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An Ecological, Anatomical and Microbiological Investigation on the Species *Galega officinalis* L. (Leguminosae) in Some Localities of Turkey

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Abstract: The present research was an ecological, anatomical, antimicrobiological investigation in plants of *Galega officinalis* L. (*Leguminosae*) collected from some localities of the Middle Black Sea region. *Galega officinalis* have not been ecological, anatomical and antimicrobiological activity. Shoot length, leaf width, number of branches, dead leaves and flowers, root/shoot ratio, total flower and root biomasses, flower and root nitrogen RE_1, RE_2, RE_3, RE_4 were determined. In addition to this plants were examined according to Ellenberg's Scale. In anatomical studies, the crude alcohol plant extracts were more effective against bacteria than yeast. In anatomical studies, the cross sections of the root, stem and leaf were examined. The root is perennial. The leaves are dorsiventral and amphistomata.

Key words: *Galega officinalis* L.(Leguminosae), biomass, anatomy, ecology, microbiology

INTRODUCTION

The genus *Galega* L. is a member of the family Leguminosae. *Galega* is represented by *Galega officinalis* L. (Goatsrue) species in Turkey. This plants widespread in Black Sea Region of Turkey[1] *G. officinalis* L. is used as an ornamental and medicinal plant and occurs as a weed[2]. However, its fodder value is thought to be impaired by the bitter - tasting quinazoline alkaloid vasicine. Plants containing vasicine have long been used in folk medicine and a number of pharmalogical activities have been described[3]. In addition to Goatsrue contains a poisonous alkaloid known as galegin[4].

Phenotypic plasticity in plants can be defined as the adaptive capability of a plant population under genetic control in response to a changing environment. Plants show considerable character variation and phenotypic plasticity when introduced to different environments[5]. Phenotypic plasticity ensures that a plant adjust to new environments[6]. Some species can be considerably adaptable to new environments. This can be primarily due to the similarity of ecological conditions, the most important of which are climatic factors[7].

G. officinalis species which were collected from six natural populations of Middle Black Sea region phenotypic plasticity, Reproductive Effort (RE), some morphological, ecological, anatomical features and microbiological activities were determined. However, this plant was examined according to Ellenberg's Scale. Indicator values of vascular plants with respect to distinct environmental factors such as soil acidity, soil moisture, nutrient supply, or temperature and other climatic conditions, have been developed by Ellenberg[8].

Studies on the anatomy of this genus are limited. Metcalfe and Chalk[9] and Watson and Dallwitz[10] explained the characteristic properties of the Legüminosae family. But the detailed anatomical structure, ecological properties and antimicrobiological activity of *G. officinalis* have not been studied. Therefore the purpose of this research was investigate of *G. officinalis*.

MATERIALS AND METHODS

Plant material: *G. officinalis* samples were collected from different localities in the Black Sea of Turkey during the generative phase (June 2002-August 2003). The localities are listed below.

- (Asarcık- Samsun): Mountain side, 550 m.
- (Engiz-Samsun): Road side, 30 m
- (Bafra-Samsun): The edge of Lakes, 50 m.
- (Çarşamba-Samsun): Field side, 15m.
- (Bolaman-Ordu): The side of stream, 5 m.
- (Perşembe): Roadside, 150 m.

Ecological studies: *G. officinalis* samples were collected from different localities in Ordu and Samsun in order to determine reproductive effort, which is defined as the ratio of reproductive biomass and nitrogen to total plant biomass and nitrogen[11].

All the plant specimens were collected from 0.25×0.25 m. quadrats located at random in each of the six

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populations. The shoot length, number of branches, living leaves, dead leaves, flowers, leaf length and width and root.shoot were determined for every ten plants. All the collected material was dried at 60°C for 72 h and weighed according to the class of the plant material. N % was determinated by Kjeldahl's method[12]. Differences in morphological and ecological traits were assessed with a one-way ANOVA test[13].

G. officinalis samples were investigated from ecological behaviour (L= Light Figure, T=Temperature Figure, K= Continentally, F= Moisture Figure, R = Reaction Figure, N = Soil Nitrogen Figure, S = Salt Figure) to Ellenberg's indices.

Anatomical studies: For anatomical analysis, cross-sections of root, stem and leaves were used. Photographs of them were taken with a Nikon FDX-35 microscope.

Microbiological studies

Preparation of extracts: Fresh leaves and shoots were dried at 45°C for 5-6 h. The extracts were prepared according to the methods described by Holopainen *et al.*[14]. with slight modifications. Dried leaves and shoots were extracted with 95% ethanol (50 g 1/5 ethanol) at room temperature. The extracts were kept at 4°C for a day and filtered through 45 μm membrane filter and then the solution was dried with an evaporator. The crude extracts were stored at -20°C until used.

Test strains and culture media: Strains of bacteria and fungus were obtained from ATCC (American Type Culture Collection, Rockville, Maryland). Antimicrobial activities of *Galega officinalis* the crude extracts of plant were assayed against *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 25923), *Esherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 10145), *Candida albicans* (ATTC 60192) and *Aspergillus* niger The species of bacteria were grown in Mueller Hinton Agar (Merck) and Mueller Hinton Brot (Merck). *C. albicans* and *A.* niger were grown in Sabouraud Dextrose Broth (Difco) and Sabouraud Dextrose Agar (Oxoid). The concentration of bacterial suspensions were adjusted to 10^8 cells mL and fungal suspension to 10^7cells mL.

Antibacterial assay: Antibacterial activity was measured using methods of diffusion disc plates on agar[17]. In order to test antibacterial activity, the extract of *Galega officinalis* was dissolved in 70%. Twenty milliliter of Mueller Hinton Agar medium (Merck) was poured into each 15 cm petri dish. All bacterial strains were grown in Mueller Hinton Broth medium (Merck) for 24 h at 37°C. Growth was adjusted to OD (600 nm) of 0.1 by dilution with Mueller Hinton Broth medium (Merck). One hundred microliter of suspension with approximately 10^8 bacteria per milliliter was placed in petri dishes, over agar and dispersed. Then, sterile paper discs (6 mm diameter) were placed on agar to load 10 μL extract (40 mg mL^{-1}). For bacteria, as positive control Amiktodalin and cefozin 10 μL (40 mg mL^{-1}) and as negative control 10 μL (70% alcohol) were used. Inhibition diameters were determined after incubation at 37°C for 24 h. All tests were made in triplicate.

Antifungal assay: *C. albicans, A. niger* were grown in Sabouraud Dextrose Broth (Difco) at 27°C for 48 h and Sabouraud Dextrose Agar (Oxoid) was employed in agar diffusion experiments. Tested fungal suspension were adjusted to 10^7 cells mL as explained above. One hundred units of nystain were used as a positive control and alcohol as a negative control. Inhibition zones were determined after incubation at 27°C for 48 h. All tests were made in triplicate.

Minimal Inhibition Concentration (MIC) for assay: The agar dilution method, describe by Vander Berghe and Vietinck[18] was used for the antibacterial screening with slight modifications. The dilution in agar-96 well microtitre plates were used. The crude extracts were dissolved in 70% ethanol and final concentration of 40, 20, 10, 5, 2.5 and 1.25 mg of extracts mL^{-1} from the solutions 200 and 100 μL Mueller Hinton Broth medium (Merck) were transferred into each well of the tissue culture plate in triplicate. After solidification each well was inoculated with 5 μL of freshly prepared bacterial suspension of 10^8 bacterial mL and incubated at 37°C for 24 h. The bacterial growth was assessed by a stereo microscope after the incubation period.

Antifungal assay was made the same method but only instead of Mueller Hinton Broth medium (Merck) and Mueller Hinton Agar medium (Merck) used to Sabouraud Dextrose Broth (Difco) and Sabouraud Dextrose Agar (Oxoid).

RESULTS

Ecological properties: Table 1 and 2 show the differences between different populations of *G. officinalis* according to morphological, ecological parameters and reproductive efforts. As shown in Table 3, the six natural populations of *G. officinalis* differed in terms of number of flowers, flower nitrogen, above ground biomass, flower biomass ($p<0.01$), leaf width, leaf length, number of dead leaves, root biomass, RE_1, RE_2 ($P<0.05$), There was no statistically significant differences between populations with respect to the shoot length, number of branches, root nitrogen, RE_3 and RE_4. Reproductive effort shown in Table 4 and high correlation coefficients were also observed in RE_1, RE_2, RE_3 and RE_4 (Table 5).

Ecological behaviour models of *G. officinalis* according to Ellenberg's indices are followed:

L	T	K	F	R	N	S
7	6	6	6	7	8	0

L Light Figure
(7) Plant generally in well lit place, but also occurring in partial shade.
T Temperature Figure
(6) Between 5 and 7
(5) Indicator of fairly warm conditions, from lowland to montane but especially in submontane- temperate sites.
(7)Warmth indicator, in warm lowland sites and colline levels.
K Continentally
(6) Subcontinental, mainly in the East of Central Europe and adjoining parts of Eastern Europe.
F Moisture Figure
(6) Indicator of moist-site.
R Reaction Figure
(7) Weakly acid soils.
N Soil Nitrogen Figure
(8) Plant often found in places rich in available nitrogen.
S Salt Figure
(0) Glycophyte

Anatomical properties

Root: Periderm is multilayered. Primer cortex is 5-8 layered and parenchymatic. Parenchymatic cells are 8-20 x10-55 μ. There are sclerenchymatic cells (10-30x10-35) on the phloem. Cambium cells are distinguishable. Xylem is composed of sclerenchymatic cells and tracheids. Primary pith rays are 2-6 layered. In the pith a primer xylem tissue is present in Table 6 and Fig. 1.

Stem: Epidermis is single layered. Parenchyma tissue is 7-12 layered and cells are 8-20x10-30 μ and with chloroplast. Endodermis is present. There are sclerenchymatic tissue on the vascular bundles. Primary pith rays are 2-6 layered. Xylem is composed of intensive sclerenchymatic cells and tracheids. Diameter of tracheay cells are 5 to 20 μ. Primary pith rays are 2-5 layered. The pith consists of large parenchymatic cells. They are 20-70 μ. Middle part of mature stem is empty (Table 6 and Fig. 2).

Leaf: There is a single layered epidermis on the upper and lower surface of the leaf. Stomata type is anisocytic and anomocytic. Lamina is dorsiventral and amphistomata. Palisade parenchyma cells are 2-3 layered, 30-60x10-20 μ and spongy parenchyma cells are 4-5 layered, 15-25x15-37.5 μ. There are large and small collateral vascular bundles (Table 6 and Fig. 3).

Microbiological: The results of the antibacterial and antifungal screening of extracts from *Galega officinalis* plant is reported in Table 7 and 8. The crude alcohol extracts of *G. officinalis* showed 9 mm per 10 μL inhibition zone against *E. coli* and 17 mm per 10 μL inhibition zone against *P. aeruginosa*. However the crude alcohol extracts of *Galega officinalis* showed 20 mm per 10 μL inhibition zone against *B. subtilis, S. aureus, C. albicans* and *A. niger*. The crude alcohol extracts of *G. officinalis* is more effective against bacteria than yeast.

The crude extracts of *G. officinalis* required a MIC for *B. subtilis* and *P. aeruginosa* of 2.5 mg mL concentration. The crude extracts of *G. officinalis* required a MIC for *S. Aureus*, A. niger of 5% mg mL concentration and MIC for *C. albicans* of 10% mg mL concentration. The concentration of the crude extracts of *G. officinalis* used in study was not fount to required a MIC for *E. coli* (Table 8).

Table 1: Mean values of morphological parameters

Locality	Shoot length (cm)	Leaf width (cm)	Leaf length (cm)	No. of branches	Dead leaves	No. of flowers
1	53.4	3.1	4.1	4	13	60
2	83.2	1.42	5.5	5	7	74
3	46.5	0.9	2.6	4	12	60
4	63.5	1	2.6	5	8	70
5	53.4	1.3	3.1	5	12	75
6	58.7	1.4	6	6	13	60

Table 2: Mean values of ecological parameters

Locality	Leaf nitrogen (%)	Flower nitrogen (%)	Root nitrogen (%)	Above ground biomass	Root biomass	Flower biomass
1	4.10	4.90	2.10	7.00	1.30	1.03
2	3.25	3.50	1.40	16.06	1.43	1.84
3	3.50	3.80	2.10	5.05	1.32	1.07
4	3.40	4.60	1.05	6.74	0.76	2.12
5	4.08	3.90	2.10	7.35	4.45	1.14
6	3.00	4.00	1.60	5.70	1.25	1.04

Table 3: Comparison of morphological and ecological parameters using one-way ANOVA

Parameter	Significance
Shoot length	0.935 (NS)
Leaf width	0.040 (*)
Leaf length	0.024 (*)
No. of branches	0.837 (NS)
No. of dead leaves	0.043 (*)
No. of flowers	0.000 (**)
Leaf nitrogen	0.028 (*)
Flower nitrogen	0.002 (**)
Root nitrogen	0.126 (NS)
Above ground biomass	0.003 (**)
Root biomass	0.048 (*)
Flower biomass	0.000 (**)
RE_1	0.0423 (*)
RE_2	0.0268 (*)
RE_3	0.071 (NS)
RE_4	0.0656 (NS)

*p<0.05, **p<0.01, NS: Non significant

Table 4: The values reproductive effort (RE)

Locality	RE_1	RE_2	RE_3	RE_4
1	0.13	0.10	0.57	0.44
2	0.05	0.027	0.55	0.54
3	0.06	0.05	0.5	0.50
4	0.32	0.23	0.58	0.60
5	0.17	0.14	0.56	0.56
6	0.18	0.11	0.53	0.52

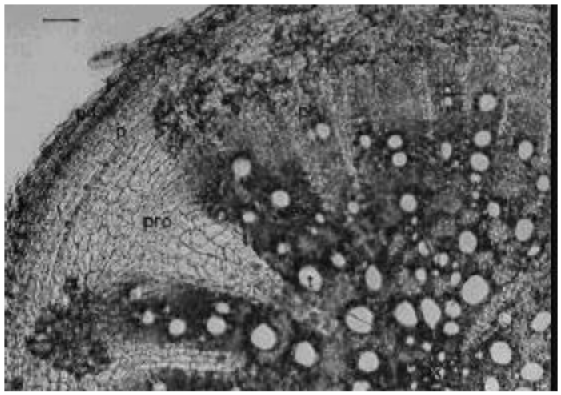

Fig. 1: *G. officinalis*. Cross-section of root. pd) peridermis, p) parenchyma, prö) pith ray, c) cambium, ph) phloem, sx) secondary xylem, t) trachea, px) primary xylem (Bar:100 µ)

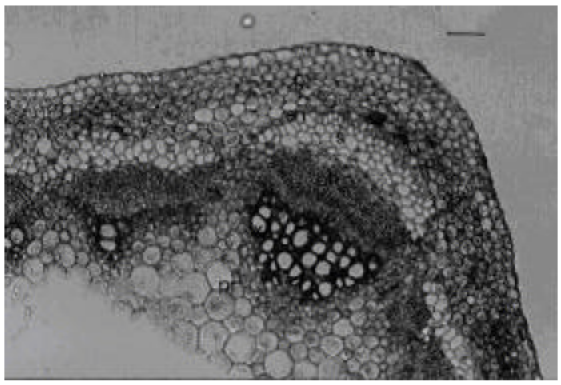

Fig. 2: *G. officinalis*. Cross-section of stem. e) epidermis, p) parenchyma, en) endodermis, s) sclerenchyma, c) cambium, ph) phloem, x) xylem, pr) pith region (Bar:100 µ).

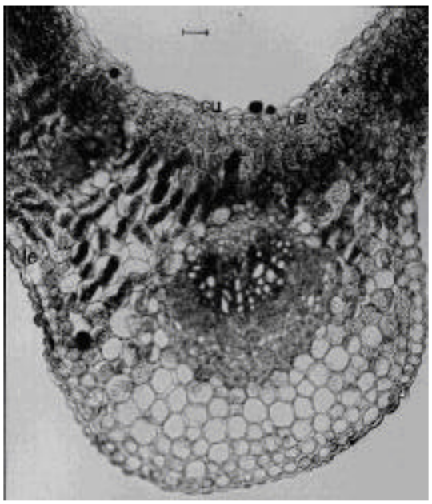

Fig. 3: *G. officinalis*. Cross-section of leaf. cu) cuticle, ue) upper epidermis, pp) palisade parenchyma, sp) spongy parenchyma, v) vasculer bundle, le) lower epidermis (Bar: 50 µ).

Table 5: Correlation coefficients in values of Reproductive effort (RE) (r =.532* = p<0.05; r =.661** = p<0.01)

Locality 1	RE_1	RE_2	RE_3	RE_4
RE_1	_			
RE_2	.862**	_		
RE_3	.894**	.545*	_	
RE_4	. 754**	.755**	1**	-
Locality 2	RE_1	RE_2	RE_3	RE_4
RE_2	_			
RE_3	.982**	_		
RE_4	.189	.371	_	
RE_1	.982**	.786**	.866**	-
Locality 3	RE_1	RE_2	RE_3	RE_4
RE_1	_			
RE_2	- .891**	_		
RE_3	- .971**	.756**	_	
RE_4	-.971**	.786**	1**	-
Locality 4	RE_1	RE_2	RE_3	RE_4
RE_2	_			
RE_3	.982**	-		
RE_4	.817**	.693**	-	
RE_1	-.999**	-.990**	-.789**	-
Locality 5	RE_1	RE_2	RE_3	RE_4
RE_1	_			
RE_2	.923**	_		
RE_3	-.321	- .661**	_	
RE_4	-.321	.996**	-.723**	-
Locality 6	RE_1	RE_2	RE_3	RE_4
RE_1	_			
RE_2	.023	_		
RE_3	.55*	- .866**	_	
RE_4	.711*	-.996**	1**	-

DISCUSSION

In this study statistically significant differences were observed in the number of flowers, flower biomass, flower nitrogen, RE_1, RE_2 values of *G. officinalis* (Table 3). This situation indicates that reproductive parts have a great effect on phenotypic plasticity. It was found that no significant differences in six populations shoot length, ber of branches, root nitrogen, RE_3 and RE_4 parameters.

Table 6: Anatomical measurements of *Galega officinalis*

		Breadth (μ)		Length (μ)	
		Min	Max	Min	Max
Root	Periderm cells	15	65	10	40
	Parenchyma cells	10	55	8	20
	Diameter of trachea	7.5	50	-	-
	Sclerenchyma cells	10	35	10	30
Stem	Epidermis cells	7	15	5	12
	Sclerenchyma cells	10	30	8	25
	Parenchyma cells	10	30	8	20
	Diameter of trachea	10	50	-	-
	Diameter of pith cells	20	70	-	-
Leaf	Cuticle	4	5	-	-
	Upper epidermis cells	10	35	8	25
	Lower epidermis cells	8	30	6	25
	Palisade paren. Cells	10	20	30	60
	Spongy paren. Cells	15	37.5	15	25

However, leaf length and width, the number of dead leaves, the number of flowers, flower biomass, flower nitrogen, above ground and root biomass, RE_1, RE_2 differed significantly in the six populations (Table 3). This might allow a more efficient dispersal of seeds and lead to a greater likelihood that some seeds will reach appropriate safe sites, as hypothesized by Janzen[17]. This may indicate that reproductive structures play an important role in the phenotypic plasticity[5]. Similar results were obtained *Commelina communis* L. (*Commelinaceae*)[5]. The majority of correlation coefficients between values of reproductive effort are statistically important as shown in Table 5 . Consequently, suggest that the phenotypic plasticity of *G. officinalis* is quite high from high correlation coefficients for reproductive effort (Table 5).

RE_1, RE_2, RE_3 and RE_4 values were higher in the Çarşamba and Peşembe localities than the other localities (Table 4). It has been suggested that higher RE values are related to the increased number of flowers[18] Present results fully support this hypothesis (Table 1). RE_3, RE_4

Table 7: Results of antimicrobial screening of *Galega officinalis* plant extracts

				Microorganisms					
Plant species and family	Part used	Collection time	Collection site	*E.c.*	*P. a.*	*B.s.*	*S.a.*	*C.a.*	*A.n.*
Galega officinalis L.	Fr, Lf	August	Ordu	9	17	20	20	20	20
Legüminasae		2003	(Persembe)						
Amiktodalin				35	37	45	48	ND	ND
Cefozin				33	20	42	36	ND	ND
Nystatin				ND	ND	ND	ND	16	15
70% alcohol				-	-	-	-	-	-

Part used: Fr, flower; Ft, fruit; Lf, leaf; Sd, seed; St, stem;
Microorganisms: E.c.: *E. coli*; P.a.: P. aeruginosa; B. s.: B. subtilis; S. a.: S. aureus, C.a, C. albicans and A.n.: A. niger

Table 8: The MIC values of plant extracts (% g mL^{-1}) From *Galega officinalis*

				Microorganisms					
Plant species and family	Part used	Collection time	Collection site	*E.c.*	*P. a.*	*B.s.*	*S.a.*	*C.a.*	*A.n.*
Galega officinalis L.	Fr,Lf	August	Ordu	-	0,5	0,5	1	2	1
Legüminasae		2003	(Perşembe)						

Part used: Fr, flower; Ft, fruit; Lf, leaf; Sd, seed; St, stem;
Microorganisms: E. c.: *E. coli;* P.a.: *P. aeruginosa;* B. s.: *B. subtilis;* S. a.: *S. aureus,* C.a: *C. albicans and* A.n.,: *A. niger*

values are given Table 4 are not different among in the localities. It has been suggested that some convergences and divergences in the reproductive strategies of different populations may occur[19]. High plasticity may allow plant species to maintain dominance in various environments by increasing the number of tolerable habitats[5].

G. officinalis had a secondary structure of root. Periderm was multilayered. There were sclerenchymatic cells on the phloem. Xylem was composed of sclerenchymatic cells and trachea. There is single layered epidermis on the stem. There are sclerenchymatic tissue on the vascular bundles. Özörgücü *et al.*[20] pointed out that there were sclerenchymatic cells on the stem. The same results were seen in *Astragalus barba-jovis* DC. var. *barba-jovis* (Fabaceae)[21]. The leaf was dorsiventral and amphistomata. Palisade parenchyma cells are 2-3 layered and spongy parenchyma cells are 4-5 layered. Stomata type was anisocytic and anomocytic. Watson and Dallwitz[10] have stated that there are usually anomocytic stomata. Özörgücü *et al.*[20] pointed out that there were anomocytic, anisocytic and paracytic stomata in this family.

The antimicrobial activity of extracts of *Galega officinalis* against bacteria was more effective than against fungus, which is similar to the results of Avato *et al.*[22] and Zavala *et al.*[23]. A further study is thought to be examined as detail properties of compounds of fractions.

In this study, anatomical structure, ecological properties and antimicrobiological activity of *G. officinalis* studied.

REFERENCES

1. Davis, P.H., 1969. Flora of Turkey and East Aegean Islands, Edinburg University Press, 3: 41-42.
2. Varis, E., 1986. Goat's rue (*Galega orientalis* Lam.), a potential pasture legume for temperate conditions. J. Agric. Sci. Finland, 58: 83-101.
3. Duke, A.J., 1987. CRC Handbook of Medicinal Herbs, CRC Press, Boca Raton, pp: 677.
4. Vırkajärvı, P., I. Laasko and K. Lindström, 1991. Phytochemical Screening of Secondary Metabolites of *Goat'srue* (*Galega orientalis* Lam). University of Helsinki, Publication No. 29.
5. Kutbay, H.G. and F. Uçkan, 1998. Phenotypic plasticity in Turkish *Commelina communis* (*Commelinaceae*) L. populations. Turk. J. Bot., 22: 199-204.
6. Schlichting, C.D. and D.A. Levin, 1984. Phenotypic phyticity of annual Phlox test of some hypotheses. Am. J. Bot., 78: 1712-1723.
7. Dunn, C.P. and R.R. Sharitz, 1991. Population structure, biomass allocation and phenotypic plasticity in Murdannia keisak. Am. J. Bot., 78: 1712-1723.
8. Ellenberg, H., H.E. Weber, R. Düll, V. Wirth, W. Werner and D. PauliBen, 1992. Zeigerwerte von Pflanzen in *Mitteleuropa scripta*. Geobotanica, 18: 257.
9. Metcalfe, C.R. and L. Chalk, 1979. Anatomy of Dicotyledons I. London: Oxford University Press, pp: 276.
10. Watson, L. and M.J. Dallwitzs, 1991. The families of angiosperm: Automated descriptions, with interactive identification and information. Retrieval. Aust. Syst. Bot., 4: 681-695.
11. Reekie, E.G. and F.A. Bazzaz, 1987. Reproductive effort in plants. Am. Naturalist, 129: 876-896.
12. Bayraklı, F., 1987. Toprak ve Bitki Analizleri. Ondokuz Mayıs Üniversitesi,. Ziraat Fakültesi Yayınları, 17, Samsun.
13. Schaefer, R.L. and R.B. Anderson, 1989. The Student Edition of Microsta and Minitab User's Manual, Addision Wesley Publishing Company Inc, California, New York.
14. Holopainen, M., L. Jabordar, T. Seppanen-Laukso, I. Laakso and V. Kauppinen, 1988. Antimicrobial activity of some finnish ericaceous plants. Acta Pharmaceutia Fennica, 97: 197-20.
15. Ronald, M.A., 1990. Microbiologia. Compania Editorial Continental S.A. de C.V., Mexico, D F., pp: 505.
16. Vander Berghe, D.A. and A.J. Vietinck, 1991. Screenin Methods for Antibacterial and Antiviral Agents from Higger Plants. In: DEy, P.M. and J.B. Harborne (Eds.). Methods in Plant Biochemistry. Academic Pres, London.
17. Janzen, D.H., 1981. Inter- and- intra-crop variation in seed weight of Costa Rican Ateleia herbert-smithii Pitt (Leguminosae). Bresnia, 14/15: 311-323.
18. Weiner, J., 1985. Size hierarchies in experimental populations of annual plants. Ecology, 66: 743-752.
19. Christy, E.J. and R.R. Sharitz, 1980. Characteristics of three populations of a swamp annual under different temperature regimes. Ecologia, 61: 454-460.
20. Özörgücü, B., Y. Gemici and I. Türkan, 1991. Comparative Plant Anatomy. Izmir: Ege University Press No. 129.
21. Kandemir, N., H. Korkmaz and A. Engin, 1996. The Morphological and Anatomical Properties of *Astragalus barba-jovis* DC. var. *barba-jovis* (Fabaceae). Turk. J. Bot., 20: 291-297.
22. Vato, P., P.M. Vitali and A. Tava, 1997. Antimicrobial activity of polyacetylenes from *Bellis perennis* and synthetic derivatives. Planta Med., 63: 503-507.
23. Avala, S.M.A., G.M.S. Perez and G.R.M. Perez, 1997.Antimicrobial screening of some medicinal plants. Phytother. Res., 11: 368-371.