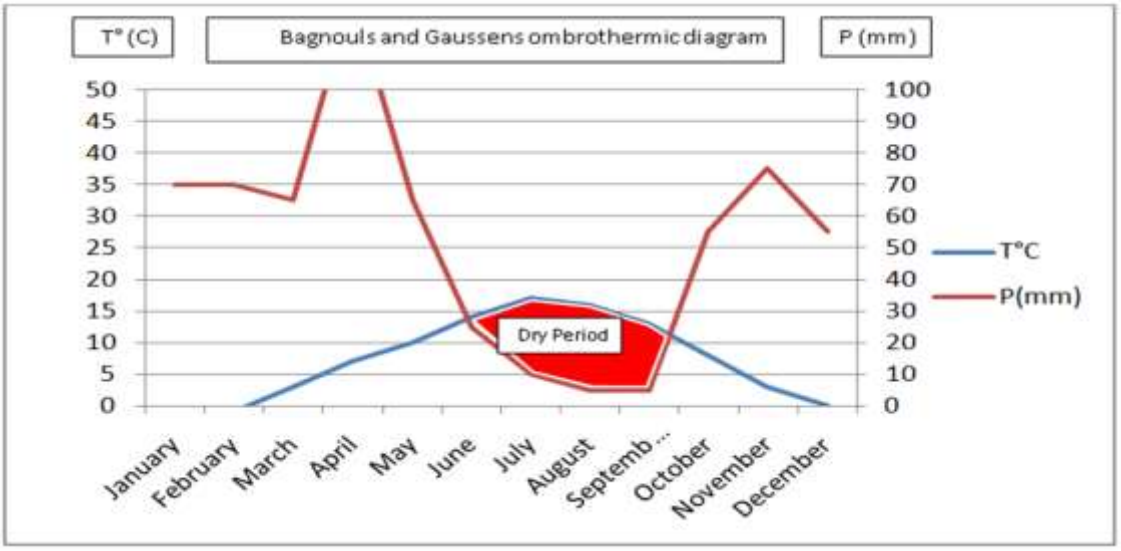

Fig .7.Ombrothermic diagram of Bagnouls and Gaussen for the Tehran station (P=2T)

2-4-6- Xinjiang region:

Table.7.Climatic data from the Xinjiang meteorological station, world meteorological organization and china meteorological administration (WMO ID: 51463) (August 2023) (1991-2020)

Month	January	February	March	April	May	June
T (°C)	-13.5	-9.6	-0.5	9.5	16	21
P (mm)	6	6	16	30	38	36
Month	July	August	September	October	November	December
T (°C)	23.9	22.2	16.2	8.2	-1.9	-10.1
P (mm)	38	30	19	22	11	5

P (mm): Precipitation in millimeters
T°C: Temperature in degrees Celsius

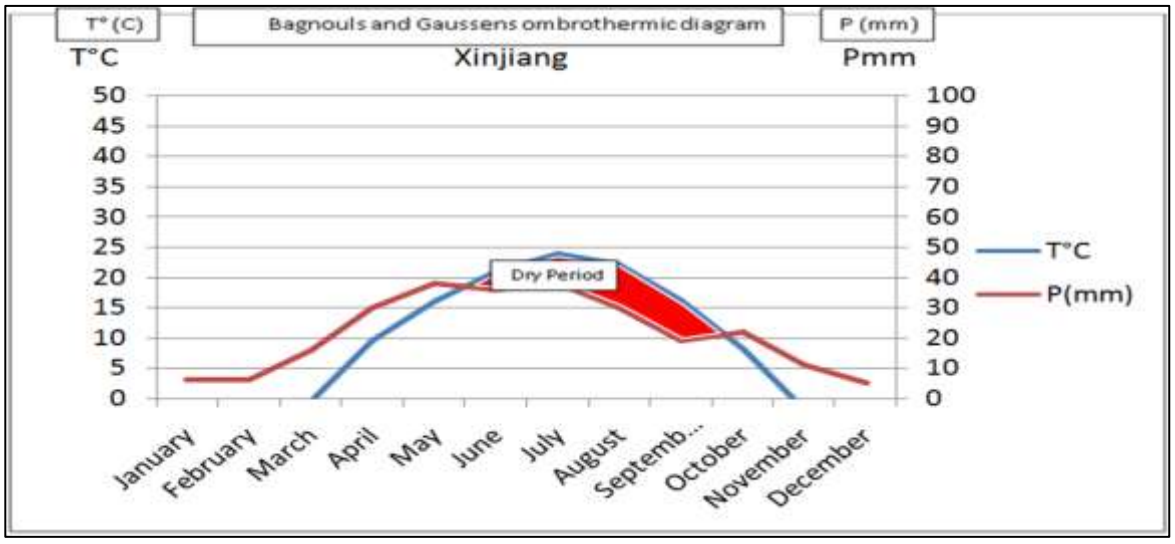

Fig.8.Ombrothermic diagram of Bagnouls and Gaussen for the xinjiang station (P=2T)

The observed spatial variability in β-carboline alkaloid content, particularly harmine and harmaline, across the investigated populations of *Peganum harmala* (Naâma, Setif, Djelfa, Toledo, Agadir, Tehran, and Xinjiang) underscores the strong modulatory role of environmental drivers on plant secondary metabolism.

Ombrothermic diagrams constructed using the Bagnouls and Gaussen approach reveal pronounced inter-regional differences in the duration and intensity of the dry period. Regions such characterized by approximately seven months of aridity, are subject to sustained hydric deficit conditions. Such persistent water limitation constitutes a major ecological constraint known to reprogram plant metabolic fluxes toward enhanced synthesis of secondary metabolites, particularly alkaloids.

Under arid and semi-arid conditions, the combined effect of drought stress and elevated temperature induces oxidative and physiological stress, triggering the upregulation of defense-related biosynthetic pathways. In this context, β-carboline alkaloids are widely recognized as part of the plant's adaptive chemical arsenal, contributing to tolerance against abiotic stressors (water deficit, high irradiance). The relatively elevated alkaloid levels recorded in xeric environments such as Toledo and Xinjiang are consistent with this stress-induced metabolic activation model.

In contrast, regions exhibiting comparatively more favorable hydric regimes, such as Setif and Tehran, display reduced environmental stress intensity, which may account for the lower accumulation of these secondary metabolites. The attenuation of abiotic stress likely reduces the selective pressure for intensified chemical defense investment, resulting in a more moderated metabolic output.

Thermal variability also appears to play a significant role. Xinjiang, in particular, is characterized by extreme continentality with marked seasonal thermal amplitudes. Such conditions may act as an additional selective pressure, promoting metabolic plasticity and enhancing secondary metabolite accumulation. Furthermore, high solar irradiance in arid ecosystems is known to influence photoprotective and stress-response pathways, potentially contributing to increased β-carboline biosynthesis.

Nevertheless, alkaloid variability cannot be exclusively attributed to climatic forcing. Additional determinants, including edaphic properties (soil texture, pH, organic matter content), ontogenic stage of the seeds, and methodological variability associated with extraction and analytical procedures (HPLC, GC-MS), may introduce significant quantitative fluctuations. These factors must therefore be considered as potential sources of experimental and ecological variance in comparative phytochemical studies.

Collectively, the present findings demonstrate that β-carboline alkaloid biosynthesis in *Peganum harmala* is governed by a multifactorial interplay between climatic stressors, environmental heterogeneity, and methodological constraints. The eco-geographical framework adopted herein provides a robust interpretative basis for understanding phytochemical variability.

2-5. Pedological Study of the Research Area:

According to the pedological analysis conducted at the Public Works Laboratory of the West (LTPO) located in the region of Tlemcen, the soil where*Peganum harmala* grows is predominantly sandy.

It is characterized as shallow, wind-transported, with low organic matter content, moderate salinity, and a basic pH (pH = 8.4; **[11]**).

The organic matter content in the El Kasdir region is approximately 0.6% (LTPO Tlemcen, 2019).

2-6. Extraction of Harmine and Harmaline Alkaloids from *Peganum harmala*seeds:

Following the protocol of **Herraiz and al. (2010) [1],** the dried seeds collected from our study area, El Kasdir, were ground using a ball mill. The resulting powder was divided into several batches and stored in amber glass vials. These vials were kept in a dark, clean, and secure location (a laboratory cupboard or closed cabinet) at a stable ambient temperature (between 18 and 25°C). Since the storage period in our study was not prolonged, these measures were sufficient to preserve the stability of the light-sensitive chemical compounds.

0.5 g of dried, ground seeds were homogenized in 20 ml of an aqueous solution containing perchloric acid ($HClO_4$) and methanol (CH_3OH) in a 1:1 molar ratio ($HClO_4$-methanol 1:1) using an Ultra-Turrax homogenizer. The mixture was then centrifuged at 10,000 rpm for 10 minutes. The supernatant was collected, and the extraction process was repeated twice with the residual pellet. The combined supernatants were designated for chromatographic analysis to identify and quantify the two major alkaloids of *Peganum harmala*, namely harmine and harmaline.

2-7.Quantitative analysis of Harmine and Harmaline alkaloids extracted from *Peganum harmala* seeds:

2-7-a. By High-Performance Liquid Chromatography (HPLC):

A suitable dilution was used for injection in HPLC-DAD with fluorescence detection. HPLC-DAD, or High-Performance Liquid Chromatography coupled with a Diode Array Detector, is a powerful analytical method used to separate, identify, and quantify the various chemical components in a complex mixture.

In the present study, this technique was employed to analyze the β-carboline alkaloids (harmine and harmaline) extracted from the seeds.

The concentration of the two major alkaloids, harmine and harmaline, was determined using calibration curves constructed with harmaline (absorbance at 355 nm) and harmine (absorbance at 254 nm) standards. Five different samples from the El Kasdir region were homogenized and analyzed by HPLC.
The analysis of β-carboline alkaloids was performed by RP-HPLC with a UV diode array and fluorescence detection using an HPLC 1050 system (Hewlett Packard) equipped with a 1100 diode array detector (DAD) and a 1046A fluorescence detector. A Nova-pak C18 column (150 mm × 3.9 mm internal diameter, 4 µm; Waters, Milford, MA, USA) was used for chromatographic separation. The chromatographic conditions were as follows: 50 mM ammonium phosphate buffer (pH 5.2) (buffer A) and 20% A in acetonitrile (buffer B).
The gradient was programmed from 0% (100% A) to 32% B over 8 minutes, and to 90% B at 12 minutes. The flow rate was 1 ml/min, the column temperature was maintained at 40°C, and the injection volume was 20 µl.
Harmine was detected at 254 nm and harmaline at 355 nm. Compound identification was performed using UV spectrometry, fluorescence, and mass spectrometry.
The identification of β-carbolines in *Peganum harmala* by HPLC-ESI mass spectrometry was carried out using a Zorbax SB-C18 column (150 mm x 2.1 mm internal diameter, 5 µm; Agilent Technologies) with an 1100 series HPLC-MSD system (Hewlett-Packard) in positive ion electrospray mode. The eluents were: (A) 0.5% acetic acid and (B) 0.5% acetic acid in acetonitrile; using a gradient of 80% B over 30 minutes at a flow rate of 0.25 ml/min. The temperature was 40°C, the mass range was 50-700 m/z, and the cone voltage was set to 100 V.

2-7-b. By Gas Chromatography / Mass Spectrometry (GC/MS):
The same protocol mentioned previously, from Herraiz and al. (2010), was employed.
The analysis was performed using Gas Chromatography coupled with Mass Spectrometry (GC/MS) with an Agilent Technologies 7820A GC and 5977B MSD system.
Specifications:
Column: ZB-5MSi (60 m × 250 µm, 0.25 µm film thickness)
Temperature program: 60-300°C, ramped at 5°C/min
Carrier gas: Helium at a constant flow of 1.0 mL/min
Injection volume:1 Ml

3. RESULTS:
3-1.Quantitative analysis of Harmine and Harmaline:
The quantitative analyses of harmine and harmaline alkaloids extracted from the seeds of *Peganum harmala* are presented in Table 8.

Table 8.Concentration of the major alkaloids, harmine and harmaline, extracted from the seeds of *Peganum harmala* in our present study area.

	El Kasdir (Naâma)
Harmaline (mg/g)	Sample 01: 31.3
	Sample 02: 35.1
	Sample 03: 31.7
	Sample 04:30.8
	Sample 05: 34.6
Mean	32.7 ± 1.7
Harmine (mg/g)	Sample 01: 20.22
	Sample 02: 22.33
	Sample 03: 20.34

	Sample 04: 20.32
	Sample 05: 21.60
Mean	20.96 ± 1.18

3-2. Chromatographic Analysis of our results:

3-2-a. Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) with Diode Array Detector (DAD)

Representative chromatograms of the seeds from our study area are shown below. The two major alkaloids were identified as harmaline and harmine based on their retention times, DAD spectra,. Co-elution with authentic harmaline and harmine standards was also performed for confirmation (Figures 9 and 10)

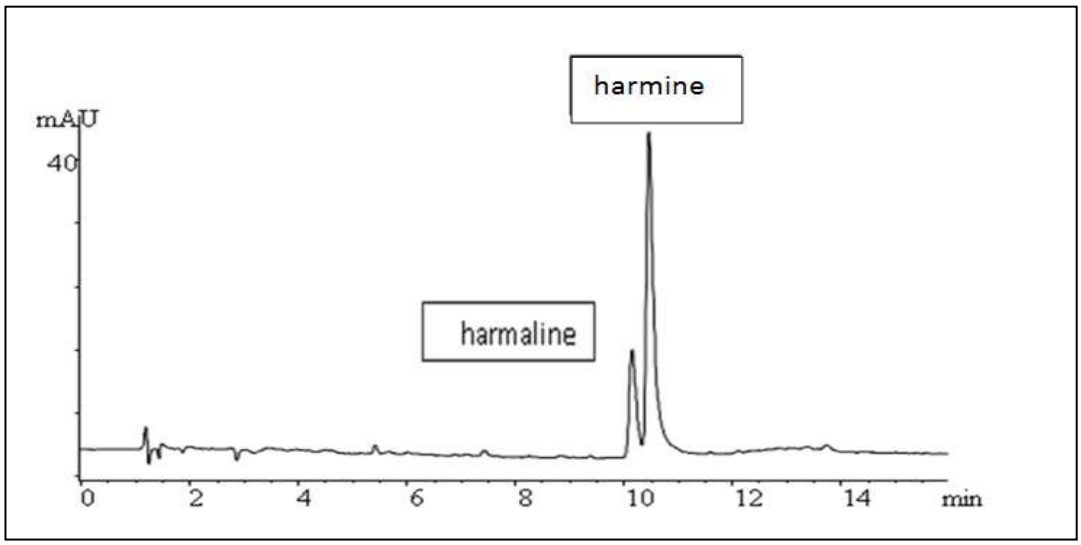

Fig.9. HPLC chromatogram (254 nm) of alkaloids from seeds of the El Kasdir region after sample preparation and dilution.

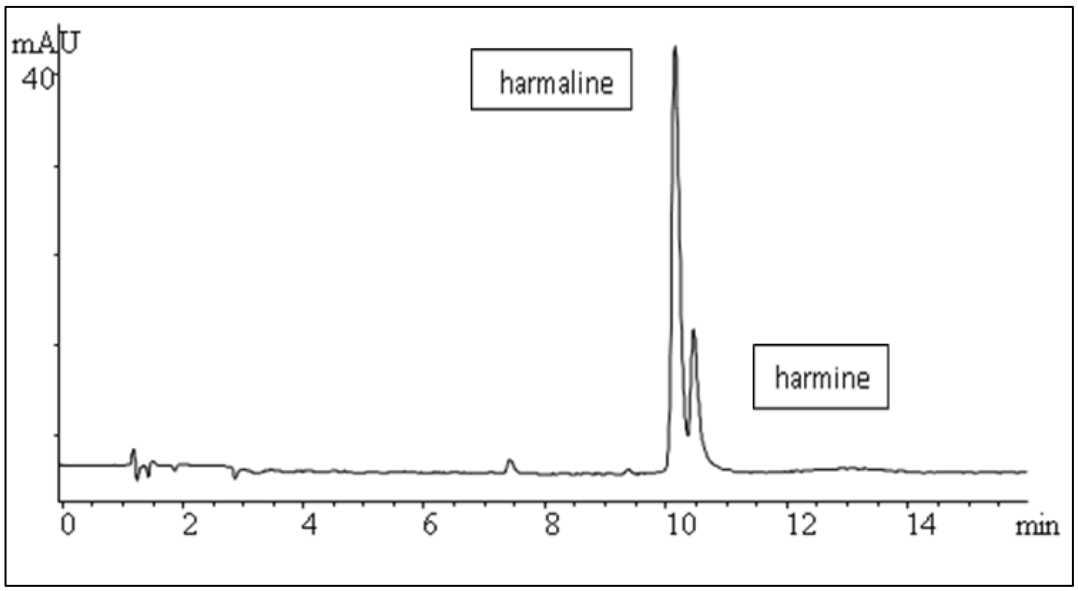

Fig.10. HPLC chromatogram (355 nm) of alkaloids from seeds of the El Kasdir region after sample preparation and dilution.

The major β-carboline alkaloids were identified by electrospray mass spectrometry and UV-VIS (DAD) as harmaline UVmax ~375 nm and harmine, UVmax ~245 and 322 nm) (Figure 11 and Figure 12).

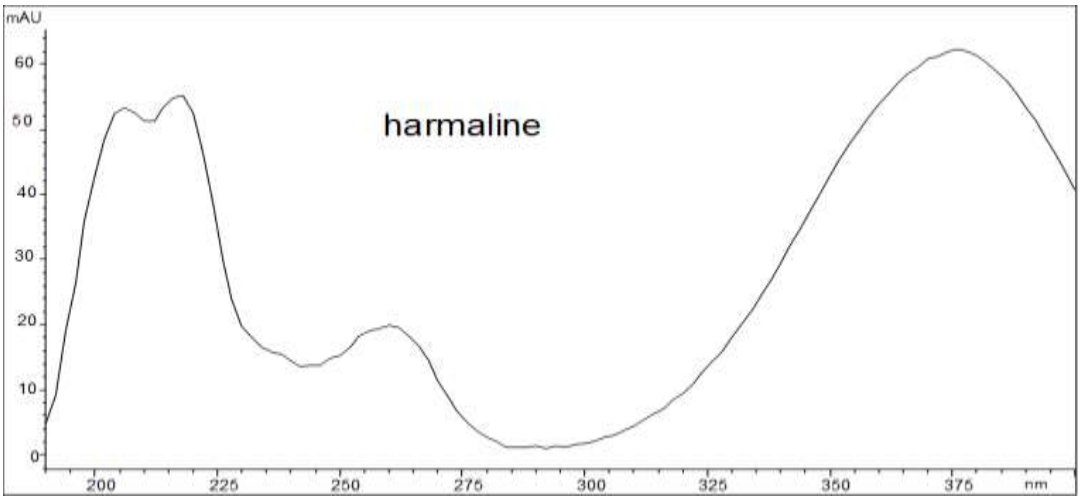

Fig.11. DAD spectra of harmaline in the chromatograms of *Peganum harmala* seeds from the El Kasdir region.

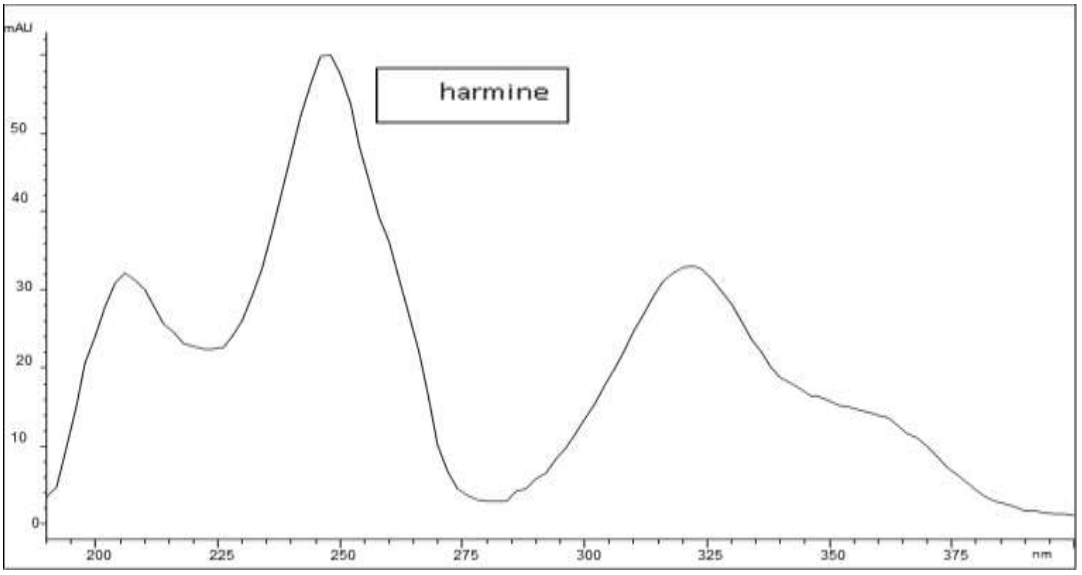

Fig.12. DAD spectra of harmine in the chromatograms of *Peganum harmala*seeds from the El Kasdir region.

3-2-b. Gas Chromatography Coupled with Mass Spectrometry (GC/MS):

Following GC/MS chromatographic analysis, the results are as follows (Figure 14).

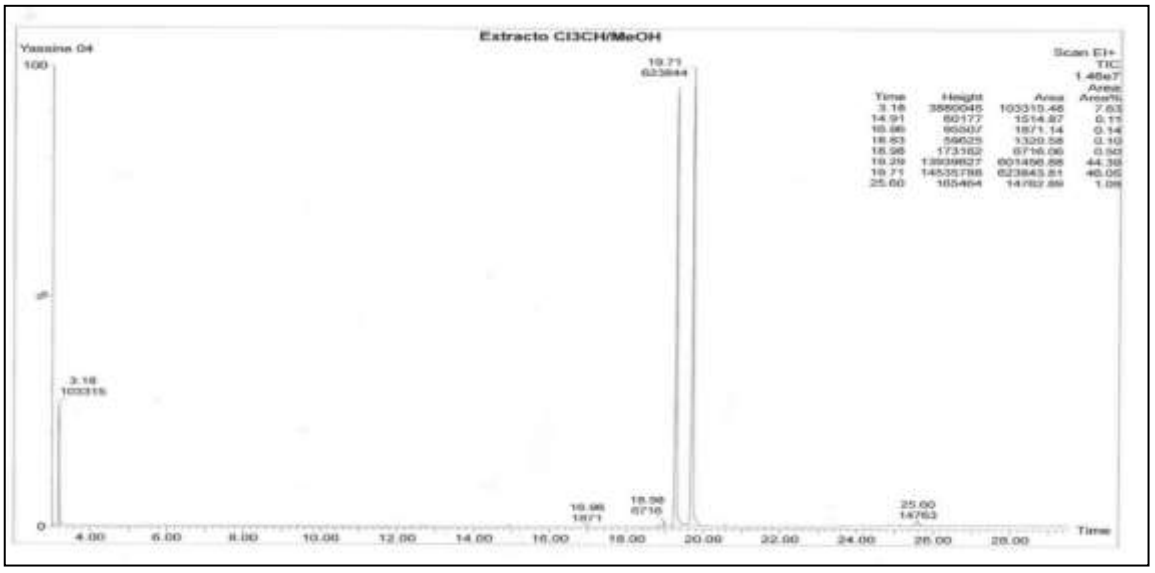

Fig.13.GC/MS analysis of the total alkaloids from *Peganum harmala*from seeds of the El Kasdir region

This chromatogram clearly shows that the two primary alkaloids present in the *Peganum harmala* seeds are harmaline (44.39%) and harmine (46.05%), corresponding to retention times of 19.29 and 19.71 minutes, respectively. These two major alkaloids constitute over 90% of the seed's components.
The analysis also indicates the presence of another alkaloid in a minimal quantity, identified as tetrahydroharmine (heptaflorine, THH) at 0.5%, with a retention time of 18.98 minutes.

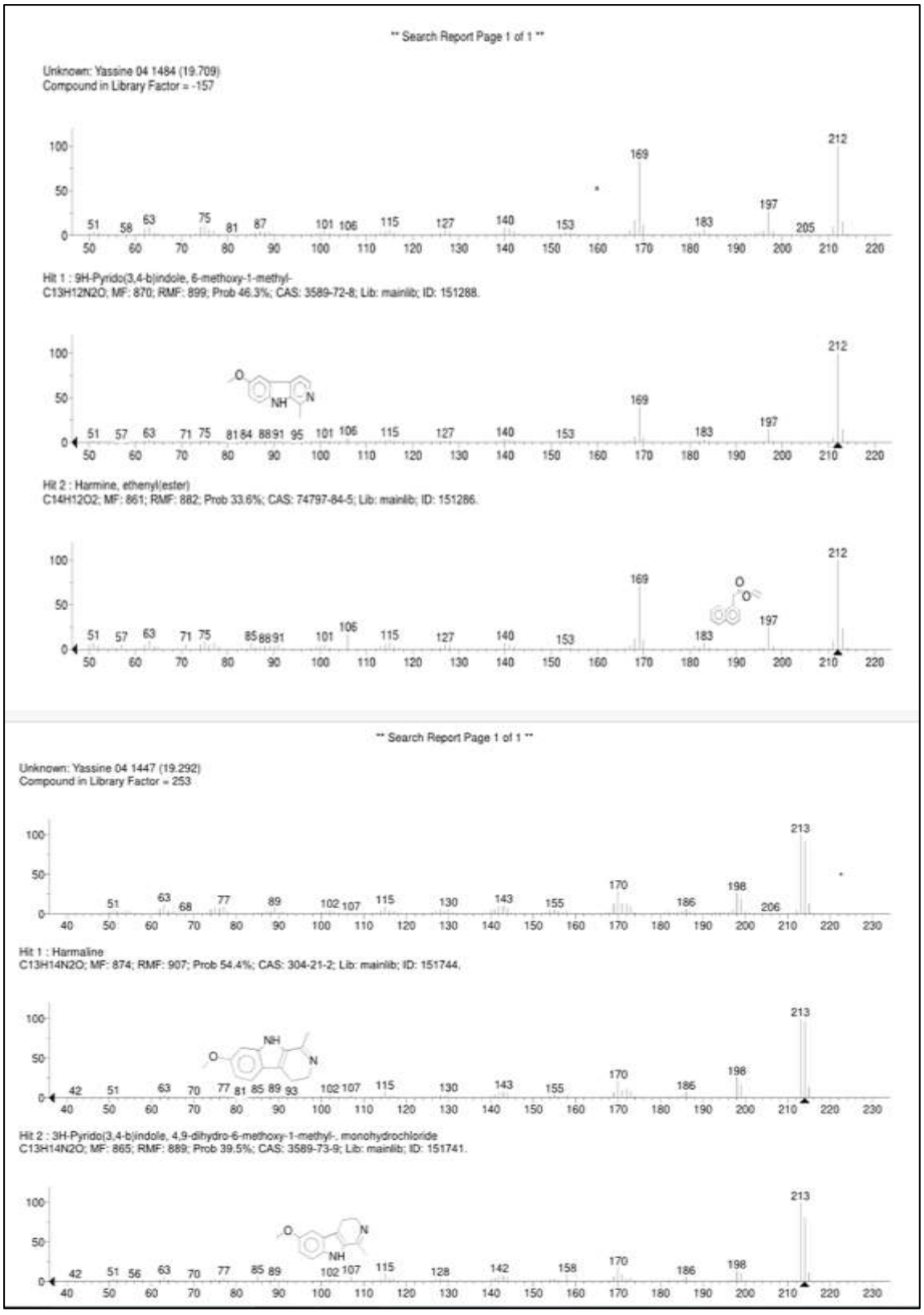

Figure 14,demonstrates the presence of harmine and harmaline in our samples, along with their chemical structures.Fig.14. GC/MS analysis showing the presence of harmine and harmaline in the seeds of *Peganum harmala* extracted from our study area(El Kasdir)

4. DISCUSSION:

The primary objective of this work was to conduct a quantitative comparative study. We compared our results on the extraction of the alkaloids harmine and harmaline from *Peganum harmala* seeds in the El Kasdir region of Naama with other studies in the literature that have previously investigated this plant for various research purposes. Different motivations have driven researchers to focus on these two major alkaloids, harmine and harmaline. In this study, we specifically focus on the sections of their work detailing alkaloid extraction.

This comparative study goes beyond a simple academic exercise; it plays a key role in identifying and explaining inter-study discrepancies, validating the obtained results, and uncovering potentially unforeseen patterns.

To this end, we chose to compare our results with six (06) previous studies that focused on the major alkaloids, harmine and harmaline, of our subject plant, *Peganum harmala*.

These studies are not identical, neither in their research objectives nor in their geographic regions of study.

We intentionally selected studies from different geographical zones—namely Algeria, Morocco, Iran, Spain, and the People's Republic of China—to enable a more robust comparison and to obtain reliable results (Table 9).

Table 9. Comparative table of harmine and harmaline content across the studied regions.

Region	Country	Plant Part Analyzed	Analytical Method	Harmaline (mg/g or %)	Harmine (mg/g or %)	Reference
El Kasdir (Naama)	Algeria	Seeds	RP-HPLC and GC/MS	32.7±1.7mg/g (44.39%)	20.96±1.18 (46.05%)	Our present study
Setif	Algeria	Seeds	RP-HPLC	3.8 %	2.9 %	Bensalem and al.,2014 **[6]**
Djelfa	Algeria	Seeds	GC/MS	48.009 %	38.440 %	Sassoui and al.,2015 **[7]**
Toledo	Spain	Seeds	RP-HPLC	56.0±2.28mg/g	43.2±mg/g	Herraiz and al.,2010**[1]**
Agadir	Morocco	Seeds	HPLC	0.874±0.016mg/g	8.514±0.521mg/g	Mayad and al., 2019**[15]**
Tehran	Iran	Seeds	HPLC	0.25%	1.84%	Hemmateenejad.2006**[16]**
Xinjiang	China	Seeds	NMR	58.46mg/g	40.77mg/g	Yinping Li and al 2018**[17]**

4.1. Geographical locations of the regions included in the comparative study:

-Setif region (Algeria):

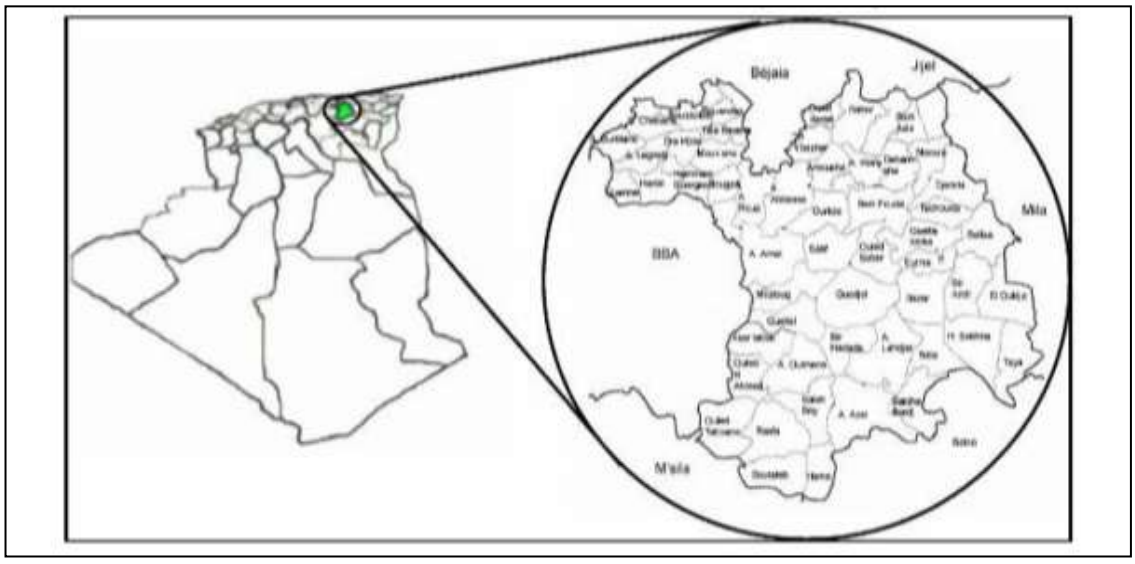

Fig. 15. Geographical location of the Setif region **(Manallah Imene, 2012). [18]**

Djelfa region (Algeria):

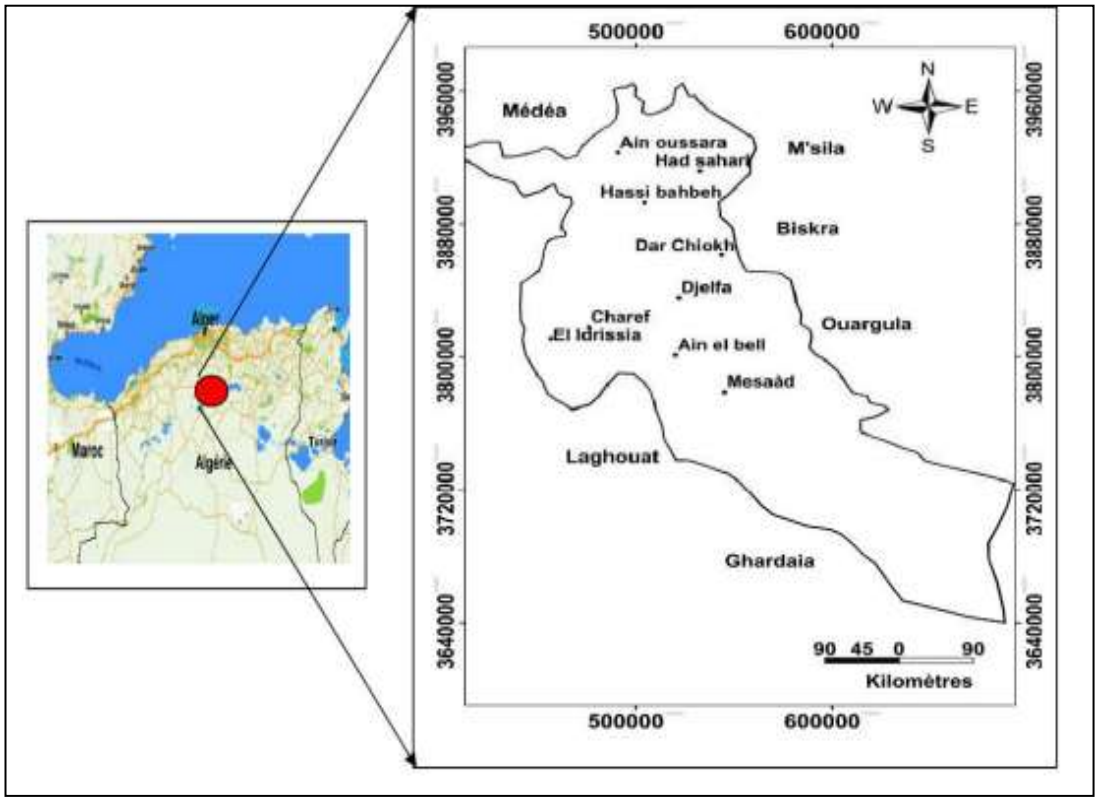

Fig. 16.Geographical location of the Djelfa region **(Koussa, M. and T. Bouziane, 2018) [19],** (Contribution of GIS to mapping water erosion risk zones in the Djelfa region, Algeria. Lebanese Science Journal, Vol. 19, No. 1, 2018).

-Toledo Region (Spain):

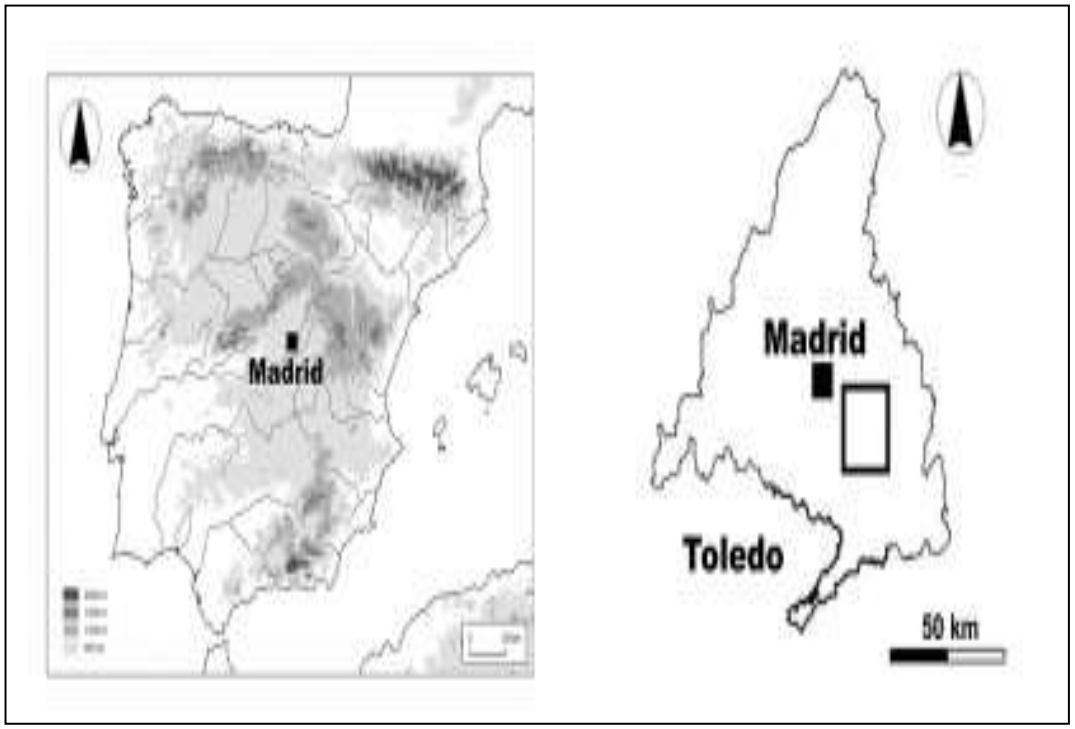

Fig. 17. Geographical location of the Toledo region **(Hugues Alexandre Blain and al., 2013).[20]**

-Agadir Region (Morocco):

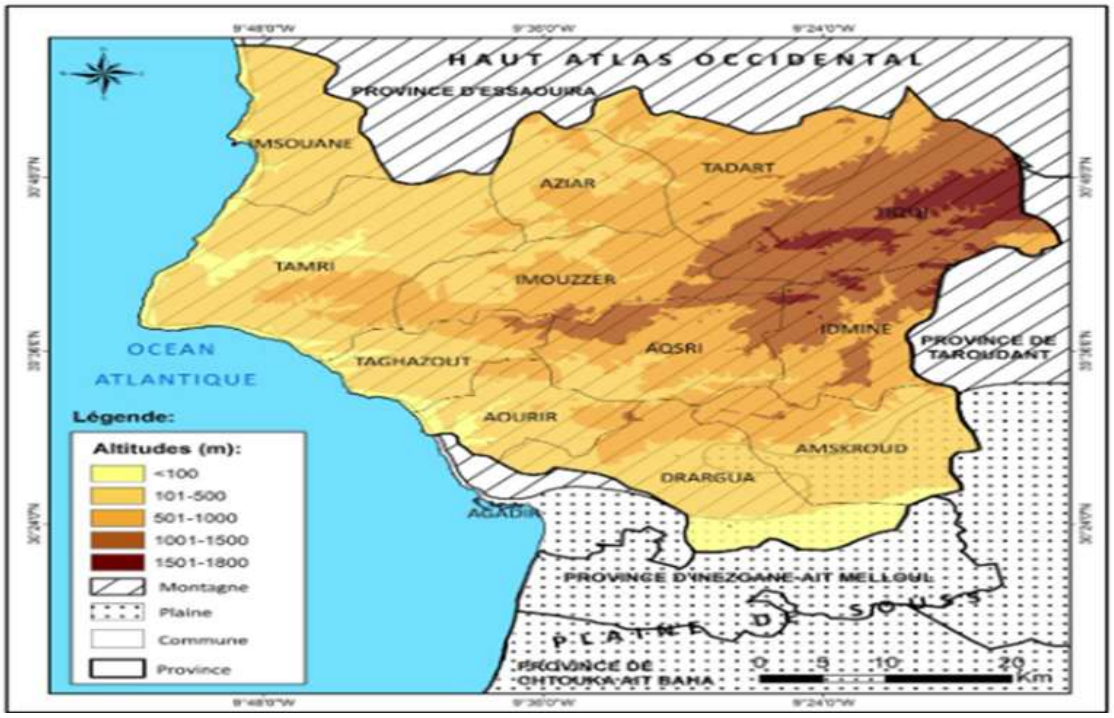

Fig.18. Geographical location of the Agadir region (Source: Ibn Zohr University, Faculty of Sciences, Department of Geology, Agadir; Practical work in Climatology, External Geodynamics module, STU2-SV2, 2018).

-Tehran region(Iran):

Fig.19. Location of Tehran (Source: **Ali Jafari and al.2025)[21]**

-Xinjiang region, (China):

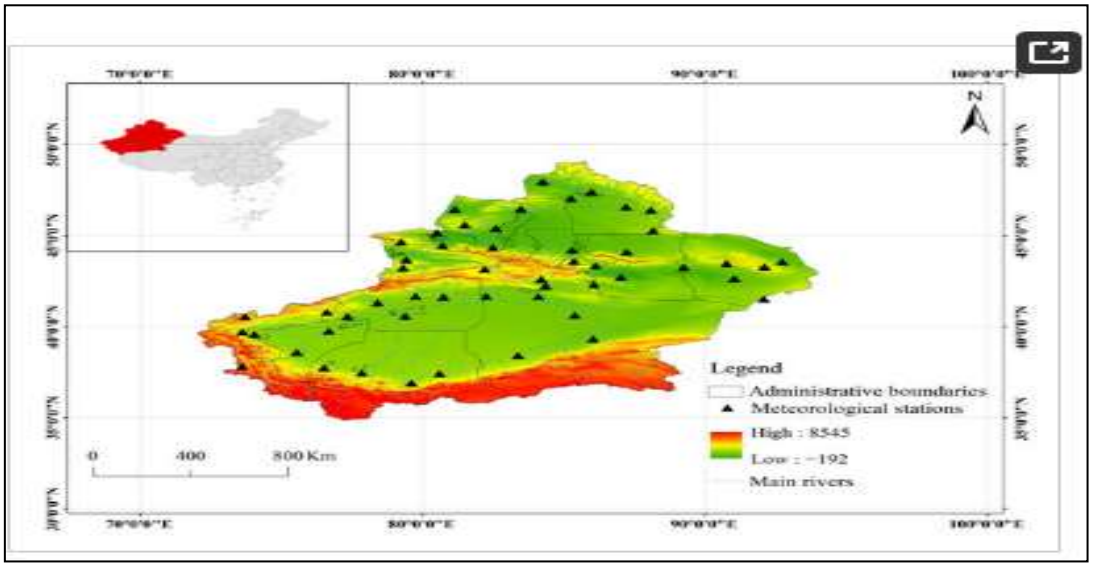

Fig.20.Map of the Xinjiang region **(Yang Yang and al., 2024).[22]**

The comparative analysis of harmine and harmaline content extracted from *Peganum harmala* seeds across several regions (Algeria, Morocco, Iran, Spain, China) reveals a marked variability in concentrations, highlighting the significant influence of climatic and eco-geographical factors.

In our study conducted in El Kasdir (Naama, Algeria)—characterized by a steppe climate with a cold, arid winter (Abdelkrim Benaradj et al., 2021)—the average concentrations of harmaline and harmine were 32.7 ± 1.7 mg/g and 20.96 ± 1.18 mg/g, respectively.

These results were compared to those obtained in Setif (semi-arid with cool winter, **[13],** where alkaloid contents were 3.8 % (harmaline) and 2.9. %(harmine), and Djelfa (semi-arid with dry winter, Mecheri Hadjer, 2018), where the contents were 48.009% and 38.44% for harmaline and harmine, respectively.

Particularly high concentrations have been reported in Toledo (Spain), an area with a lower meso-Mediterranean climate**[23]**, and Xinjiang (China), with a semi-arid continental climate (Köppen classification BSk), where peaks reached 56 mg/g (harmaline) and 43.2 mg/g (harmine) in Spain, and 58.46 mg/g and 40.77 mg/g, respectively, in China.

In contrast, much lower concentrations were observed in Agadir (Morocco), a region with a semi-arid climate featuring a mild, ocean-influenced winter (Achadmi Nadia et al., 2024), with only 0.874 mg/g of harmaline and 8.5114 mg/g of harmine.

Tehran (Iran), where the measured contents were 0.25 %(harmaline) and 1.84% (harmine). These interregional variations can largely be explained by differences in climate (precipitation, temperature, drought duration), soil type, altitude, and the physiological state of the plant at harvest. Arid climates with hot summers appear to favor the accumulation of beta-carboline alkaloids, whereas more temperate or maritime conditions lead to a decrease in their concentration.

These results confirm that alkaloid biosynthesis is highly dependent on environmental conditions, aligning with the conclusions of Nettleship and Slaytor (1974) and Kartal et al. (2003), who stated that geographical, edaphic, and climatic factors significantly influence the secondary metabolism of medicinal plants like *Peganum harmala*.

Based on our study, a clear quantitative difference is observed. The overall results suggest that the variation in alkaloid content (harmine and harmaline) can be explained by several factors, including: Environmental factors such as climate, precipitation, sunlight, and average temperature.Edaphic factors such as soil composition, humidity, and mineralization, and biological factors such as altitude and proximity to the ocean.

It is also important to emphasize that extraction methods (HPLC vs. GC/MS) as well as geographic origin also play a role in the quantitative aspect of the alkaloids.

These results confirm that alkaloid content can vary significantly depending on several biological and technical factors.

The observed differences can be attributed to eco-geographical variations, the extraction methods employed, or the physiological maturity of the plants at harvest.

CONCLUSION:

The main objective of this study was to perform a comparative quantitative analysis of the two major β carboline alkaloids, harmine and harmaline, extracted from the seeds of Peganum harmala collected in the El Kasdir region (Naama, Algeria). Our results, obtained by HPLC DAD and GC/MS, show average concentrations of 32.7 ± 1.7 mg/g for harmaline and 20.96 ± 1.18 mg/g for harmine, representing together more than 90 % of the total alkaloid fraction.

By comparing these values with six previous studies carried out in different geographical zones (Sétif and Djelfa in Algeria, Toledo in Spain, Agadir in Morocco, Tehran in Iran and Xinjiang in China), a marked variability in alkaloid content is clearly observed. The highest levels were recorded in China (58.46 mg/g harmaline) and Spain (56.0 mg/g harmaline), while the lowest were found in Morocco (0.874 mg/g harmaline). Our results from El Kasdir fall into an intermediate range.

Several factors explain this quantitative heterogeneity. Climatic conditions play a major role: arid and semi arid climates with hot, dry summers and cold winters (such as in El Kasdir, Djelfa, Tehran and Xinjiang) seem to promote the accumulation of harmine and harmaline. In contrast, the mild, ocean influenced winter of Agadir is associated with much lower concentrations. Edaphic factors (sandy, low organic matter, basic soils in our study area) as well as altitude and proximity to the sea also influence secondary metabolism. Finally, methodological differences (HPLC vs. GC/MS, extraction protocols, plant maturity at harvest) cannot be overlooked and may partly explain the discrepancies between studies.

From a broader perspective, our work confirms that Peganum harmala is a species with high chemical plasticity, whose alkaloid profile is strongly shaped by environmental and geographical conditions. This finding has practical implications: for pharmaceutical or industrial applications, it is essential to carefully select the plant's origin and to standardise extraction and analysis methods. Future research could extend this approach to other alkaloids (e.g., tetrahydroharmine, detected at 0.5 % in our samples) and investigate the influence of seasonal variations, water stress or soil mineral composition on the biosynthesis of these bioactive compounds. In addition, a deeper chemotaxonomic study, combining ecology and metabolomics, would help to better understand the adaptive role of β carbolines in arid environments.

In summary, this study not only provides new quantitative data for the El Kasdir region but also highlights the importance of an integrative, multi site approach when characterising medicinal plants. The observed differences are not artefacts but reflect real ecological and methodological effects that must be taken into account in any future use of Peganum harmala as a source of harmine and harmaline.

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