

1-1-2000

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BAŞLAR, SÜLEYMAN (2000) "An Investigation on *Chrozophora tinctoria* (L.) Rafin. Distributed in WestAnatolia," *Turkish Journal of Botany*. Vol. 24: No. 2, Article 3. Available at: <https://journals.tubitak.gov.tr/botany/vol24/iss2/3>

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An Investigation on *Chrozophora tinctoria* (L.) Rafin. Distributed in West Anatolia*

Süleyman BAŞLAR

Dokuz Eylül University, Buca Education Faculty, Department of Biology, Izmir-TURKEY

Received: 29.07.1998

Accepted: 19.10.1999

Abstract: The morphological characteristics of the root, stem, leaf, flower, seed and fruit of *Chrozophora tinctoria* (L.) Rafin. (*Euphorbiaceae*) distributed in West Anatolia were determined, and biometric measurements were taken from samples collected from 42 different localities. This plant is a monoecious, annual herb, 28-63.50 cm. and stellate hairy. Its leaves are alternate and their shapes vary from rhombic-ovate to ovate-lanceolate. The leaves are bifacial and amphistomatous. Their stomatas are of the amaryllis and parasitic types. The development of the root, stem, and leaves of the plant were observed when grown under various fertilizer, soil, lime and watering conditions, and their biometric measurements were taken. The plant did not show any sign of growth or development under shade and lime conditions. Fertilizer inhibited growth and development of the root while stimulating the stems and leaves. IBA and Pokon hormones were not effective in vegetative propagation. The plant grows under various conditions at altitudes of 0-1650 m.

Key Words: *Chrozophora tinctoria* (L.) Rafin., morphology, anatomy, dye plant, vegetative propagation, cultivation.

Batı Anadolu'da Yayılış Gösteren *Chrozophora tinctoria* (L.) Rafin. Üzerinde Bir Araştırma

Özet: Batı Anadolu'da yayılış gösteren *Chrozophora tinctoria* (L.) Rafin. (*Euphorbiaceae*)'nin kök, gövde, yaprak, çiçek, tohum ve meyvesinin morfolojik özellikleri tesbit edildi ve 42 farklı lokaliteden alınan örneklerin biometrik ölçümleri yapıldı. Bu bitki monoik, annual otsu, 28-63.50 cm. ve stellat tüylüdür. Yapraklar alternattır. Yaprak şekli rombik-ovattan ovat lanseolata kadar değişir. Yapraklar bifasiyel ve amfistomatiktir. Stomalar amaryllis ve parasitik tiptedir. Farklı gübre, toprak, kireç ve sulama koşullarında gözlenerek biometrik ölçümleri yapıldı. Bu bitki gölge ve kireçli ortamlarda büyüme ve gelişme göstermedi. Gübre, kökün büyüme ve gelişmesini inhibe ederken, gövde ve yaprağınkini ise stimüle etti. IBA ve Pokon hormonları vejetatif üretimde etkili olmadı. Bu bitki Türkiye'de 0-1650 m. arasında, değişik ortamlarda yetişmektedir.

Anahtar Sözcükler: Akbaş bitkisi, morfoloji, anatomi, boya bitkileri, vejetatif üretim, kültür.

Introduction

Turkey has a rich flora due to its geographical position and climatic features. For this reason, many studies of the Turkish flora have been carried out. For deeper knowledge of *Chrozophora tinctoria* (L.) Rafin. (*Euphorbiaceae*), and in order to make use of its economic potential, it should be investigated autecologically, morphologically and anatomically. Ecological investigations of plants distributed naturally in Turkey is important from the point of view of economic evaluation. Besides autecological studies, it has been known for centuries that dye substances can be produced from natural plants existing in the flora of Turkey, and also from stone, soils, mines and animals (1). Dye substances can be obtained from different organs such as

the flower, fruit, leaf, stem and root of dye-plants (2). In addition, non-flowering plants can provide natural dye substances (3). The natural dyes obtained from these plants include three main colours; red, yellow and blue. It is possible to obtain other colors from mixtures of these colors (4). Nearly 150 kinds of plant are used in the production of natural dyes (5). There has been a great increase in the number of autecological studies of plants used in natural dye production (6,7,8). *C. tinctoria*, which grows widely in West Anatolia, the area of our study, was used as the research material in this study because of its dye value. All plants used in dyeing are referred to as dye plants.

Our study material *C. tinctoria* grows as a ruderal plant and is found in cultivated areas. This plant, which

* This paper is part of a Ph. D. Thesis supervised by Prof. Dr. H.H. MERT.

belongs to the family *Euphorbiaceae*, is called the turnsole plant. Dye substances can be obtained from all its organs (9,10). In some parts of Anatolia, the plant known as "Akbaş" (White head) is a manual type (11). Very few studies, most of which have been related to ecological levels, have been done on the plant *C. tinctoria* (12,13).

Materials And Methods

The specimens of *C. tinctoria* were collected from different localities in West Anatolia and identified taxonomically with the help of Flora of Turkey and East Aegean Islands (14).

Collection Sites

MANİSA: 1. Akhisar entrance (DEBB* 293), 2. Süleymanlı Village exit (DEBB 294), 3. Kırkağaç (DEBB 295), 4. Soma; Turgutalp (DEBB 296), 5. Kula (DEBB 297), 6. Turgutlu- Avşar (DEBB 298); BALIKESİR: 7. Savaştepe, Karaçam (DEBB 299), 8. Ayvalık; Altınova (DEBB 300), 9. Burhaniye; Karaağaç (DEBB 301), 10. Edremit (DEBB 302), 11. Bandırma; Aksakal (DEBB 303), 12. Bandırma; Erdek, Gelinönü (DEBB 304), 13. Balıkesir; Susurluk (DEBB 305); ÇANAKKALE: 14. Lapseki (DEBB 306); İZMİR: 15. Kınık (DEBB 307), 16. Bergama; Bakırçay (DEBB 308), 17. Kemalpaşa (DEBB 309), 18. Beydağ; Çiftlik (DEBB 310), 19. Tire; Gökçe (DEBB 311), 20. Torbalı; Aslanlar (DEBB 312), 21. Menemen; Türkel (DEBB 313), 22. Aliağa; Kalabak (DEBB 314), 23. Aliağa; Çaltıdere (DEBB 315), 24. Foça; Bağarası (DEBB 316), 25. Urla - Çeşmealtı (DEBB 317), 26. Çeşme; Ilıca (DEBB 318), 27. Çamlık - Aydın (DEBB 319); MUĞLA: 28. Milas - Selimiye (DEBB 320), 29. Bodrum - Turgutreis (DEBB 321). 30. Yatağan; Maden (DEBB 322), 31. Ula (DEBB 323), 32. Ula-Ataköy (DEBB 324), 33. Köyceğiz; Doğuşbelen (DEBB 325), 34. Fethiye; Hisarönü (DEBB 326); DENİZLİ: 35. Acıpayam; Darıveren (DEBB 327), 36. Kale; Kavakdede (DEBB 328), 37. Buldan (DEBB 329); AYDIN: 38. Kuşadası (DEBB 330), 39. Söke (DEBB 331), 40. Sultanhisar (DEBB 332), 41. Koçarlı (DEBB 333), 42. Germencik - Ortaklar (DEBB 334).

Specimens collected from 42 different localities for biometric measurements were taken during the flowering season. The mean and standard deviation values of the measurements were calculated according to Rummel (15).

Plant material of *C. tinctoria*, collected from different localities (İzmir; Buca, Tekel garden, Manisa; Kırkağaç city

entrance and Aydın; Kuşadası city entrance) was fixed in 70% alcohol, and then anatomical sections of root, stem and leaf were taken. An Olympus microscope was used to photograph the sections after staining with "sartur" reactive.

Growth of Plants in Different Media

a) 75% fertilizer + 25% soil b) 50% fertilizer+ 50% soil. c) 25% fertilizer + 75% soil. d) 25% lime + 75% soil. e) 50% lime + 50% soil.

Seeds were left to germinate in pots containing sand. After reaching 15 cm in height, 3 seedlings were transplanted to post containing the five different mixtures given above. These were allowed to grow under two different conditions, light and shade The pots were irrigated once a day, once every two days, once every four days and once every six days, and these, conditions were replicated. Measurements of the plants were taken in the flowering season. The standard deviation was calculated and this figure is given in Tables 1, 2 and 3.

The cuttings taken from the roots, shoots, stem and the parts between the roots and stem were used for vegetative propagation. The length of the cuttings were 1, 3, 5, 7 and 10 cm. These were left to grow in water, sand, soil and fertilizer. Indol-3-butric acid (IBA; $C_{12}H_{13}NO_2$ mol: 203.24 gr/mol MERC) and pokon implant hormone were applied before the cuttings were grown. Hormonal treatment was given after 1, 4, 7 and 10 hours. IBA was applied with hormonal concentrations of 10, 20, 30, 40 and 50 ppm.

Results and Discussion

Up to the second part of the 19th century, most plants used in dye manufacturing were taken from Turkey. Turkey fulfilled up to two thirds of the world's requirements in terms of madder root production. We selected the above ground parts of *C. tinctoria* due to its dye characteristics. This plant is present in the West Anatolian region and is used for obtaining the colour red and its tones. For this reason we carried out morphological, anatomical and autecological studies on this species.

Morphology

C. tinctoria, which belongs to the family *Euphorbiaceae*, is an annual plant and is the only species of the *Chrozophora* genus found in Turkey. Our phenological observations showed that the flowering

* DEBB: Dokuz Eylül University, Buca Education Faculty, Department of Biology, Herbarium.

Table 1. Biometric measurements of *C. tinctoria*.

Plant parts	No of meas.	Width		Length		mean±s.d.	
		Min	Max	Min	Max	Min	Max
Plant	42	10.00 cm	61.30 cm	38.59±14.06	28.00 cm	63.50 cm	48.81±9.29
Root	42	3.00 mm	9.00 mm	5.14±1.85	7.30 cm	32.60 cm	17.33±6.01
Stem	42	2.00 mm	12.00 mm	6.14±2.73	2.50 cm	7.00 cm	4.75±1.43
Leaf	42	1.20 cm	5.50 cm	2.75±1.10	1.80 cm	7.30 cm	4.45±1.49
Petiole	43	–	–	–	1.00 cm	10.00 cm	4.99±2.35
Pedicele	42	–	–	–	3.00 cm	30.00 cm	14.64±7.87
Calyx (female)	43	0.25 mm	1.25 mm	0.96±0.21	2.50 mm	4.00 mm	3.12±0.43
Calyx (male)	42	0.50 mm	1.50 mm	0.98±0.27	2.50 mm	4.00 mm	3.11±0.61
Corolla (female)	42	1.00 mm	1.50 mm	1.19 ± 0.21	2.75 mm	5.00 mm	3.88±0.69
Corolla (male)	42	1.00 mm	1.50 mm	1.25±0.23	3.00 mm	4.75 mm	3.66±0.53
Stigma	41	–	–	–	0.75 mm	2.00 mm	1.30±0.38
Style	40	–	–	–	0.75 mm	1.50 mm	1.06±0.202
Ovary	43	–	–	–	2.0 mm	3.75 mm	3.04±0.346
Anther	42	0.50 mm	1.00 mm	0.73±0.17	1.0 mm	1.75 mm	1.22±0.23
Filament	42	–	–	–	0.45 mm	1.25 mm	0.98±0.15
Seed	43	3.00 mm	4.00 mm	3.72±0.45	3.00 mm	6.00 mm	4.51±0.59
Fruit	41	0.30 cm	1.20 cm	0.58±0.20	0.30 cm	0.80 cm	0.55±0.12

season is from June to September. In the morphological studies, the measurements of the flower, seed and fruit, as well as the position of the leaves and other parts of the plant were noted. The average of these measurements and the standard deviations were calculated (Table 1). All the values are in full conformity with Davis (14).

Herbs. Stems ascending, often becoming woody at base; leaves alternate, rhombic-ovate to ovate-lanceolate, apex acute or obtuse, base cuneate to shallowly cordate, shallowly repand-dentate. Inflorescences paniculate, male flowers with 5 sepals; sepals linear-lanceolate stellate pubescent outside, glabrous inside; petals, 5, yellowish, elliptic-lanceolate, with lepidote scales outside, pubescent inside, with simple hairs; stamens, 3–12, antipetalous. Sepals and petals of female flowers similar to male sepals; ovary with dense lepidote scales; style stellate-pubescent outside, papillose inside, homostylous; stigma bifurcate. Anthers basifixed, open lengthwise, with parallel theca; base obtuse. Fruit schizocarpic, 3-lobed, often becoming sparingly tuberculate. Seeds c. 4 mm., pale grey. Placentation free-central.

Anatomy

Most previous anatomical investigations have been carried out on the other species of the family *Euphorbiaceae* (16). In many species of this family, it is

reported that there are laticiferous cells and laticiferous vessels (17,18). In our anatomical examination, none of these structures were observed in *C. tinctoria*. These results show conformity with findings for other members of the *Euphorbiaceae* family (16).

In the root cross-section of *C. tinctoria*, it was observed that the epidermis had been destroyed by the development of seconder structure. Following this, the cortex, which consisted of sclerenchyma and parenchyma, was observed. Sclerenchyma cells were dispersed in the cortex. The next structures after the cortex were the phloem and xylem. The xylem covered a wide area. The endodermis and pericycle could not be seen clearly (Fig 1). There was no pith in the centre of the root due to the excess development of the xylem. These findings are in agreement with those of Metcalfe & Chalk (16), except that these authors did not report the sclerenchyma in the cortex.

In the stem anatomy of *C. tinctoria*, it was observed that there was one layer of epidermis. Under the epidermis, there was a cortex consisting of collenchyma and parenchyma cells. It was observed that the collenchyma was interspersed with chlorenchyma at some points. The sclerenchyma groups which were among the cortex parenchyma cells surrounded the vascular system

Table 2. Biometric measurements of root of *C. tinctoria* grown under different conditions.

Med-ium	Light con-dit.	Sample No	Irrigation day	Number of roots	ROOT					
					min	max	Length (cm) mean±S.D	min	max	Width (mm) mean±S.D.
Fertil-izer 75%+Soil 25%	Light	1	1	3	6.2	8.4	73.±0.8	2	3	2.66±0.4
		2	2	3	7.4	15.8	11.0±3.5	3	4	3.33±0.4
		3	4	3	6.3	9.7	7.7±1.4	2	4	3±0.8
		4	6	3	7.4	13.2	10±2.4	3	4	3.33±0.4
	Shade	5	1		Plants	shifted	to	shade		
		6	2							
		7	4							
		8	6							
Fertil-izer 59%+ Soil 50%	Light	9	1	3	7.2	12.2	9.4±2.0	3	4	3.66±0.47
		10	2	3	6.5	10.1	8.46±1.48	2	4	3±0.8
		11	4	3	6.8	11.2	9.46±2.05	3	4	3.33±0.47
		12	6	3	7.5	14.3	10.66±2.79	3	5	4±0.8
	Shade	13	1		Plants	shifted	to	shade		
		14	2							
		15	4							
		16	6							
Fertil-izer 25%+ Soil 75%	Light	17	1	3	7.3	14.8	11.1±3.06	3	5	4±0.8
		18	2	3	7.4	15.3	11.9±3.3	4	6	5±0.8
		19	4	3	6.6	9.4	8.13±1.15	3	4	3.66±0.47
		20	6	3	6.3	11.2	8.93±2.0	3	4	3.33±0.47
	Shade	21	1		Plants	shifted	to	shade		
		22	2							
		23	4							
		24	6							

from the outside and they were not clearly separated from the central cylinder. It was observed that the area up to the vascular system showed the character of the primary stem structure. After this structure came the phloem. It was observed that a secondary structure had developed in the xylem. There was a parenchymatic pith in the centre of the stem (Fig 2). These findings are in agreement with those of Metcalfe & Chalk. However, these authors did not report chlorenchyma in the cortex.

The leaf anatomy of *C. tinctoria* showed the cuticle and epidermis covered with stellate hairs; in addition, there was a single layer of palisade with small intercellular spaces which was rich in chlorophyll, and a narrow region of spongy parenchyma with wide intercellular spaces (Fig. 3). These results are in agreement with those of Metcalfe & Chalk (16). The leaves were bifacial and amphistomatous. Their stomata were of the amaryllis and parasitic type (Figs. 4, 5).

Culture Experiments on Plant Growth and Development

The growth and development of the root, stem and leaf of *C. tinctoria* was observed under various soil, light and watering conditions. *C. tinctoria* seedlings 10 cm in height were collected from the wild and transplanted under different conditions (Tables 2, 3 and 4). *C. tinctoria* was grown under conditions of 75% fertilizer + 25% soil, 50% fertilizer + 50% soil and 25% fertilizer + 75% soil, under daylight with different watering programmes: once a day, once every two days, once every four days and once every six days. Plants kept under shade became dried in spite of the same watering and soil conditions (Tables 2, 3 and 4; Figs 6, 7 and 8). *C. tinctoria* plants which were grown in a mixture of 25% lime+75% soil and 50% lime+50% soil under either daylight or shade and had the same watering programmes did not show any sign of growth. Root growth was less in 75%

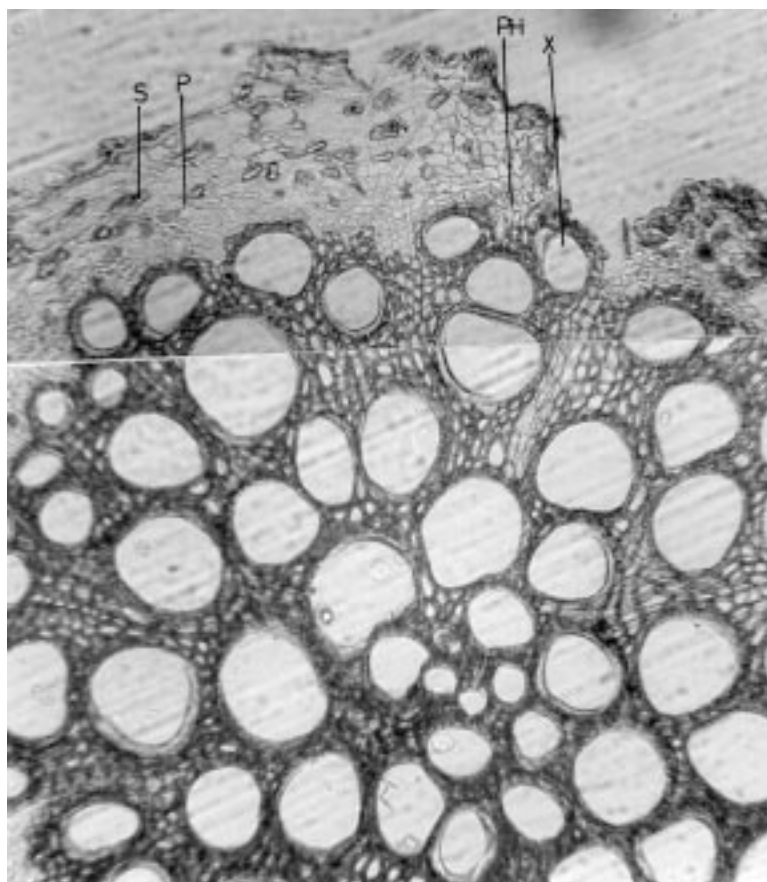


Figure 1. The cross section of the root of *C. tinctoria* (10x6.3). P- Parenchyma, S- Sclerenchyma, PH- Phloem, X-Xylem.

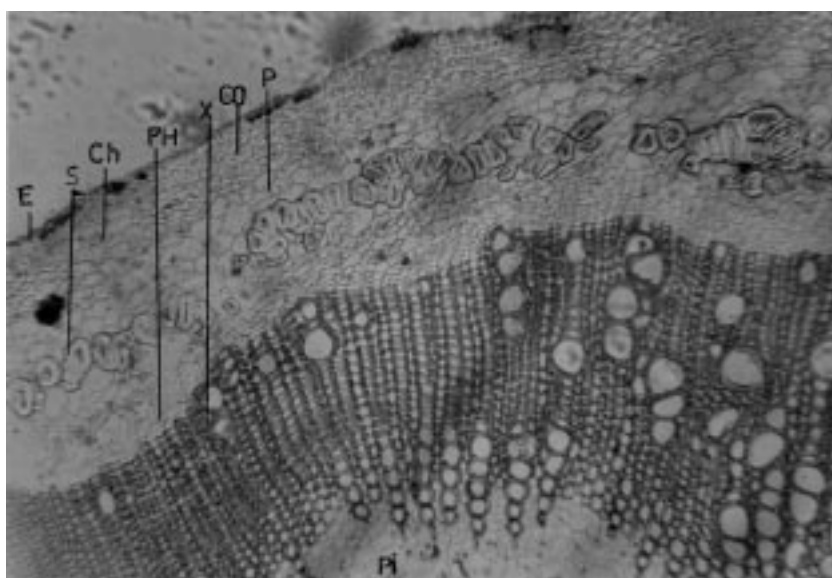


Figure 2. The cross section of the stem of *C. tinctoria* (10x6.3) E- Epidermis, PH- Phloem, X-Xylem, CO-Collenchyma, S- Sclerenchyma, Ch- Chlorenchyma, P- Parenchyma, Pi- Parenchymatic Pith.

fertilizer+25% soil than in 75% soil+25% fertilizer or 50% fertilizer+50% soil (Fig.6). Stem and leaf growth,

however, was greatest in 75% fertilizer+25% soil (Figs 7 and 8). This result shows that root growth and

Table 3. Biometric measurements of stem of *C. tinctoria* grown under different conditions.

Medium	Light condit.	Sample No	Irrigation day	Number of steams	Stem					
					min	max	(cm) mean±S.D	min	max	(mm) mean±S.D.
Fertilizer 75%+ Soil 25%	Light	1	1	3	46.8	63.8	55±6.9	4	6	5±0.8
		2	2	3	41.2	53	47.2±4.8	3	6	4.66±1.24
		3	4	3	38.7	50.6	44.3±4.87	3	5	4±0.8
		4	6	3	33.2	46	39.5±5.2	2.0	3.5	2.83±0.6
	Shade	5	1		*	Plants	shifted	to	shade	
		6	2							
		7	4							
		8	6							
Fertilizer 59%+ Soil 50%	Light	9	1	3	47.2	65	55.13±7.3	3.5	6	4.6±1.02
		10	2	3	43.4	57.4	50±5.7	3.0	5	4±0.8
		11	4	3	37.4	47.3	42±4.07	2.5	4	3.16±0.62
		12	6	3	32.7	40.0	36.9±3.09	2.5	3	2.83±0.23
	Shade	13	1		*	Plants	shifted	to	shade	
		14	2							
		15	4							
		16	6							
Fertilizer 25%+ Soil 75%	Light	17	1	3	44.4	61	53.4±6.85	2.5	5	3.6±1.02
		18	2	3	41.5	50.3	46.5±3.7	2.5	4	3.3±0.6
		19	4	3	38.6	43.8	41.06±2.13	2.0	3	2.5±0.4
		20	6	3	30.3	38.6	34.6±3.3	1.5	2	1.83±0.2
	Shade	21	1		*	Plants	shifted	to	shade	
		22	2							
		23	4							
		24	6							

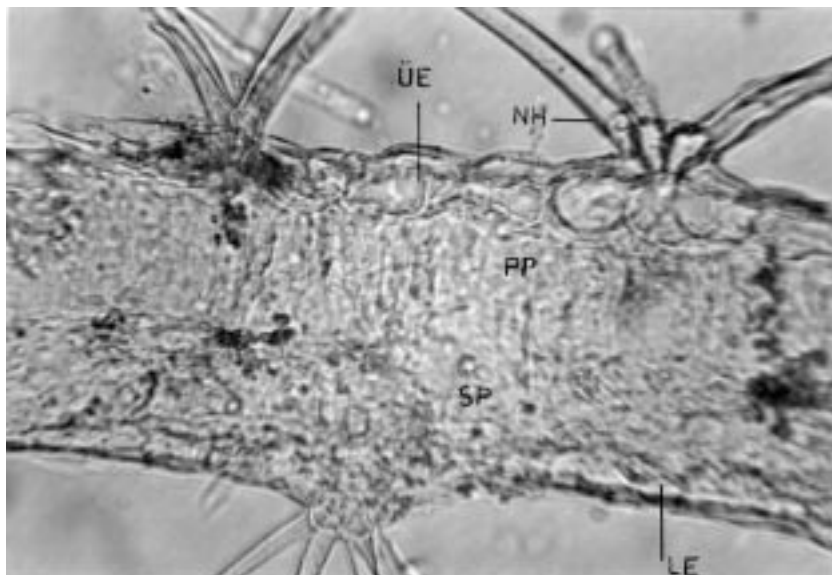
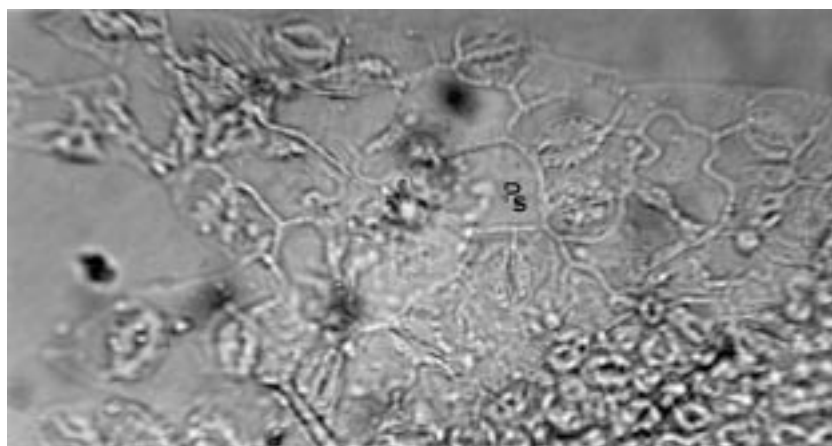


Figure 3. The cross section of the leaf of *C. tinctoria* (40x6.3) NH-Non-glandular hair, Pp-Palisade parenchyma, Sp-Spongy parenchyma, EU-Upper Epidermis, LE-Lower Epidermis.

Table 4. Biometric measurements of leaf of *C. tinctoria* grown under different conditions.

Medium	Light condit.	Sample No	Irrigation day	Number of leaves	Leaf					
					min	max	(cm) mean±S.D	min	max	(mm) mean±S.D.
Fertilizer 75%+ Soil 25%	Light	1	1	3	5.0	5.3	5.14±0.11	2.7	3.9	3.14±0.6
		2	2	3	5.1	5.3	5.2±0.08	3.6	4.3	3.63±0.81
		3	4	3	5.2	5.8	5.2±0.08	2.5	3.1	2.69±0.61
		4	6	3	5.1	6.0	5.55±0.27	2.0	2.7	2.20±0.66
	Shade	5	1		*	Plants	shifted	to	shade	
		6	2							
		7	4							
		8	6							
Fertilizer 59%+ Soil 50%	Light	9	1	3	3.6	4.1	3.71±0.25	1.6	2.2	1.80±0.41
		10	2	3	3.4	3.9	3.62±0.15	1.7	2.4	1.98±0.21
		11	4	3	3.6	4.0	3.80±0.13	1.7	2.6	2.12±0.33
		12	6	3	4.2	5.3	4.83±0.33	1.5	2.3	1.91±0.22
	Shade	13	1		*	Plants	shifted	to	shade	
		14	2							
		15	4							
		16	6							
Fertilizer 25%+ Soil 75%	Light	17	1	3	2.0	2.6	2.32±0.17	1.3	1.7	1.52±0.13
		18	2	3	1.6	2.1	1.87±0.16	1.4	1.8	1.68±0.11
		19	4	3	2.0	3.4	2.55±0.42	1.0	1.5	1.39±0.12
		20	6	3	3.7	4.7	3.93±0.29	1.1	1.6	1.44±0.12
	Shade	21	1		*	Plants	shifted	to	shade	
		22	2							
		23	4							
		24	6							

Figure 4. Upper epidermis with stomata in the transverse section of the leaf of *C. tinctoria* (40x6.3). Ps-Paracytic stomata.

development are inhibited in 75% fertilizer+25% soil while stem and leaf growth and development are stimulated. In our opinion, 75% fertilizer, which contains

organic and inorganic substances, exerts osmotic pressure. Due to this force, the plant could not intake water and nutrients and, consequently, root growth was

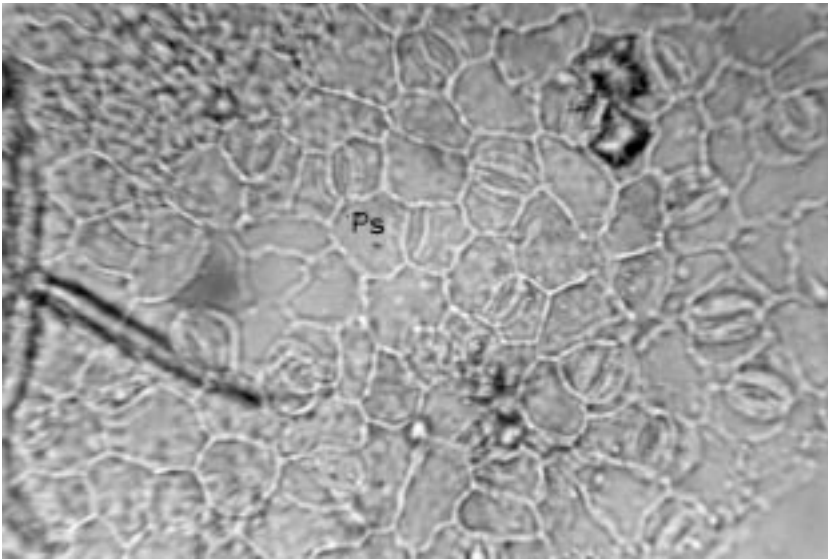


Figure 5. Lower epidermis with stomata in the transverse section of the leaf of *C. tinctoria* (40x6.3). Ps-Paracytic stomata.

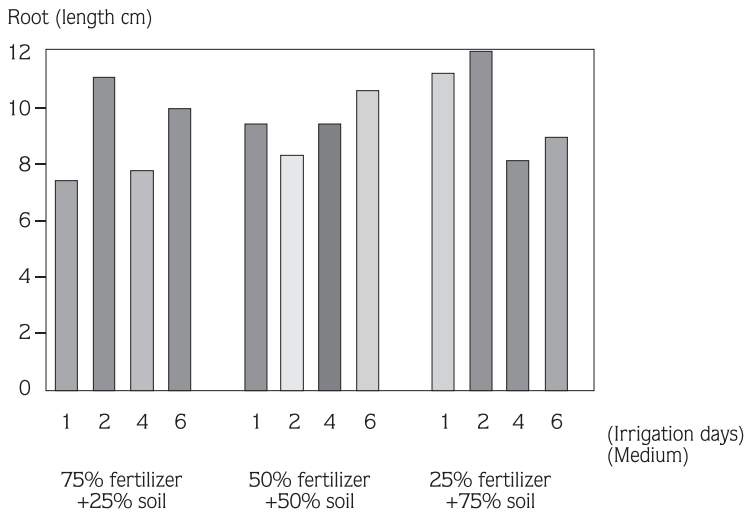


Figure 6. Root development of *C. tinctoria* grown under various conditions.

inhibited. *C. tinctoria* did not show any growth in 25% lime+75% soil and 50% lime+50% soil under any watering conditions. Studies on the effects of light on growth and development showed that *C. tinctoria* did not grow in shade.

Vegetative Propagation

Materials taken from the stem, shoot and the parts between the root and stem of *C. tinctoria* were used for propagation studies. They were planted in sand under greenhouse conditions. The growth of material with

nodes was observed in soil, fertilizer and distilled water. There was no positive response. In the second set, materials of different lengths were taken and different amounts of IBA hormone and pokon hormone used by farmers were applied. No significant results were obtained from the application of these hormones in vegetative propagation.

Ecological Distribution

C. tinctoria grows in ecologically different habitats. It was observed that it grows in macchie, phrygana, *Pinus*

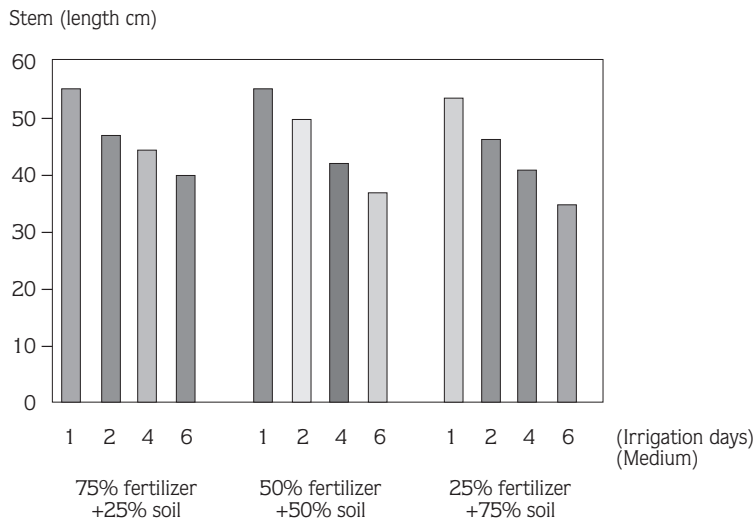


Figure 7. Stem development of *C. tinctoria* grown under various conditions.

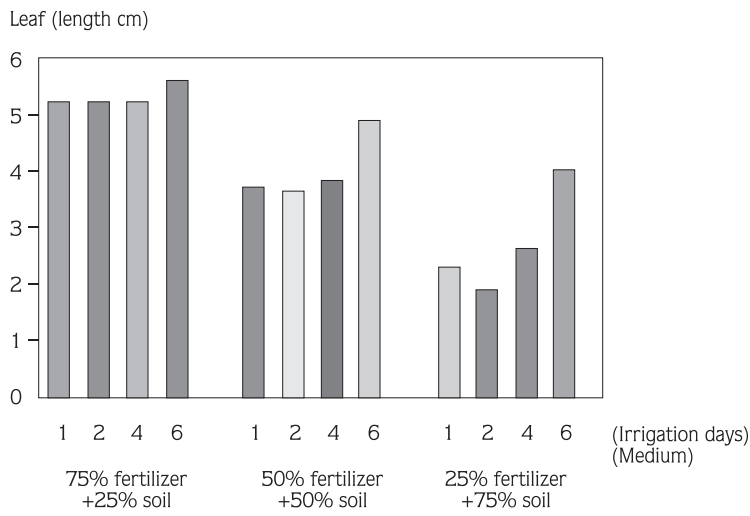


Figure 8. Leaf development of *C. tinctoria* grown under various conditions.

brutia Ten. forests, stony sites, saline steppes, fields, path sides, on rubble sites and on fallow soils. In Turkey it grows at altitudes of 0-1650 m.

There have been few autecological studies on plant species which grow in Turkey and are used for dyeing. Consequently, autecological studies were carried out on *C. tinctoria*, which is used as a source of dyeing material in carpets, kilims and in other crafts in West Anatolia. Our

autecological findings should help during the cultivation of *C. tinctoria* in future and this should benefit the economy of Turkey.

Acknowledgement

I would like to express my thanks and gratitude to Prof.Dr. H.Hüseyin MERT for his valuable help and suggestion in the preparation of the present thesis.

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