

STUDIES ON THE MORPHOLOGY, ANATOMY AND ECOLOGY OF *ANACAMPTIS PYRAMIDALIS* (L.) L. C. M. RICHARD (ORCHIDACEAE) IN TURKEY

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Abstract

Morphological, anatomical and ecological characteristics of *Anacamptis pyramidalis* (L.) L. C. M. Richard in Turkey were investigated in this study. Plant materials of *A. pyramidalis* were provided from 21 native populations between 2007 and 2009 in Turkey, and their localities were recorded. *A. pyramidalis* samples were evaluated within 19 morphological, 20 anatomical, and 18 soil characters & habitat properties. The findings of this study are as follows: The plant length was minimum 248 mm and maximum 655 mm, underground part length minimum 23 mm and maximum 140 mm, and number of leaves change between 2 and 14. The leaves were tetrastichous, had no trichomes. In surface section of the leaves, the cuticle thickness (abaxial and adaxial), epidermis cell size (abaxial and adaxial) stomata dimensions and stomata index were measured. The epidermal cells' rows were parallel to the midrib. In cross sections of the leaves, upper epidermis was larger than lower epidermis. Vascular bundles were collateral and consist of xylem, phloem and sclerenchyma cells. Raphide bundles were observed in the mesophyll tissue and leaf midrib had lacunas. Chlorenchyma had scattered homogeneously. According to habitat definition of *A. pyramidalis* it grows from sea level to 1600 m whereas the most common habitat of *A. pyramidalis* are meadow, macchie and gaps in the forest lands. *A. pyramidalis* are grown in stony soils and also are rich in clay, and medium in organic carbon. pH of the soils differ from 6.77 to 7.54.

Introduction

The monospecific genus *Anacamptis* (Orchidaceae) was established by the French botanist Louis Claude Marie Richard (1754-1821) in 1817, based upon *Anacamptis pyramidalis* (L.) Rich., the well-known Pyramidal Orchid (Wood & Ramsay, 2004). Based on the recent molecular studies, some species of *Orchis* genus was put in *Anacamptis* (Aceto *et al.*, 1999; Bateman *et al.*, 2003) and *Anacamptis* consist of 11 species and 25 taxa under 7 sections (Kretzschmar *et al.*, 2007). While Renz & Taubenheim (1984) ordered the genus with only one species and Kreutz (2009) defined the genus with 16 taxa in Turkey. But Stace (2010) claimed the extension of *Anacamptis* through inclusion of *Orchis laxiflora* and *O. morio* incorrect. The latter 2 species are completely different in appearance from *A. pyramidalis*, and it is difficult to see how the new *Anacamptis* and *Orchis* can now be readily recognised, still less keyed (Stace, 2010). According to Clapham *et al.*, (1962), Genders (1994) and Niemela & Baur (1998) *A. pyramidalis* could be found in most of Europe, including Britain, south and east to North Africa and West Asia in grassland, on chalk or limestone and on calcareous dunes (Štajner *et al.*, 2010). Distribution of the species is noted as; Mediterranean area: North & Central Europe, Russia, Crimea, Caucasia, Northwest & North Iran. It has quite wide distribution range consisted from squares A1 (A), A1 (E), A2 (A), A2(E), A3, A4, A5, A6, A7, A9, B1, B2, B3, B4, B6, B7, B8, B9, C1, C2, C3, C6, C8, C9. in Turkey. The habitat of the species could be described as rocky slopes in macchie and phrygana, meadows, grassy open wodland, olive-groves, on calcareous and schistose soil, sea level-1750m. (Renz & Taubenheim, 1984).

According to Flora of Turkey the species was defined within those generations: Tubers were roundish and

undivided. Stem consisted of leaves with linear-lanceolate, densely distributed near base. Spikes were dense, pyramidal, and oblong. Flowers were small. Sepals and petals were almost equal to each other. Labellum was flat and has fan-shaped, 3-lobed with 2 prominent longitudinal parallel ridges at base. Spur was long and filiform. Column was short and anther is obtuse. Both pollinia were attached to a single transversely strap-shaped viscid gland, enclosed in a pouch. Ovary was cylindrical, subsessile, twisted, and glabrous (Renz & Taubenheim, 1984). The flowers were pollinated by a variety of butterflies and moths in a very precise mechanism. (Delforge, 2006). *A. pyramidalis* had nectarless longer and narrower spurs. By this way it was pollinated by lepidopteran family. Spurs of *A. pyramidalis* lacked papillae and exhibit moderately developed parallel striations (Bell *et al.*, 2009). It was detected that *A. pyramidalis* showed a perforate to rugulate exine characteristic in the study of pollen micromorphology features of *Orchidinae* subtribe (Lumaga *et al.*, 2006).

The novelty seen in *f. fumeauxiana* is that the additional spurs developed on the lateral sepals (!), not on petals (lip and petals form inner whorl, sepals form outer whorl). This kind of mutation in the genus *Anacamptis* was not previously reported (Kowalkowska, 2010). *A. pyramidalis* species are called as "Cam salebi, Peynir Cicegi, Peynircik, Yogurtcuk, Tavsan otu in Turkey (Sezik, 1969; Baytop, 1997; Tuzlacı, 2006). The drug known as 'salep' used to make from dried tubers of several species of orchids and related genera such as the *A. pyramidalis*. Salep is a Turkish word (Greek 'salapi' Arabic word 'sahlab') refers to the tubers of terrestrial orchids. Tubers contain a nutritious starch-like polysaccharide called glucomannan (Baytop, 1968; Sezik, 1967, 1982, 1991, 2002; Sezik & Baykal, 1988; Güler, 2005; İşler, 2005, Çağlar, 2006; Hossain, 2011; Štajner *et*

al., 2010). Štajner *et al.*, (2010) indicated that the aboveground part of the plant exhibited higher antioxidant activity. It has high quality “salep” but dwindling rapidly from nature which is alarming need of its conservation (Sezik, 1984, Tecimen *et al.*, 2010). On the other hand *A. pyramidalis* is not covered by lists the species under risk in “Red Book of Turkey” while placed in the CITES list (Ekim *et al.*, 2000; Anon., 2011). Besides the Pyramidal Orchid is a strictly protected plant in some countries as Czech Republic and Slovak Republic. This species is simultaneously protected by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (Hoskovec, 2007).

There are limited studies related to site factors effects on morphological and anatomical features of Orchidaceae family which are attributed as important characters in Turkey. The species is confronted to be extinction under subsisted collection pressure since it offers an economically value to the collectors which requires the reproduction trials inevitably. However studies on determination of soil micro-fungus had been in Turkey in recent years (Kara *et al.*, 2010). Throughout the perspective of making benefit from that species without collecting from the field the habitat data of the species should be overcome. Within the current study we intended to determine habitat, morphological and anatomical properties of the species in detail so as to provide base knowledge for further reproduction studies and give courage to the future researchers.

Material and Methods

Plant materials of *A. pyramidalis* were taken from 21 native populations between 2007 and 2009 in Turkey, and their localities were recorded (Table 1, Fig. 1). Samples are stored in the Faculty of Forestry Herbarium of

Istanbul University (ISTO No: 35097-35106). Besides the habitat properties of 171 records from previous studies and Herbarium records of EGE, ISTE and ISTF were collected and reviewed (Alpınar, 1979; Altundağ, 2005; Aytepe, 2005; Çelik *et al.*, 2004; Çiçek, 2001; Demirelma, 2006; Doğru, 2002; Duran, 2002; Düşen, 2001; Güler, 2005; İkinci, 2000; İşler, 2005; Kaya, 2004; Kutbay, 1993; Orhan, 2006; Özbek, 2004; Özen, 1993; Sahanç, 2001; Sarı, 2003; Serin & Ertuğrul, 1999; Sezer, 2006; Sezik & Baykal, 1988; Tugay, 1997; Türkmen, & Düzenli, 1998; Uçar, 2002; Varol, 2003; Varol, 2004; Yılmaz, 2004; Kreutz, 2009). *A. pyramidalis* samples were evaluated within 19 morphological, 20 anatomical, and 18 soil characters & habitat properties. Morphological characters of investigated *A. pyramidalis* species were plant length, underground part, tuber length and width, leaf number, longest leaf length and width, shortest leaf length and width, bract length, the elements of flower were as; dorsal and lateral sepal length, labellum length and width, petal length, spur length, ovary length and caudiculum length, polinium length measurement results were presented as average, minimum, maximum values and number of the measured samples. Leaves of samples were fixed in 70% alcohol for light microscopy. Anatomical characters were obtained from samples of the middle part of the lamina of the first leaf. Anatomic observations were performed on the cross-sections of leaves, and surface sections of leaves taken by free-hand. All sections were stained with safranin and mounted with entellan (Vardar, 1987; İnce, 1989; Ruzin, 1999). The well-staining sections were photographed on Leica DFC 295 color camera type, Leica DM 2500 light microscope. In surface section of the leaves, the cuticle thickness (abaxial and adaxial), epidermis cell size (abaxial and adaxial) stomata dimensions and stomata index were measured. All measurement and observations were determined on 10 staining sections.

Table 1. Site properties and locations of sampling points in *A. pyramidalis*.

Sample	Sample		Altitude (m)	Slope (%)	Aspect	Locality
	Sampling points	Plant				
1	1	X	16	60	N	Aydın - Söke - Milas
2	5	X	722	20	SW	Aydın - Nazilli - Kızılcıca village
3	5	X	83	10	S	Bartın - Amasra Road Kaman village
4	5	X	19	20	E	Bartın - Amasra Road Uzunöz village
5	5	X	77	10	S-SE	Bartın - Orduyeri
6	5	X	534	5	N	Kastamonu vicinity
7	5	X	693	0	0	Kastamonu - Muratbaşı
8	5	X	561	45	SE	Kastamonu - Küre Mountains
9	5	X	218	10	N-NE	Kastamonu - Çatalzeytin
10	5	X	520	5	E	Kastamonu - Ziraatler village
11	10	X	887	2	S	Muğla- Yerkesik - Ören
12	10	X	385	40	E	Aydın- Nazilli - Karacasu
13	9	X	176	0	0	Tekirdağ- Istranca-Saray
14	1	X	1115	0	0	Kastamonu - Kırıkk village
15	4	-	1400		NW	Eskişehir- Çataçık
16	6	-	1209	0	0	Kastamonu - Masref
17	-	-	1591	0	0	Kastamonu - Araç
18	10	-	1200	0	0	Kastamonu - Taşköprü - Değirmendere
19	2	-	1213	0	0	Kastamonu - Taşköprü
20	5	-	130		S	İzmir - Çayağzı village
21	-	-	53	0	0	İzmir Çeşme - Alaçatı village

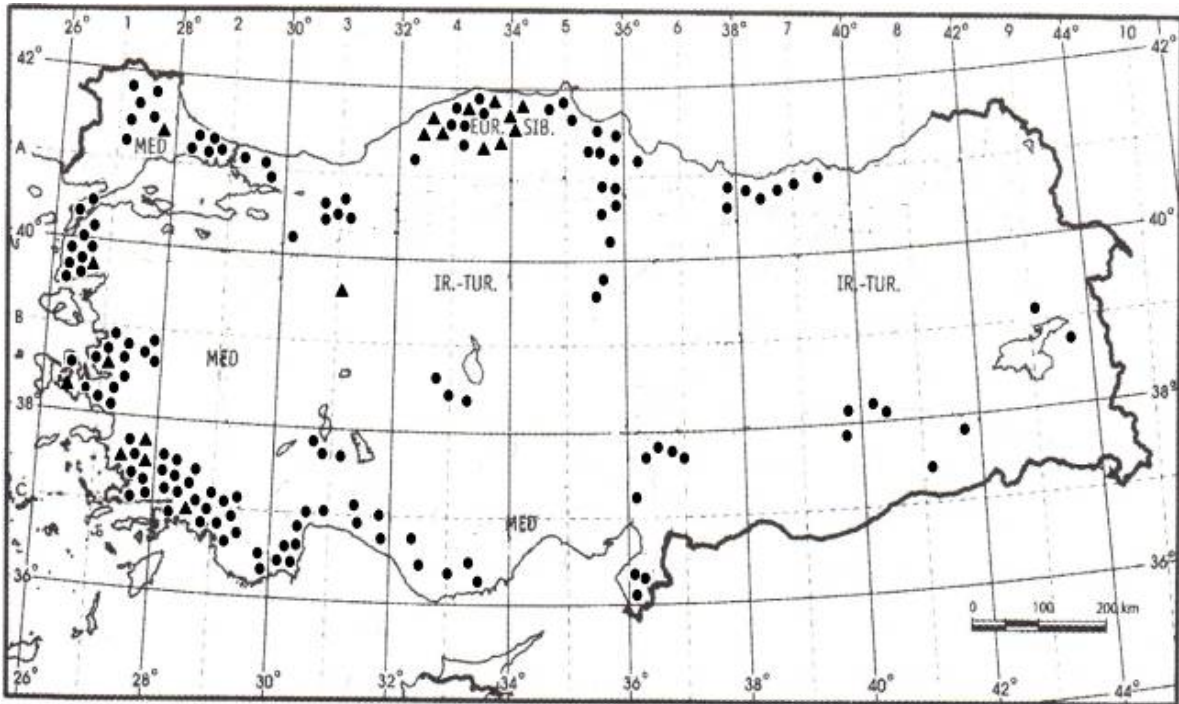


Fig. 1. ● Distribution of *Anacamptis pyramidalis* records; ▲ Distribution of collected samples of *A. pyramidalis*.

Soil samples were taken from 0-5 and 5-15 cm depths for investigations of soil properties of *A. pyramidalis*. Soil volume weight (g/l), fine soil weight (g/l), skeleton weight (g/l), sand rate (%), silt rate (%), clay rate (%) (Irmak, 1954; Gülçür, 1974), pH (Mc Lean, 1982), organic carbon (Corg) (%) (Nelson and Sommers, 1982), total nitrogen (Nt) (%) (Jackson, 1962; Bremner & Mulvaney, 1982) and C:N rates were detected. The variants used for ecological evaluation on habitats of *A. pyramidalis* were admitted as altitude, habitat type, decline, slope position and surface stoniness. Morphological characteristics and soil properties were presented by descriptive statistics. Person correlation coefficients were calculated in SPSS 13 (Kalıpsız, 1981; Özdamar, 2002).

Results and Discussion

Morphological features: Vegetative and generative morphological characteristics were measured on mature tuber, leaf and flower collected from the field. The measurements were applied on species collected from 21 localities and further statistical evaluations were executed.

The findings of this study were as follows: The stem was slender. The plant and underground part lengths were between 248-655 mm and 23-140mm respectively, and number of leaves were 2-14 with bract-like leaves above. Length of longest basal leaf was 65-230mm. Width of longest basal leaf was between 6-22mm. Spike was many flowered. Bract length was 4-18mm and lanceolate acuminate. Flowers were carmin or dark to pale rose-pink, sometimes white. Dorsal sepal length was 4-7mm, lateral sepal length was 4-9mm. Labellum was 5-15 x 4-10mm (width x length). Petal length was 4-7mm. Spur length was 8-21mm and horizontal or descending. Ovary length was 6-16mm. Caudiculum was 1-1.5mm, polinium was 1mm (Table 2).

Morphological characters such as underground part length, bract length, labellum width, ovary length, caudiculum and polinium were initially detected within the framework of this study. Although our results are generally in line with the description of Flora of Turkey, some remarkable differences are depicted here. It was reported that the length of the plant were maximum 80cm, spur length were 10-13mm (Renz & Taubenheim, 1984). In our study, the plant is maximum 65.5cm, spur length were 21 mm. *A. pyramidalis* is an evolutionarily plastic species (Prete *et al.*, 1991), with variable spur length and diameter. Our study results also supported that. According to Bateman & Rudall (2006) there are some morphological variation among flowers within an inflorescence some European orchids. They stated that *A. pyramidalis* has labellum length at 4.483mm (mean), labellum width at 6.743mm (mean) and spur length at 9.477mm (mean). Consistent with our results the ranges belonged to those 3 morphological characteristics are wider for *A. pyramidalis* in Turkey.

Leaf anatomy

Surface section: It was observed that surface section of the leaves of all investigated samples was glabrous. The epidermal cells were placed parallel to the midrib. Shapes of epidermal cells were narrowly rectangular on abaxial and broadly rectangular on adaxial leaf surface. The size of epidermal cells were 812.82 x 229.54µm on abaxial and 133.63 x 229.09µm on adaxial. Stomata cells were abaxially located and tetracytic type. Shape of stomata cells were more or less elliptical. Stomata cell size was 53.75 x 85.00µm and stomata index was 32.75 % (Table 3, Fig. 2b,c).

Table 2. Morphological characters of *A. pyramidalis* plant size, tuber, leaves and flowers.

Characters	N	Min	Max	Mean	Std. Dev.
Length (mm)	103	248	655	395.10 ± 10.24	103.93
Underground part (mm)	103	23	140	57.75 ± 1.98	20.10
Tuber length (mm)	99	12	52	22.06 ± 0.71	7.09
Tuber width (mm)	99	6	40	15.41 ± 0.53	5.28
Leaf number	103	2	14	6.71 ± 0.23	2.33
Length of longest leaf (mm)	93	65	230	112.80 ± 3.09	29.77
Width of longest leaf (mm)	93	6	22	11.81 ± 0.29	2.77
Length of shortest leaf (mm)	43	45	180	84.19 ± 4.13	27.11
Width of shortest leaf (mm)	43	5	19	10.74 ± 0.45	2.92
Bract length (mm)	102	4	18	9.86 ± 0.33	3.30
Dorsal sepal length (mm)	102	4	10	5.42 ± 0.10	1.04
Lateral sepal length (mm)	102	4	9	6.30 ± 0.10	0.98
Labellum length (mm)	101	4	10	6.54 ± 0.12	1.19
Labellum width (mm)	102	5	15	7.91 ± 0.18	1.80
Petal length (mm)	102	4	7	5.18 ± 0.08	0.83
Spur length (mm)	91	8	21	14.25 ± 0.31	2.93
Ovary length (mm)	102	6	16	10.77 ± 0.20	1.97
Caudiculum	102	1	1.5	1.01 ± 0.01	0.07
Polinium	102	0.5	1	1.00 ± 0.00	0.05

Cross section: It was observed that cuticle covered both abaxial and adaxial. Cuticle layers were slightly striate on the midrib and smooth on the margins of abaxial surface, even more smooth on adaxial surface. Cuticle thickness was

20µm on abaxial and 3.35µm on adaxial. Abaxial epidermal cells consisted of squared or round-shaped of cells, whereas adaxial epidermal cells were rectangular shaped. The chlorenchyma contained thin walled, ovate and round-shaped, 9-11 layered spongy parenchymatic cells, however it had 13-15 layers only on midrib. Vascular bundles were wider on midrib than on margins. Midrib had lacunas close to adaxial surface. Raphide bundles were occasionally observed in the mesophyll tissue (Table 4, Fig. 2a). However the mesophyll of *A. pyramidalis* generally consist of 6-8 rows Del Prete *et al.*, (1991) affirmed that the mesophyll of *A. pyramialis* is composed of rounded parenchymatous cells, rich in chloroplasts. We detected difference between the rows of cells in mesophyll.

Table 3. Leaf anatomical characters of *A. pyramidalis* (surface).

Taxa	Epidermal cells shape		Epidermal cells size (width-length)		Stomata (width-length)	
	Abaxial	Adaxial	Abaxial (µm)	Adaxial(µm)	Size (µm)	Index (%)
<i>A. pyramidalis</i>	Narrowly rectangular	Broadly rectangular	812.82 x 229.54	133.63 x 229.09	53.75 x 85.00	32.75

Table 4. Leaf anatomical characters of *A. pyramidalis* (cross-section).

Taxa	Cuticular type		Cuticular thickness		Epidermal cells shape		Chlorenchyma cells layered		In Mezophyll	
	Abaxial	Adaxial	Abaxial (µm)	Adaxial (µm)	Abaxial	Adaxial	General	On midrib	Lac.	Rap.
<i>A. pyramidalis</i>	striate	smooth	20	3.35	squared-circulary	rectangular	9-11	13-15	+	+

Lac.: Lacunae, Rap.: Raphide

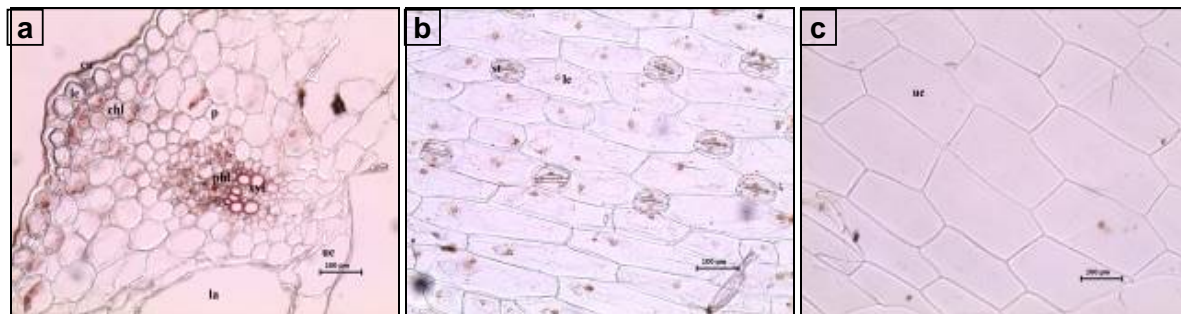


Fig. 2. Transverse section of the *A. pyramidalis* leaf (a); abaxial surface of leaf (b); adaxial surface of leaf (c). cu: cuticle, chl: chlorenchyma, ue: upper epidermis, le: lower epidermis, p: parenchyma, xyl: xylem, phl: phloem, st: stomata, la: lacuna

Ecological features: The distribution of the species is quite wide such as containing Amasya, Antalya, Aydın, Balıkesir, Bartın, Bilecik, Bolu, Çanakkale, Denizli, Diyarbakır, Eskişehir, Giresun, Hatay, Isparta, İstanbul, İzmir, Kahramanmaraş, Kastamonu, Kırklareli, Kocaeli,

Konya, Manisa, Mardin, Mersin, Muğla, Ordu, Osmaniye, Samsun, Siirt, Sinop, Şırnak, Tekirdağ, Trabzon, Van ve Yozgat provinces of Turkey (Fig. 1). The altitudinal distribution of the species reaches up to 1750m asl beginning from sea level. Its density of presence

decreases with increased altitude of which R^2 is 0.63 where it is almost rare after 1400 m asl indicating selection of habitats at lower altitudes (Fig. 3). The widest presence of the species was detected under forest lands with openings and with an ascending rank it was seen at maquis lands, olive and nut farm lands whereas barely individual were noticed at abandoned lands. Whilst, it has been distributed to olive farmlands at Aegean and Mediterranean Regions it has distributed to nut farmlands

at Black Sea Region. *A. pyramidalis* mainly distributed to grassland habitats in Estonia and UK of (Kull & Hutchings, 2006) nonetheless in Belgium and in Netherlands calcareous grasslands are more commonly selected (Jacquemyn *et al.*, 2005). On the other hand in Europe and in Asia open situations, taller grassland or open grassy woods were also had been observed (Rasmussen, 1995).

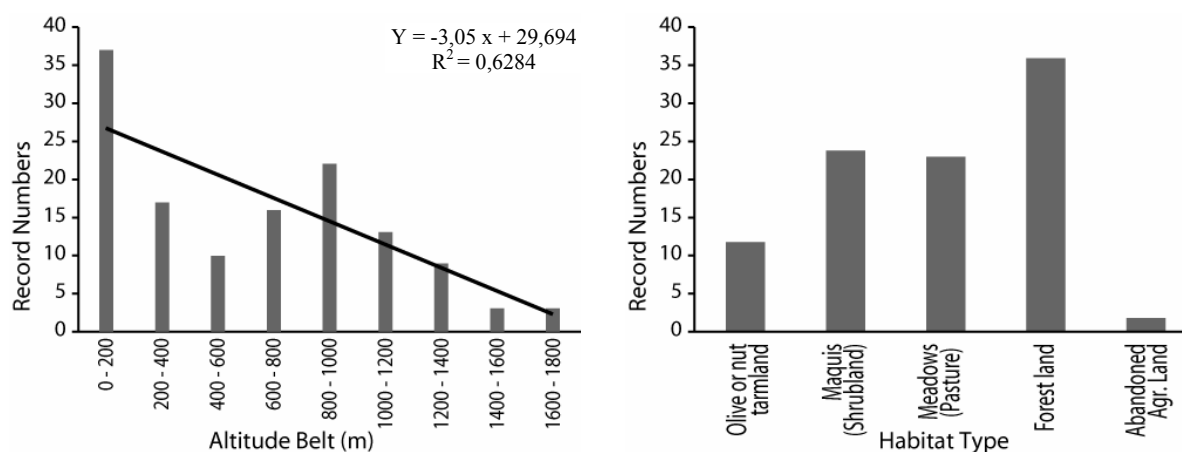


Fig. 3. Record numbers of *A. pyramidalis* % values a: according to altitude, b: habitat selections.

The results of soil analysis are as following. Soil volume weight at upper soil depth is 1019.96 g l^{-1} , from aspect of soil texture its clay content rate is 28.92% and ranks at mid-textured soils (Çepel, 1988) (Table 5). pH of the soils have average value of 7.14 which means alkaline soil, the soils reveal higher than medium of humus as organic matter content (Çepel, 1978), the total nitrogen content of the soils is 0.220% and has 13.72 C:N ratio. At 5-15 cm soil depth stoniness slightly increased, from aspect of soil texture and its clay content it ranks at mid-

textured soils (Çepel, 1988). pH of the soils have average value of 7.27 which ranks at below steps of alkaline class, soils have average humus as organic matter content (Çepel, 1978), the total nitrogen content of the soils is 0.080% and has 50.82 C:N ratio (Table 5). Tsiftsis *et al.*, (2008) has stated that the *A. pyramidalis* ranged between the altitudes 120–1300m and at soil pH sorted between 4.94–7.99 and organic matter content at % 0.78–23.94 (Tsiftsis *et al.*, 2008).

Table 5. Descriptive statistics of characteristics of soil and sampling points.

	Variable	Symbol	N	Min	Max	Mean	Std. Dev.
0-5cm	Volume weight (g/lt)	T1	13	763.00	1176.20	1019.96 ± 13.80	120.34
	Fine soil weight (gr/lt)	T2	13	481.00	1099.70	761.83 ± 20.92	182.41
	Stone weight(gr/lt)	T3	13	28.50	603.20	258.13 ± 18.57	161.90
	Sand (%)	T4	13	19.15	74.83	45.69 ± 2.09	18.19
	Silt (%)	T5	13	6.57	51.21	25.39 ± 1.23	10.76
	Clay (%)	T6	13	7.53	52.15	28.92 ± 1.47	12.84
	pH	T7	13	6.77	7.54	7.14 ± 0.02	0.16
	Corg (%)	T8	12	0.73	6.19	2.77 ± 0.21	1.67
	Nt	T9	13	0.08	0.59	0.22 ± 0.02	0.14
	C/N	T10	12	4.81	44.47	13.72 ± 1.22	9.93
5-15cm	Volume weight (g/lt)	T11	13	1003.10	1477.40	1231.52 ± 18.46	160.97
	Fine soil weight (gr/lt)	T12	13	578.90	1154.00	790.40 ± 20.75	180.87
	Stone weight(gr/lt)	T13	13	110.80	898.50	441.06 ± 25.01	217.99
	Sand (%)	T14	13	17.30	65.40	39.38 ± 1.79	15.61
	Silt (%)	T15	13	12.94	60.82	28.29 ± 1.26	10.97
	Clay (%)	T16	13	8.58	54.15	32.28 ± 1.41	12.32
	pH	T17	13	7.03	7.41	7.27 ± 0.01	0.11
	Corg (%)	T18	12	0.00	2.83	1.57 ± 0.12	0.94
	Nt	T19	13	0.01	0.18	0.08 ± 0.01	0.05
	C/N	T20	12	4.12	318.09	50.82 ± 11.19	85.98
	Altitude	Y1	13	16.00	1115.00	428.55 ± 33.97	296.13
	Slope	Y2	13	0.00	60.00	14.54 ± 1.85	16.10
	Surface stoniness	Y4	13	0.00	65.00	12.09 ± 1.28	11.18

This study reveals detailed morphological, anatomical and ecological report on *A. pyramidalis* in Turkey. The measurements related to morphological characteristics were obtained within a wide variance range. Anatomical properties were given as digital definitions indigenous to species itself. We found that the species is spread at various habitat types which could be attained to the account of variation of variables.

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