Original contributions

F. Nadjafi, A. Koocheki, P. Rezvani Moghadam and B. Honermeier

First experiments on cultivation of *Nepeta binaludensis* Jamzad – an example of domestication of a highly endangered medicinal plant of Iran

Introduction

The cultivation of medicinal plants is useful to meet current and future demands for a large volume production of plant-based drugs and herbal remedies, and also to reduce pressure on wild populations (21). Cultivation can have the potential to save wild populations of medicinal plants and their genetic diversity. However, many species are difficult to cultivate because of specific biological features or ecological requirements such as seed dormancy, slow growth rate, special soil requirements, low generation rates, susceptibility to pests etc. (17). A thorough understanding of their reproductive and growth biology as well as the identification of biological constraints leading to reduced fitness or even extinction is therefore increasingly necessary. Understanding of the biological, phytochemical and ecological characteristics of species in their common habitat is also essential for their conservation biology as well as to predict their behavior under artificial cultivation (14). In case of domestication beyond their normal ecological range or in case of changed ecosystem conditions, many of the wild medicinal and aromatic plants tend to behave differently. So the species' response to different agronomical parameters such as planting date, irrigation, soil condition, fertilization, plant density, harvesting time, etc. in new ecological conditions on the farm should be studied. Different researches are focused on the effect of agronomical conditions like irrigation and water deficiency (6,7,13) and soil fertilization (19) on growth, yield and chemical components of different medicinal plant species.

The genus of *Nepeta* represents 67 species, which are found wild in different regions of Iran (9). *Nepeta binaludensis* Jamzad is an endemic and rare perennial aromatic herb belonging to the Lamiaceae which grows in a limited area in the Binalud mountains in northeast Iran (5). This plant is widely used in traditional medicine as an antispasmodic, nervine, and diuretic (10). Eucalyptol is the main component of its essential oil. Over-exploitation and unsustainable herbal collection of this plant by local gatherers and the limited abundance in the habitat make the plant an endangered species (10).

The objective of the present research was to study the domestication performance of *N. binaludensis* as a method to conserve the wild species. In the domestication process, the effect of the agronomical factors fertilization, irrigation and row spacing on growth, yield and chemical components of this plant should be determined.

Abstract

Nepeta binaludensis Jamzad is a rare and highly endangered ethnomedicinal plant of the Lamiaceae family which grows in the northeast of Iran. A field experiment was conducted with biennial cultivation of the plant in 2006 and 2007 at the University of Mashhad to evaluate its domestication performance. Different experiments were set up to clarify the effects of cultivation on the establishment, phenology, yield and the morphological and phytochemical properties of the plant. These experiments included irrigation intervals (7, 14, 21 and 28 days), cow manure application (10, 20, 30 and 40 t/ha) and row spacing of 50 and 75 cm and in-row spacing of 25 and 50 cm. The results indicated that *N. binaludensis* can be well established in an agricultural system. The highest biomass (1 105 $g \cdot m^{-2}$), plant height (50 cm), plant diameter (48 cm), essential oil yield (5.1 $g \cdot m^{-2}$) and 4aα,7α,7aα-nepetalactone concentration (14.8 %) were observed at an irrigation interval of 7 days in the second year of the study. The highest amount of eucalyptol (1,8-cineole) was obtained at an irrigation interval of 21 days in both years of the study. The effects of cow manure on biomass, essential oil content and chemical components were not statistically significant. The whole growth period of *N. binaludensis* was nearly 206–214 days which was equivalent to 2 792-2 826 growing degree days (GDD). Plant distances did not affect the biomass and essential oil content significantly.

Keywords

Domestication, essential oil, irrigation, *Nepeta binaludensis,* organic fertilization, spacing, yield

Material and Methods

Plant material

Ripe seeds of *N. binaludensis* Jamzad were collected from its native habitat in the Binalud Mountains, Dowlat Abad (latitude: 36°, 18'N; longitude: 58°, 5' E), in the Northeast of Iran. The seeds were placed in a mixture of 2 peatmoss, 2 sand and 1 loamy soil (v/v) and grown in a glasshouse of the Ferdowsi University of Mashhad at 16 h light and 8 h dark, 24±2 °C day/15±2 °C night, conditions. A mist irrigation system was used. The two months old plants with a height of 12–15 cm in the 8–12 leaves stage were used for farm experiments after one week of hardening outside the greenhouse in the shade. They were cultivated on the farm in the mid of May 2006.

Local conditions

Three field experiments were carried out in the growing seasons 2006 and 2007 at the experimental station of the College of Agriculture, Ferdowsi University of Mashhad (latitude: 36°, 15' N, longitude: 59°, 28' E, altitude: 928 m). The climate in Mashhad is semidry and precipitation occurs from November to May. The annual mean air temperatures in 2006 and 2007 were 15.4 °C and 14.6 °C respectively. The annual rainfalls in 2006 and 2007 were 279.8 mm and 153 mm respectively. Soil samples were taken at the beginning of May with an auger (core size of 80 mm) from a depth of 0–15 cm and 15–30 cm to analyze the physico-chemical properties. The experimental soil was a loamy soil with pH 7.4, EC 2.8 dS·m^{-1}, total N 33.5 ppm, P 4.3 ppm and K 212.7 ppm.

Cow manure experiment

The experimental design was randomized in complete blocks with three replicates where the plot size was 4 m^2. Four levels of cow manure (10, 20, 30 and 40 t/ha) were applied in the mid of December in 2005 and 2006. The physico-chemical properties of the used cow manure were pH 8.1, P_2O_5 0.8 kg·t^{-1}, K_2O 3.4 kg·t^{-1}, N total 11.08 kg·t^{-1} and organic carbon 54%. In order to prevent the lateral spread of water, plots were surrounded by dikes with a distance of 2 m. Young plants at a height of 15 cm and in the 8–12 leaves stage were planted on 17 May 2006 into 50 cm wide rows at a 40 cm plant to plant spacing. The young plants were irrigated immediately after planting and a weekly irrigation interval (51 mm at each irrigation time) was used. Weeds were controlled by hand when needed. The whole plants were cut at full flowering on 18 August 2006 and on 17 May and 18 September 2007.

Irrigation regime experiment

The field experiment was conducted as a complete randomized block design with four irrigation treatments (irrigation in 7, 14, 21 and 28 days intervals) and three replicates. 25 t·ha^{-1} cow manure were applied in the mid of December in 2005 and 2006. Plots (4 m^2) were irrigated uniformly (51 mm per application) up to field capacity in each irrigation treatment. Furrows in each plot were irrigated by a hose (4 cm diameter) with a counter on it. In order to prevent the lateral spread of water, each plot was surrounded by dikes with a distance of 2 m. Young plants were transplanted on 17 May 2006 into 50 cm wide rows at a 40 cm plant to plant spacing. The young plants were irrigated immediately after transplanting, and irrigation was applied every week. Irrigation treatments started on 24 June in the first year (after establishment of the young plants) and 28 April in the second year (after three preceding irrigations). The whole plants were harvested at full flowering by one cut on 18 August 2006 and by two cuts on 17 May and 18 September 2007.

Plant row arrangement experiment

A two-factorial experiment based on a randomized complete block design with three replicates was set up with all combinations of row spacing of 50 and 75 cm and in-row spacing of 25 and 50 cm. In 2006, 25 t·ha^{-1} cow manure were applied in the mid of December. The plants were transplanted to the field on 13 April 2007, followed by immediate irrigation, and then further irrigation every week (51 mm per application). Plants were cut on 6 July 2007 at full flowering. No data is available for 2006 because of total plant damage by hail.

Observations

Plant height and plant diameter (the area a plant covers) were recorded at different times. For this purpose, five plants were randomly selected from each plot and marked. To record the fresh and dry biomass yield, 1 m^2 was randomly selected from each plot and plants were cut just above the lignified stem parts at the full flowering stage. The samples were weighed before and after drying. Drying was conducted at 55 °C for 72 hours using a Binder lab heater.

To record the development stages (*Tab. 1*) on a weekly basis, five plants were selected and marked randomly from each plot. The developmental stages were determined when 50 % of all plants in each treatment reached that stage.

Phenological stages were recorded and Growing Degree Days (GDD) were also

Erste Versuche zum Anbau von *Nepeta binaludensis* Jamzad – ein Beispiel für die Inkulturnahme einer stark gefährdeten Arzneipflanze in Iran

Zusammenfassung

Nepeta binaludensis Jamzad ist eine seltene ethnomedizinisch genutzte Pflanze aus der Familie der Lamiaceae, die in der Provinz Khorasan im Nordosten Irans vorkommt. In den Jahren 2006 und 2007 wurde in der Forschungsstation der Agrarhochschule Ferdowsi der Universität Mashhad ein zweijähriger Feldversuch durchgeführt, um die Möglichkeit der Inkulturnahme von *N. binaludensis* zu untersuchen. Um den Einfluss agronomischer Bedingungen auf die Etablierung, den Entwicklungsverlauf, den Ertrag sowie auf die morphologischen und phytochemischen Eigenschaften der Pflanze zu prüfen, wurden ein Bewässerungsversuch (7, 14, 21 und 28 Tage Bewässerungsintervall), ein Düngeversuch mit Wirtschaftsdünger (10, 20, 30 und 40 t Rinderdung/ha) und ein Standraumversuch (Reihenweite: 50 cm und 75 cm, Pflanzenabstand in der Reihe: 25 und 50 cm) durchgeführt. Die Ergebnisse zeigten, dass diese Wildpflanzenart gut in einem landwirtschaftlichen System etabliert werden kann. Die höchsten Biomasseerträge (1 105 g·m^{-2}), Pflanzenlängen (50 cm), Pflanzendurchmesser (48 cm), Erträge an ätherischem Öl (5,1 g·m^{-2}) und Gehalte an 4aα,7α,7aα-Nepetalacton (14,8 %) wurden bei einem Bewässerungsintervall von 7 Tagen im zweiten Jahr erreicht. Der höchste Gehalt an Eukalyptol (1,8-Cineol) lag bei einem Bewässerungsintervall von 21 Tagen in beiden Studienjahren vor. Die Anwendung von Rinderdung hatte keinen statistisch gesicherten Einfluss auf die Prüfmerkmale. Die Länge der Wachstumsperiode von *N. binaludensis* betrug 206 bis 214 Tage, was 2 792 bis 2 826 Wachstumsgradtagen entspricht. Die unterschiedliche Standraumzumessung der Pflanzen hatte keinen Einfluss auf die Biomasse und auf den Gehalt an ätherischem Öl.

Schlagwörter

Ätherisches Öl, Bewässerung, Ertrag, Inkulturnahme, organische Düngung, *Nepeta binaludensis*, Standraum

Tab.1: Definition of phenological stages in *Nepeta binaludensis*
Tab. 1: Definition der Entwicklungsstadien von *N. binaludensis*

Phenological stages (Entwicklungsstadien)	**Definition** (Definition)
Initial growth	Growth of new stems from the base of the plant
Budding	One flower bud was observed
Start of flowering	One open flower was observed
Full flowering	100 % of the plants were in flowering
Start of seed setting	Visible brownish seeds were observed
Full seed set	Dark brown seeds were observed in whole parts of spikes. Leaves were completely yellow.
Fully mature	Plants were completely dried

calculated for every stage on climatology data using the following equation: Growing Degree Days (GDD) (18):
$GDDs = \Sigma (T_{max} + T_{min}/2 - T_b)$
with GDDs = Growing Degree Days, T_{max} and T_{min}= daily maximum and minimum temperatures, and T_b= base temperature.
The considered base temperatures for seed germination and initial growth of plants were 8 °C and 12 °C, respectively (11).

Phytochemical study
Essential oils were obtained from dried above-ground parts (complete plant at flowering stage) by hydro-distillation for 3 hours, using a clevenger-type apparatus (3). Then the slightly yellow oils were separated, dried over anhydrous sodium sulphate and stored in sealed containers under N_2 atmosphere and freezing (-20 °C). The essential oils were analyzed for the qualitative and quantitative identification of their components by gas chromatography (GC) and GC-mass spectrometry (MS).
The GC analyses were performed using a Thermoquest-Finnigan Trace Gas Chromatograph equipped with a flame ionization detector (FID). Separations were achieved on a DB-1 fused silica column (30 m·0.25 mm i.d., film thickness 0.25 μm; temperature programming 60–250 °C at 4 °C/min; injector temperature 250 °C; detector temperature 280 °C; carrier gas N_2 at a rate of 1.1 ml·min^{-1}).
GC/MS analyses were carried out with a Thermoquest-Finnigan Trace GC fitted with a DB-1 fused silica column (60 m·0.25 mm i.d., 0.25 μm film thickness) interfaced with a Thermo-Finnigan quadruple mass detector and a computer equipped with Wiley 7 and Nist1.7 spectra libraries. Column temperature was 60–250 °C at 5 °C·min^{-1}; injector temperature was 250 °C, volume injection was 0.1 μl, split ratio was 1:50, carrier gas was helium at 1.1 ml·min^{-1} with an ionization voltage of 70 eV, ionization current was 150 μA, ion source temperature was 250 °C and mass range was from 35–465 amu.
The amount of the individual compounds was computed from the GC peak areas without using correction factors. The oil components were identified by their GC retention indices, obtained with reference to a n-alkenes series on a DB-1 column, by comparison of their mass spectra and fragmentation patterns (1) in relation to previously published data on essential oil components of this species (16) and by computer matching with Wiley 7 and Nist1.7 Mass Spectral Database.

Statistical analyses
The data were analyzed by one-way ANOVA using the statistical analysis system (SAS) (2000) and means were compared by Duncan's multiple range test at the 5 % probability level.

Results and Discussion

Essential oil composition
The compound 1,8-cineole was found to be the major component of the essential oil of *N. binaludensis* followed by 4aα,7α,7aα-nepetalactone (*Tab. 2*). Similar results were reported by Rustaiyan and Nadji (16) for *N. binaludensis* harvested from the natural habitat, while Nadjafi et al. (10) reported 1,8-cineol as the main essential oil compound of wild gathered *N. binaludensis*; they did, however, not find nepetalactone among the essential oil components.

Effect of manure application
The application of different cow manure levels had no significant effect on the yield, essential oil content and oil composition, plant height and plant diameter of *N. binaludensis* in two research seasons (*Tab. 3* and *4*). *N. binaludensis* grows in its wild habitats in poor soils with low nutrient availability which can explain its behavior under farm conditions (10). Efficient utilization of animal manure requires thorough understanding of the relationship between crop responses and availability of nutrients in the soil. Most studies about the effect of manure are conducted with crops which are selected for high input cropping systems; it seems that wild species do not respond in the same way to fertilization because of their adaptation to low nutrient conditions. That is, for instance, the case in *Curcuma aromatica* Salisb., a wild medicinal species in India, which did not significantly respond to different levels of manuring in yield, essential oil content and plant ingredients (19).

Tab. 2: Composition (%) of the essential oils from the aerial parts of *N. binaludensis* cultivated under field conditions
Tab. 2: Zusammensetzung (%) des ätherischen Öls in der oberirdischen Biomasse von *N. binaludensis*, angebaut unter Feldbedingungen

Compounds (Komponenten)	RI*	Percentage (Anteil)
α-thujene	929.0	0.1
α-pinene	938.2	1.3
sabinene	966.2	0.1
β-pinene	977.9	4.5
myrcene	981.3	0.1
p-cymene	1 018.0	0.3
1,8-cineole	1 033.0	63
γ-terpinene	1 050.0	0.3
linalool	1 080.0	0.7
trans-hydrate sabinene	1 106.0	0.0
trans-carneol pino	1 125.0	0.1
pinocavon	1 140.0	0.1
α-terpineol	1 148.0	2.2
borneol	1 151.0	0.0
p-cymene-8-ol	1 405.0	1.4
alchol fenchyl	1 176.0	5.3
4-acetyl-1-methtyl-cycloe	1 188.0	0.4
cuminaldehyde	1 212.0	0.0
carvacrole	1 276.0	0.0
4aα,7α,7aα nepetalactone	1 338.0	19
4ab,7α,7ab nepetalactone	1 361.0	0.3
lactone dyhydronepeta	1 381.0	0.1
germacrene d	1 475.0	0.1

* RI = The retention Kovats indices were determined on DB-1 fused silica column.
(* RI =Retentionsindex, dieser wurde mit einer Quarzglassäule (DB-1) bestimmt.)

Tab. 3: Effect of different levels of cow manure application on herbage yield, essential oil content and components of *N. binaludensis*
Tab. 3: Einfluss unterschiedlicher Mengen an Rinderdung auf den Krautertrag sowie auf Gehalt und Zusammensetzung des ätherischen Öls von *N. binaludensis*

Manure amounts (Stalldunggaben) (t/ha)	Herbage wet yield (Krautertrag, frisch) (g·m^{-1})		Herbage dry yield (Krautertrag, trocken) (g·m^{-1})		Essential oil (Äther.Öl) (%)		Essential oil yield (Äther. Ölertrag) (g·m^{-1})		1,8-cineole (%)		4aα,7α,7aα nepetalactone (4aα,7α,7aα-Nepetalacton) (%)	
	Year (Jahr)											
	1	2	1	2	1	2	1	2	1	2	1	2
10	1060a	6187a	293a	1769a	0.4a	0.6a	1a	10a	72a	70a	3a	9a
20	1186a	5589a	292a	1530a	0.4a	0.7a	1a	12a	72a	60a	4a	16a
30	1130a	5955a	302a	1803a	1.0a	0.6a	3a	8ab	71a	71a	4a	8a
40	911a	5302a	320a	1451a	0.8a	0.7a	2a	5ab	70a	65a	4a	13a

Mean values followed by the same letter are not significantly different at $p \leq 0.05$
(Mittelwerte mit denselben Buchstaben unterscheiden sich nicht signifikant bei $p \leq 0.05$)

Tab. 4: Effect of manure application levels on plant height and plant diameter (cm) of *N. binaludensis* at different recording times
Tab. 4: Einfluss unterschiedlicher Stalldunggaben auf Pflanzenlänge und Pflanzendurchmesser (cm) von *N. binaludensis* bei unterschiedlichen Messterminen

Manure amounts (Stalldunggaben) (t/ha)	Date of recording (Termine der Datenerfassung)											
	First year (erstes Jahr)						Second year (zweites Jahr)					
	30 June		19 July		17 August		30 April		16 May		17 September	
	H*	D**	H	D	H	D	H	D	H	D	H	D
10	22.5ab	24.4a	31.9a	40.2a	52.4a	59.4a	30.5a	43.3a	51.0a	69.5a	48.6a	45.6a
20	23.4ab	25.1a	33.6a	39.7a	49.6a	59.6a	34.7a	48.7a	59.1a	69.0a	54.9a	51.6a
30	25.5a	29.0a	36.4a	43.4a	44.2a	59.7a	33.2a	48.8a	51.8a	70.0a	54.0a	50.7a
40	20.5b	24.1a	29.5a	38.4a	47.1a	52.1a	23.3a	44.1a	58.0a	65.4a	45.1a	47.3a

*H = height, D** = Diameter, (*H = Wuchshöhe, D** = Durchmesser)
Mean values followed by the same letter are not significantly different at $p \leq 0.05$. Mean values comparing is done separately for each recording date. Important days: 10 May 2006 – first year, planting of seedlings, 18 August 2006 – harvesting, 11 April 2007 – second year, initial growth, 12 May 2007 – first cut, 18 September – second cut.
(Mittelwerte mit denselben Buchstaben unterscheiden sich nicht signifikant bei $p \leq 0,05$. Der Vergleich der Durchschnittswerte erfolgt separat für jeden Datenerfassungstermin. Wichtige Termine: 10. Mai 2006 – erstes Jahr, Auspflanzen der Jungpflanzen, 18. August 2006 - Ernte, 11. April 2007 – zweites Jahr, Wachstumsbeginn, 12. Mai 2007 – erster Schnitt, 18. September 2007 – zweiter Schnitt.)

Tab. 5: Effect of different irrigation regimes on herbage yield, essential oil content and components of *N. binaludensis*
Tab. 5: Einfluss unterschiedlicher Bewässerungsregime auf Krautertrag sowie Gehalt und Zusammensetzung des ätherischen Öls von *N. binaludensis*

Irrigation regimes (Bewässerungsregime) (Days) (Tage)	Herbage wet yield (Krautertrag, frisch) (g/m^2)		Herbage dry yield (Krautertrag, trocken) (g/m^2)		Essential oil (Äther. Öl) (%)		Essential oil yield (Äther. Ölertrag) (g/m^2)		1,8-cineole (%)		4aα,7α,7aα nepetalactone (4aα,7α,7aα-Nepetalacton) (%)	
	Year (Jahr)											
	1	2	1	2	1	2	1	2	1	2	1	2
7	1243a	4429a	327a	1105a	0.7a	0.5b	2a	5a	67c	58a	7a	15a
14	749b	2729a	206b	955a	0.4a	0.4b	1b	4ab	74ab	67a	3a	8b
21	703b	2213b	194b	628ab	0.6a	0.5b	1b	3ab	75a	68a	2b	7b
28	584b	1472b	171b	316b	0.5a	1.0a	1b	2b	70b	61a	5ab	12b

Mean values followed by the same letter are not significantly different at $p \leq 0.05$.
(Mittelwerte mit denselben Buchstaben unterscheiden sich nicht signifikant bei $p \leq 0.05$.)

Effect of irrigation regimes

The irrigation regimes had significant effect on the herbage yield of *N. binaludensis* (*Tab. 5*). The highest herbage yield was obtained under irrigation regimes of 7 days in both years. In the first year, there was no significant difference between the irrigation intervals of 14, 21 and 28 days, probably because of the late application date. In the second year, the herbage yield decreased significantly if irrigation was applied in intervals >14 days. It became apparent that *N. binaludensis* benefited from a higher soil water content in terms of biomass production and plant ingredients. This result confirms studies (10) in the natural habitat which reported that this plant mainly grows on slopes facing the north, with less sunlight and therefore lower evaporation. Other *Nepeta* species, which are growing in Tehran Province, also show a comparatively high water demand and are found in habitats with more than 400 mm annual rainfall (13).

The effect of irrigation regimes on essential oil content was not significant in the first season, but the significantly highest amount was observed with an irrigation interval of 28 days in the second season of the experiment (*Tab. 5*). An increase of essential oil percentage with water scarcity is reported in other plants like *Thymus vulgaris* (7, 15) and *Hyssopus officinalis* (7).

But the highest essential oil yield, which is an economical index for essential oil production in a country, was observed at an irrigation interval of 7 days (*Tab. 5*), probably because of the higher herbage yield under this irrigation regime. It is reported (6) that irrigation intervals of 7 and 14 days significantly increased the number of branches per plant, herbage yield and essential oil yield of rosemary (*Rosmarinus officinalis* L.) compared to a 21 days irrigation interval.

The lowest and highest amount of 1,8-cineole was achieved at irrigation intervals of 7 and 21 days, respectively, but the differences between treatments were not significant in the second year of the study (*Tab. 5*). For rosemary, water scarcity (irrigation interval of 21 days) was reported to increase the 1,8-cineol content (6), but we have seen the clear trend in *N. binaludensis* only in the first season of the study.

There was a significant difference between the 4aα,7α,7aα-nepetalactone content with respect to the irrigation interval in both years, and the highest amount of this component was observed at an irrigation interval of 7 days in both years of the study. It seems that adequate irrigation can increase these components which are important for pharmaceutical use.

The highest plant height and plant diameter were observed at an irrigation interval of 7 days (*Tab. 6*). Plants became smaller as irrigation intervals increased. These results showed that the growth of this species requires a sufficient water supply. But if the objective of cultivation is to achieve an essential oil with a higher 1,8-cineole content, the irrigation interval should be increased.

Harvesting times

The results of the irrigation and manure experiments showed that one harvest in the first year of the study (18 August) and two harvests (12 May and 18 September) in the second year of the study in the full flowering stage can be performed. It is reported (12) that in thyme (*T. vulgaris* L.), one cut in the first year and 2–3 cuts in the second year in the full flowering stage can be achieved. The current results indicated that the herbage yield, essential oil percentage and essential oil yield were higher in the second cut but the difference was not significant (data not shown). Similar to this result, the highest herbage yield and essential oil percentage of sage (*Salvia officinalis* L.) were obtained in summer cuts compared to spring cuts by Zutic et al. (22). The higher essential oil content in summer cuts were probably related to higher temperatures and long-day conditions.

Plant age

The two years old plants in the irrigation and manure experiments had a higher herbage yield, essential oil yield, and nepetalactone content compared to the one year old plants, but the 1,8-cineole content was higher in the one year old plants (*Tab. 7*). It is reported (15) that 3 years old *T. vulgaris*

Tab. 6: Effect of irrigation regimes on height and diameter of *N. binaludensis* at different recording times

Tab. 6: Einfluss unterschiedlicher Bewässerungsregime auf Höhe und Durchmesser (cm) von *N. binaludensis* zu unterschiedlichen Messterminen

Irrigation regimes (Bewässerungsregime) (Days) (Tage)	Date of recording (Messtermine)									
	First year (erstes Jahr)				Second year (zweites Jahr)					
	19 July		17 August		30 April		16 May		17 September	
	H*	D**	H	D	H	D	H	D	H	D
7	33a	35a	50a	57a	30a	46a	54a	71a	50a	48a
14	32a	33ab	45a	45b	24ab	37ab	42ab	56b	45ab	37ab
21	32a	29ab	43ab	49ab	25ab	40ab	46ab	63ab	33bc	35ab
28	26ab	26b	37b	43b	20b	31b	37b	54b	26c	24b

*H = height, D** = Diameter, (*H = Wuchshöhe, D** = Durchmesser)

Mean values followed by the same letter are not significantly different at $p \leq 0.05$. Mean values comparing are done separately for each recording date. Important days: 10 May 2006 – first year, planting of seedlings, 18 August 2006 – harvesting, 11 April 2007 – second year, initial growth, 12 May 2007 – first cut, 18 September – second cut.

(Mittelwerte mit denselben Buchstaben unterscheiden sich nicht signifikant bei $p \leq 0,05$. Der Vergleich der Durchschnittswerte erfolgt separat für jeden Datenerfassungstermin. Wichtige Termine: 10. Mai 2006 – erstes Jahr, Auspflanzen der Jungpflanzen, 18. August 2006 – Ernte, 11. April 2007 – zweites Jahr, Wachstumsbeginn, 12. Mai 2007 – erster Schnitt, 18. September 2007 – zweiter Schnitt.)

Tab. 7: Effect of plant age on herbage yield, essential oil yield and content of 1,8-cineole and 4aα,7α,7aα-nepetalactone in farm experiments on irrigation intervals and manure use

Tab. 7: Einfluss des Pflanzenalters auf Krautertrag, Ertrag an ätherischem Öl und Anteil an 1,8-Cineol und 4aα,7α,7aα-Nepetalacton in Versuchen zur Bewässerung und zur Düngung mit Stalldung

Experiment (Versuch)	Plant age (Pflanzenalter) (Years) (Jahre)	Herbage yield (Krautertrag) ($g \cdot m^{-2}$)	Essential oil yield (Äther. Ölertrag) ($g \cdot m^{-2}$)	1,8-cineole (%)	4aα,7α,7aα-nepetalactone (4aα,7α,7aα-Nepetalacton) (%)
Manure (Stalldung)	1	279.2b	1.8b	71.8a	3.7b
	2	1729.9a	9.3a	65.1b	11.6a
Irrigation (Bewässerung)	1	224.5b	1.4b	71.7a	4.5b
	2	751.3a	3.8a	63.7b	10.6a

Mean values followed by the same letter are not significantly different at $p \leq 0.05$

(Mittelwerte mit denselben Buchstaben unterscheiden sich nicht signifikant bei $p \leq 0,05$)

L. plants had a higher herbage and essential oil yield in comparison to the one and two years old plants. The effect could be related to the full plant establishment and fully-developed root system and therefore better water and nutrient uptake ability of older plants.

Phenology

N. binaludensis plants needed nearly 83 days, equivalent to 996 GDD, from seeding to transplanting into the field (*Tab. 8* and *9*). The initial growth of the plant in the first year was very slow which is common in the Labiatae family. Flowering started 70 days after transplanting in the first year of establishment (at the end of July). In the second year, plant growth started at the end of April, and flowering started at the beginning of May. If plants are harvested in the mid of May they will probably produce flowering shoots again in the mid of September. It is observed (8) that *T. vulgaris* plants grew slowly in the first year of establishment and started to flower at the end of July; from the second year onwards growth started early in spring (March), and plants flowered already from mid May to mid June. If the plants are cut at this time, the new shoots produce flowers until the end of August to early September. Seeds of *N. binaludensis* matured in late July and plants died off by mid November. The whole growth period of this species was nearly 206 days under farm conditions, equivalent to 2 792 GDD in the first year, and 214 days equivalent to 2 826 GDD in the second year of establishment.

Our investigations showed that manure application and irrigation regimes did not affect the development of the plant in the first year of plant establishment, but water stress (28 days irrigation interval) accelerated seed maturing, so that the plant died off in the mid of August. It is reported (4) that irrigation had no effect on the duration of plant development of *Nigella sativa* L. and *Plantago ovata* L. until seed formation. The differing effect of irrigation treatments on developmental duration became visible at seed formation and thus reduced the time from seed formation to maturity.

Effect of row arrangement

Row distances did not affect herbage yield, essential oil content and essential oil yield of *N. binaludensis* (*Tab. 10*). The effect of in-row distances on herbage yield and essential oil yield was significant, and the highest herbage and oil yields were obtained at the lower distance (25 cm). Essential oil content was not affected by in-row distances. The increase of plant density can have a positive effect on crop yield by decreasing weeds through higher competition of the crop, by covering the soil and also by better use of light and nutrients resulting in a higher dry matter yield. Results of experiments with thyme indicated that different row spacings had no significant effect on the oil content, but the oil yield significantly increased under lower row spacing, because of an increase in total dry matter yield (12). There was no interaction between in-row and inter-row distances. Nevertheless, the limited water supply in a semiarid environment could set a limit to the yield potential of the plant individuals. In these environments, the choice of the optimum distance between rows

Tab. 8: Phenological stages of *N. binaludensis* under field conditions (first year of life)
Tab. 8: Entwicklungsstadien von *N. binaludensis* unter Feldbedingungen (erstes Vegetationsjahr)

Phenological stage (Entwicklungsstadium)	Seed planting – seedling transfer (Aussaat – Pflanzung)	Seedling transfer – vegetative growth (Pflanzung – vegetatives Wachstum)	Vegetative growth – start of flowering (Vegetatives Wachstum – Blühbeginn)	Start of flowering – full flowering (Blühbeginn – Vollblüte)	Full flowering – start of seed setting (Vollblüte – Beginn Samenbildung)	Start of seed setting – full seed set (Beginn Samenbildung – Ende der Samenbildung)	Full seed set – final stage (Ende der Samenbildung – Vollreife)
Date (Datum)	17 February – 10 May	10 May – 30 June	30 June – 19 July	19 July – 1 August	1 August – 18 August	18 August – 20 October	20 October – 30 November
Number of days (Anzahl der Tage)	83	51	19	13	17	63	43
GDD*	996	829.4	341.7	269.3	325.3	818.6	207.8

*GDD, Growth degree days (Wachstumsgradtage

Tab. 9: Phenological stages of *N. binaludensis* under field conditions (second year of life)
Tab. 9: Entwicklungsstadien von *N. binaludensis* unter Feldbedingungen (zweites Vegetationsjahr)

Phenological stage (Entwicklungsstadium)	Initial growth – budding (Wachstumsbeginn – Knospenstadium)	Budding – flowering (Knospenstadium – Blüte)	Flowering – start of seed setting (Blüte – Beginn der Samenbildung)	Start of seed setting– full seed set (Beginn der Samenbildung – Ende der Samenbildung)	Full seed set – final stage (Ende der Samenbildung – Vollreife)
Date (Datum)	11 April – 27 April	27 April – 2 May	12 May – 5 June	5 June – 31 July	31 July – 11 November
Number of days (Anzahl der Tage)	16	15	24	56	103
GDD*	171.8	138.3	294.1	1029.6	1192.4

*GDD, Growth degree days (Wachstumsgradtage)

Tab. 10: Effect of different row and in-row distances on herbage yield and essential oil content of *Nepeta binaludensis*
Tab. 10: Einfluss unterschiedlicher Reihenweiten und Pflanzenabstände in der Reihe auf den Krautertrag und den Gehalt an ätherischem Öl von *Nepeta binaludensis*

Row distance (Reihenabstand) (cm)	Herbage wet yield (Krautertrag, frisch) ($g \cdot m^{-2}$)	Herbage dry yield (Krautertrag, trocken) ($g \cdot m^{-2}$)	Essential oil (Ätherisches Öl) (%)	Essential oil yield (Äther. Ölertrag) ($g \cdot m^{-2}$)
50	704.7a	209.3a	0.8a	1.8a
75	634.5a	168.6a	0.8a	1.3a
In-row distance (Abstand in der Reihe) (cm)				
25	862.4a	243.0a	0.8a	2.0a
50	505.8b	134.9b	0.8a	1.1a

Mean values followed by the same letter are not significantly different at $p \leq 0.05$
(Mittelwerte mit denselben Buchstaben unterscheiden sich nicht signifikant bei $p \leq 0,05$)

is therefore particularly important to save water for the crops. So one single experimental year cannot show the real effect of plant density and plant arrangement on growth parameters and yield of a perennial plant like *Nepeta* and more research should be done to achieve a better understanding of the plant reaction to density.

Conclusion

This research is the first study on the cultivation of *Nepeta binaludensis* Jamzad, an endemic and rare medicinal plant of Iran which is highly endangered due to increased harvesting and unsuitable collection practices in the wild habitat. This research provided basic information on the establishment, growing, phenology and essential oil content of this plant under agronomical factors like irrigation, manure application and planting arrangement outside its wild habitat. Results showed that this plant can be cultivated and established successfully in artificial conditions outside its natural habitat with an acceptable herbage yield and essential oil quality. This plant can be cultivated in poor soils with low nutrient content but it is a plant that requires a sufficient water supply. To achieve higher yields of herb and essential oil, lower irrigation intervals are necessary. For economical cultivation of this plant, more research in different aspects such as harvesting techniques, planting dates, fertilization, post harvest processing, economic evaluation, etc is required; the studies are already in progress.

References

1. Adams RP. Identification of Essential Oil Components by Gas Chromatography-Mass Spectrometry. Carol Stream, IL: Allured; 1995.
2. Akbarinia A, Sefidkon F, Bagher Rezaie V, Bakhsh Khaniki GH. Study the essential oil of *Nepeta pogonosperma* in natural and controlled conditions. In: Abstract book of the National Iranian Conference of Medicinal Plants. Research Institute of Forest and Rangelands, Tehran, Iran (in Persian); 2001.
3. British Pharmacopeia. Vol.2, London: HMSO; 1998. p. A137–A138.
4. Bannayan M, Nadjafi F, Azizi M, Tabrizi L, Rastgoo M. Yield and seed quality of *Plantago ovata* and *Nigella sativa* under different irrigation treatments. Ind Crops Prod 2008;27:11–16.
5. Ghahreman A. Biodiversity of Plants in Iran. Tehran University, Iran (text in Persian); 1999.
6. Kandeel AM. Effect of irrigation intervals on the growth and active ingredients of *Rosmarinus officinalis* L plants. Arab Univ J Agric Sci 2001;9(2):825–838.
7. Khazaie HR, Nadjafi F, Bannayan M. Effect of irrigation frequency and planting density on herbage biomass and oil production of thyme (*Thymus vulgaris*) and hyssop (*Hyssopus officinalis*). Ind Crops Prod 2008;27:315–321.
8. Letchamo W, Gosselin, A. Variation in photosynthesis and essential oil in Thyme. J Plant Physiol 1995;147:29–37.
9. Mozaffarian VA. A Dictionary of Iranian Plant Names. Research Institute of Forest and Rangelands, Tehran, Iran; 1996.
10. Nadjafi F, Koocheki A, Honermeier B, Asili J. Autecology ethnomedicinal and phytochemical studies of *Nepeta binaludensis* Jamzad a highly endangered medicinal plant of Iran. JEOBP 2009;12(1), 97–110.
11. Nadjafi F, Koocheki A, Rezvani Moghadam P, Rastgoo M. Evaluation of seed germination characteristics in *Nepeta binaludensis*, a highly endangered medicinal plant of Iran. Agric Sci Technol 2007;2:385–392.
12. Naghdi Badi H, Yazdani D, Ali SM, Nazari F. Effect of spacing and harvesting time on herbage yield and quantity/quality of oil in thyme, *Thymus vulgaris* L. Ind Crops Prod 2004;19(3):231–236.
13. Najaf-Purnavai M. Ecological investigation of some aromatic plants belonging to *Mentha*, *Nepeta* and *Thymus* genus in Tehran province. Iran Med Plants Res 2000;25:1–5.
14. Pushpangadan P. On conservation biology, domestication and commercial cultivation of wild medicinal and aromatic plants. In: Raych and Huri (eds). Recent Advances in Medicinal, Aromatic and Spice Crops. New Delhi: Today and, Tomorrows Printers & Publishers.1992;2:431–436.
15. Omidbaigi R. Study on aspects of thyme production and processing of its active substances. Pajouhesh-va-Sazadegi J 1998;36:67–71.
16. Rustaiyan A, Nadji K. Composition of the essential oils of *Nepeta isphanica* Boiss and *Nepeta binaludensis* Jamzad from Iran. Flavour Frag J 1999;14:35–37.
17. Schippmann U, Leaman D, Cunningham B. Impact of cultivation and gathering of medicinal plants on biodiversity: global trends and issues. In: Biodiversity and the Ecosystem Approach in Agriculture, Forestry and Fisheries. Satellite event on the occasion of the Ninth Regular Session of the Commission on Genetic Resources

for Food and Agriculture. FAO, Rome, 12-13 October 2002. Inter Department Working Group on Biological Diversity for Food and Agriculture.
18. Synder RL. Hand calculating degree days. J Agric Meteorol 1985;35:353–358.
19. Singh GRD, Meena RL, Singh MK, Kaul V, Lal B, Acharya, R, Parsad M. Effect of manure and plant spacing on crop growth, yield and oil-quality of *Curcuma aromatica* Salisb. in mid hill of western Himalaya. Ind Crops Prod 2006;24,105–112.
20. SAS Institute. SAS System, 8th ed. SAS Inst., Cary, NC.; 2001.
21. WHO, IUCN & WWF Guidelines on the Conservation of Medicinal Plants. Geneva, Switzerland;1993.
22. Zutic I, Putievsky E, Dudai N. Influence of harvest dynamics and cut height on yield components of Sage (*Salvia officinalis* L.). J Herbs, Spices Med Plants 2003;10(4):291–293.

Addresses of the authors

F. Nadjafi[1], A. Koocheki[2], P. Rezvani Moghadam[2] and B. Honermeier[3]

[1]Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, G.C. Tehran, Iran.

[2]College of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran.

[3]Institute of Agronomy and Plant Breeding, Justus Liebig University Giessen, Ludwigstr. 23, D-35390 Giessen, Germany.

Corresponding Author:

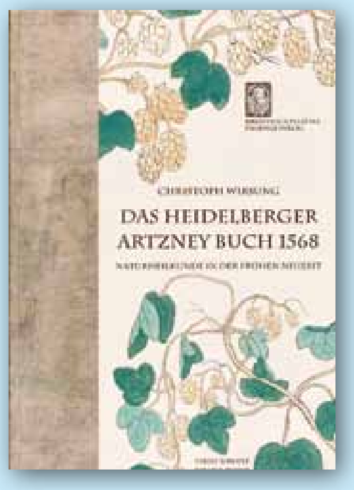
Dr. Farsad Nadjafi, Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, Tehran, Iran. Phone-Fax: +98-21-22431783 Email: f-najafi@sbu.ac.ir

Received: 01 December 2010
Accepted: 28 February 2011

Buchbesprechung

Wirsung C; Schofer U, Pfister K, Fuchs L. Das Heidelberger Artzney-Buch 1568 des Christoph Wirsung: Naturheilkunde in der frühen Neuzeit

Die Herausgeberinnen haben das von Christoph Wirsung 1568 geschriebene Heidelberger Artzney Buch aus dem Frühneuhochdeutschen ins Neuhochdeutsche übertragen und damit naturheilkundliches Wissen des Mittelalters und des 16. Jahrhunderts einem breiteren Interessentenkreis zugänglich gemacht. Exemplare des Originalbuches befinden sich u.a. in der Bibliotheca Palatina Vaticana in Rom. Der Apotheker Wirsung sammelte im 16. Jahrhundert ca. 15 000 Rezepte und wollte mit dem Druck des Artzney-Buches medizinisches Wissen auch an ärmere Bevölkerungsschichten weitergeben. Er schrieb dieses Buch deshalb nicht, wie sonst in der damaligen Schulmedizin üblich, in lateinischer Sprache, sondern in Deutsch. Der heutige Leser sollte beachten, dass diese Rezepte keine Anleitung zur Selbstmedikation sind, sondern ihre Anwendung in vielen Fällen erhebliche Risiken mit sich bringt. Das Buch ist in acht Teile gegliedert, denen eine ausführliche Einleitung vorangestellt ist, in der u.a. Ursachen von Krankheiten und der Nutzen von Arzneimitteln erläutert und Anleitungen zum Sammeln von Arzneipflanzen mit Hinweisen zur Lagerfähigkeit und Informationen zum Purgieren und zum Aderlass gegeben werden. Die ersten vier Teile sind nach dem Prinzip *„a capite ad calcem"* (vom Kopf zu den Füßen) mit Rezepten für Krankheiten von Kopf bis Hals/Hals bis Diaphragma/Zwerchfell bis Oberschenkel/Arme und Beine strukturiert. Kapitel 5 behandelt Krankheiten der Haut, Adern und Nerven, Teil 6 Fieber, Pest und Trunksucht, Teil 7 Gifte und Gegengifte und Teil 8 gibt Anleitungen zur Herstellung von Arzneimitteln. Dem therapeutisch-praktischen Abschnitt eines jeden Teils sind zunächst theoretische Erläuterungen vorangestellt. Jeder Teil schließt mit umfassenden Empfehlungen für die Lebensführung des Erkrankten ab. Die Rezepte berücksichtigen ca. 440 Stammpflanzen, 82 Drogen tierischen Ursprungs und 86 Mineralien und anorganische Verbindungen. Auf 16 ganzseitigen Farbtafeln werden Zeichnungen von Leonhart Fuchs präsentiert. Ein umfangreiches Glossar der Drogenbezeichnungen gibt Auskunft über heute übliche deutsche Bezeichnungen, botanische Namen und die von Wirsung in der Originalfassung des Buches verwendeten Bezeichnungen. Der raschen Orientierung dienen Register der Drogen, Namen und Orte. Das Faksimile des Heidelberger Artzney-Buchs 1568 ist eine wertvolle



Quelle zur Ergründung mittelalterlicher Naturheilkunde durch die pharmakologische Forschung und gibt auch dem interessierten Laien einen Einblick in die damalige naturheilkundliche Praxis.

Wirsung C, Schofer U, Pfister K, Fuchs L. Das Heidelberger Artzney-Buch 1568 des Christoph Wirsung: Naturheilkunde in der frühen Neuzeit/herausgegeben von Ulrike Schofer und Kathrin Pfister, 806 S., 16 farbige Abb.; Knittlingen: Bibliotheca Palatina Faksimile Verlag; 2011; EUR 135. ISBN 978-3-9811463-2–5.

Dr. F. Pank, f.pank@online.de