AN INVESTIGATION ON ECOLOGY AND QUANTATIVE ANALYSIS OF DYEING SUBSTANCES OF SOME DYE PLANTS DISTRIBUTED IN WEST ANATOLIA

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We certify that we have read this thesis and in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Doctor of Philosophy.

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ABSTRACT

This study was made in order to put forward the morphology, anatomy, ecology and economical importance of *Chrozophora tinctoria* L. and *Rubia tinctorum* L. distributed in West Anatolia.

The morphology of both the plants, the anatomical characteristics of the roots, stems and leaves, and the germination behaviour of seeds were examined. In addition, the physical chemical analysis of the soils where these plants grow and chemical analysis of the plants was done. Results obtained were compared using regression analysis. Plants were cultirated, different conditions and vegatative reproductive capacity determined.

It was found that both the plants generally grow on loam and clayey-loam, neutral to slightly alkaline soils poor and rich in calcium carbonate content, slightly saline, moderately rich., rich and very rich in organic matter, rich and moderately rich in phosphorus, high and very high in potassium. These plants are economically important as such dying capacity of dyes obtained from these plants was determined. They give high quality colour as red and in its tones, as such they are desired, valuable plants. İn addition, these plants are being used as drugs in medicine.

In conclusion, our study material with its large distribution area in West Anatolia, possesses a great economical patential because of the characteristics mentioned above. Thus, the preservation of *C. tinctoria* and *R. tinctorum* and their contribution through planned plantation in future has been the aim of this study. We hope this study will prove an initiator as a potential in the economy of our country.

ÖZET

Bu araştırma Batı Anadoluda yayılış gösteren *Chrozophora tinctoria L.* ve *Rubia tinctorum*'un morfolojisi, anatomisi, ekolojisi ve ekonomik önemini ortaya koymak amacı ile yapılmıştır.

Her iki bitkinin morfolojisi kök, gövde ve yaprak anatomisi; tohumlarının çimlenme özellikleri incelendi. Ayrıca yaşadıkları toprakların fiziksel ve kimyasal analizleri ve bitkilerin kimyasal analizleri yapıldı. Elde edilen sonuçlar regrasyon analiz yöntemi ile karşılaştırıldı. Bitkiler kontrallu koşullarda değişik ortamlarda yetişebilme özellikleri de incelendi ve vejitatif üreme potansiyeli saptandı.

Her iki bitkininde genellikle tınlı ve killi -tınlı bünyeli, nötr ve hafif . alkalı topraklarda yetiştiği, daha çok kireçce fakir ve zengin toprakları tercih ettiği, tuzluluk etkisinin çok az olduğu , organik madde bakımından orta, zengin ve çok zengin, fosfor bakımından orta ve zengin, potasyum bakımından ise yüksek ve çok yüksek toprakları tercih ettikleri saptandı.

Ekonomik açıdan önemli olmalarından dolayı bitkilerden elde edilen boyaların renk kuvvetleri tespit edildi. Kırmızı ve kırmızının çeşitli tonlarının kaliteli renk vermesi bakımından özellikle aranan bitkilerdir. Ayrıca bu bitkiler tıpta drog olarakta kullanılmaktadır.

Sonuç olarak geniş bir yayılış alanına sahip olan araştırma materyallerimiz yukarda saydığımız özellikleri ile ekonomik bir potansiyele sahiptir. *C. tinctoria* ve *R. tinctorum* bitkilerinin korunması ve ilerde yapılması planlanan plantasyonuna katkı sağlamak bu araştırmanın amacını teşkil etmiştir. Bundan dolayı bu potansiyelin ülkemiz ekonomisine kazandırılması yönünde yapılacak çalışmalara öncülük edeceğini ümit ediyoruz.

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CHAPTER ONE
INTRODUCTION

1.Introduction

Due to their nature, human beings have searched in several ways to look beautiful. Because of this reason they have used dyes produced from natural plants which form the basis of cosmetics industry (Harmancıoğlu, 1955). This continued until nineteenth century and in 1968 due to synthetic production of alizerine by Graeke and Leiberman, the techniques in the natural production of dyes was dropped and synthetic dying replaced these (Algan, 1976). The technology used ih the production of natural dyes was known in China as early as 3000 B.C. and amongst the Indians, Phonecians, Hebrews and Venetians in 13th Century A.D. and later was passed on to the Greeks and Romans, it was also known in Africa, Mexico and Peru (Eyuboglu et al, 1983; Tapan, 1983; Sanayii ve Ticaret Bakanlığı, 1991). Turks have used the techniques of natural dying; which was about to fade because of migrations in the Middle Age; successfully and introduced them to the world. (Eyüboğlu et al., 1983). It is known that French have learnt dying of cotton with natural root dyes from Turks in 1715 (Atayolu, 1991). Plant originated dyes are still used succesfully in several areas of arts and industy like carpets, rugs, textile, leather manufacturing, ceramics earthenware vessels and fine arts (Doğan, 1994). High quality dyes and genuine patterned Turkish goods had become famous in the Ottoman times because of the natural dying techniques and certain styles organised under the control of the government organization (Öztürk, 1982). The plantation of *Rubia tinctorum* (root dye) was carried out in Persia, Anatolia, Egypt and India in the begining and after 16 th Century, it was planted also in European countries. It is known that Ottomans have covered two thirds of world needs of root dyes in the 1700's (Eşberk and Koşker, 1945; Eşberk and Harmancıoğlu, 1951). It is stated that in the foreign trade during the Ottoman Empire, the most important customer of root dye exports, which came after the export of cereal and silk; was England (Baykada, 1992). It is seen that plantation of cotton and tobacco has taken the lead over plantation of root dye plants because of cheapness of production of synthetic dye substances as well as due to the support of the agriculture of cotton and tobacco (Enez, 1987 & Baykara, 1992). The price of root dye which was 60 DM/kg in 1870 fell down to 1 DM/kg. and in the end the cultiration of thes plant totally stopped, because synthetic dyes took the place of the naturals (Algan, 1976). Turkish carpets which are dyed with synthetic dyes have lost their importance. Before the entrance of synthetic dye substances into our country, valuable carpets and rugs were woven which were dyed with natural dyes and didn't glide and fade and had light purity. Today synthetic dyes have taken place of plant originated natural dyes. Using root dyes instead of synthetic dyes in carpet and rug production is the reason why Iran brought out world famous Persian carpets (Öztürk, 1982).

In our time every colour of synthetic dyes can be produced very simply, quickly and cheaply. This looks like, an advantage but sone problems like destroying the ecological balance and difficulties in the storage of erastes are disadvantages of synthetic dyes (Anl and Kınacı, 1985). Although the way of dying by using solvents decreases this problem mainly, any way of purifying waste water requires much care and money (Shreve and Brink, 1985). It is being seriously thought around the world now that we should return to natural dyes at least in handicrafts, because synthetic dyes are anti-hygienic and non-degradable in water. Turkish carpets dyed with world famous "Turkish Red" have succecded in taking the place amongst the most demanded carpets and outside demand has begun to increase rapidly (T.M.E, 1989).

Our country has a rich flora because of it's geographical situation and climatic features. Because of this reason many studies have been made in this connection. At the end of these studies, existence of approximately 10243 taxo has been put forth (Davis, 1966-1988). To know these plants better and to make use of them economically, they should be investigated ecologically, morphologically and anatomically. The importance of the ecological investigations on the plants distributed naturally in our country is important from the point of view of their economical evaluation. Studies undertaken in our country on the ecology of some taxo include those on *Myrtus communis* (Öztürk, 1970), *Ceratonia siliqua* (Seçmen, 1972), *Mentha species* (Öztürk & Görk, 1979), *Inula viscosa* (Pirdal, 1980), *Rubia* *tinctorum*, *Cistus laurifolius* and *Rumex obtusifolius* subsp. *subalpinus* (Başlar & Oflas, 1990) *vitex agnus-castus* (Doğan, 1994) and *Spartium junceum* (Mert at al., 1995). Besides these autecological studies, it is known since the ancient times that dye substances could be produced from some natural plants existing in the flora of our country and from stone, soils, mines and animals (Arlı, 1982). There is no doubt that the main source of production of the dye substances increases the value of these natural plants. The dying substances considered above are produced from different organs like flower, fruit, leaf, stem and root of plants which are called dye-plants (Harmancıoğlu, 1973). It is reported that natural dying substances are also raised from non-flowering plants (Eyüboğlu et al., 1983). It is seen that the natural dyes obtained from these plants include three main colours; red, yellow and blue. It possible to have the colors from a mixture of these colors, as they can be produced from only one plant (Uğur, 1988). Nearly 150 kinds of plants are used in the production of dyes naturally in our country (Mert et al., 1992). There is a great increase in the autocological studies of the plants used in natural dye production (Algan, 1976; Enez, 1981, Arlı, 1982; Başlar & Oflas, 1990; Mert et al., 1995). *Rubia tinctorum* and *Chorozophora tinctoria* which grow widely in West Anatolia; the area of our study; were used as the research material in our study because of their dye value and medicinal importance. All the plants used in dying in different styles are called as "dye plants". Because widely used and high quality dye is produced from *Rubia tinctorum* known as "root dye", other plants which are used in dying have taken same name. This plant is called plant grass, tongue-bleeder, red-paint, red-root, sticky-grass and egg paint in different parts of our country (Başlar & Oflas, 1996). The world famous dye called "Turkish red" or "Edirne red" which is used in Izmir carpets, silk textiles of Anatolia and Syria and cotton products of Thessellia and Macedonia is produced from *Rubia tinctorum* (Baytop, 1974; Başlar & Oflas, 1996; Baykara, 1992). It is known that dying substances are obtained from the roots and rhizoms of this plant (Algan, 1976). Some workers have mentioned that dying substance is obtained from the roots of this plant because they do not know the rhizome of this plant (Hegi, 1906, Eşberk & Köşker, 1943-1951, Engler, 1964). It is reported that a grey colored dye is obtained from the fresh fruits of *Rubia tinctorum* (Sanayii ve Ticaret Bakanlığı, 1991). It is also reported that the dying substance which is produced from the underground suckers of this plant includes pseudo-purparin, rubiadin, minjistin, alizarin and purparin (Baylav, 1963; Enez, 1987). It is also known that this plant is used as a diuretic (Tanker, 1985), against stones (Blomeke et al., 1991) and fodder (Baykara, 1992).

The other type of plant chosen in our study material *Chrozophora tinctora* grows as a ruderal plant in plantation areas. This plant belongs to the family *Euphorbiaceae* and is called as turnsole plant. The dye substances can be produced from all it's organs (Baylav, 1963; Mert et al., 1993). The plant which is known as "Akbaş" (White head) in some parts of Anatolia is an annual type of this plant (Baytop, 1994). Although many studies have been made on the morphology and anatomy of *Rubia tinctorum*, these studies have been done mainly on the family level (Hegi, 1906; Bonnier, 1934; Krause, 1939; Raymond, 1941; Brauner & Hasman, 1945; Engler, 1964; Algan, 1976; Başlar & Oflas, 1996). It is also reported that biochemical researchs have been made on *Rubia tinctorum* (Hawaka et al., 1984; Sato et al., 1990). Very little studies have been made on the plant *Chrozophora tinctoria* (Saovedra et al., 1988; Hidalgo et al., 1990). These studies are mostly on the ecological level.

In the light of the facts cited above and side effects of synthetic dyes in order to evaluate the potential of the production of natural dyes and dying substances the autological studies on *Rubia tinctorum* and *Chrozophora tinctoria* have been carried out in this study. We think that the results of our study will enlighten the possibilities for the plantation of these plants from now on and add to the countries economical potential.

CHAPTER TWO
MATERIAL AND METHODS

2. Material And Methods

2. 1 Localities

The specimens of *Rubia tinctorum* and *Chrozophora tinctoria* collected from different localities in west Anatolia were identified taxonomically with the help of flora of Turkey and East Aegean Islands (Davis, 1987).

Chrozophora tinctoria;

<u>MANİSA</u>

1. B1. Akhisar entrance, railway side.
2. BI. Süleymanlı village exit, road side.
3. BI. Fallow field between Kırkağaç and Soma.
4. BI. Soma; Turgutalp town exit, fallow field.
5. B2. Near Kula-Eşme road, around söğüt river.
6. BI. Turgutlu - Avşar village , tabacco field.

BALIKESİR

7. BI. Savaştepe, Karaçam village; next to Soma road.
8. BI. Ayvalık; Altınova, next to cemetery
9. BI. Burhaniye; Karaağaç town, fallow field
10. BI. Edremit; City exit, olive field
11. AI. Bandırma; Aksakal entrance, cotton field.
12. AI. Bandırma; Erdek, Gelinönü environs.
13. B2. Balikesir; Susurluk river side,

ÇANAKKALE

14. AI. Lapseki City entrance, peach field.

İZMİR

15. BI. Kınık entrance , tabacco field.

16. BI. Bergama Bakırçay environs

17. BI. 2 km. to Kemalpaşa, road side

18. B2. Beydağ; Çiftlik village

19. BI:Tire; Gökçe town exit, tabacco field.

20, BI. Torbalı; Aslanlar village entrance, olive field.

21. BI. Menemen, Türkeli village, peach field.

22. BI. Aliağa, Kalabak village, İncirbükü plateau.

23. BI. Aliağa; Çaltıdere village, Kalabak environs, sea side

24. B1. Foça; Bağarası village entrance, tabacco field side.

25. BI. Urla - Çeşmealtı, road side.

26. BI. Çeşme; Ilıca Imamoğlu river environs

27. BI. Çamlık -Aydın road exıt, road sıde

MUĞLA

28. CI. Milas-Selimiye village, Dedekuyu environs, melon field

29. CI. Bodrum-Turgutreis after monuement, road side.

30. C2. Yatağan; Maden tabacco fields

31. C2. 4 km to Ula, cotton field

32. C2. Ula; next to Ataköy cemetery, corn field.

33. C2. Köyceğiz; Doğuşbelen exit, corn field

34. C2 Fethiye; Hisarönü

DENİZLİ

35. C2. Acıpayam; Darıveren village, fallow field

36. C2. Kale; kavakdede

37. B2. Buldan; 9 km west, road side

AYDIN

38. CI. Kuşadası; old road entrance, fallow field

39. CI. Söke; 5 km after Ağaçlıköy, fallow field

40. C2. Sultanhisar city exit, 3 km west, road sıde .

41. CI. 3 km to Koçarlı, fallow field

42. Germencik-Ortaklar village, fig orchard.

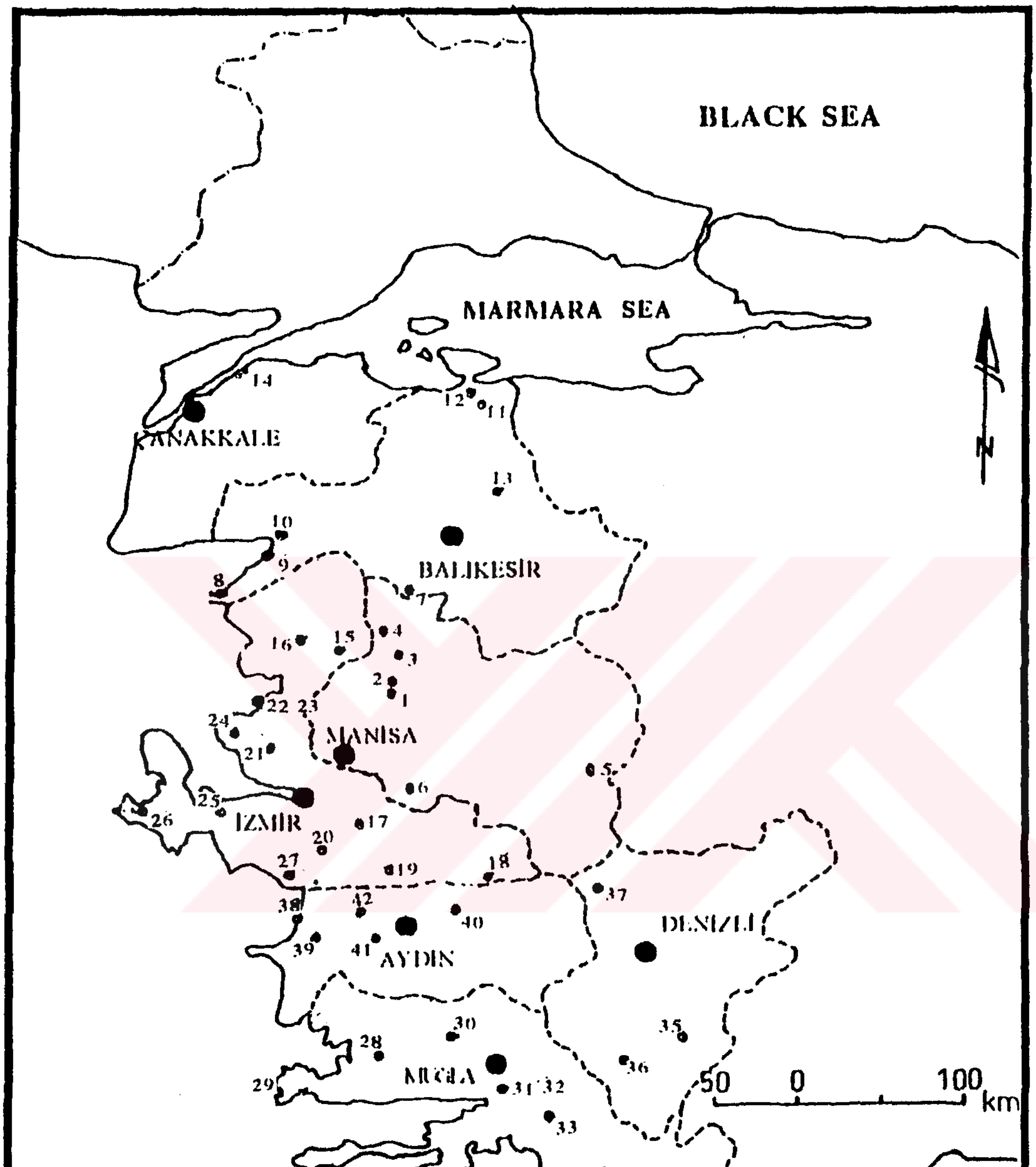

Figure 2.1: Study area of *C.tinctoria* in West Anatolia.

R. tinctorum ;

MANİSA

1. BI. Üçpınar; Üçpınar exit road side
2. BI. Beydere
3. BI. Akhisar; City entrance, railway side
4. BI. Kırkağaç; 3 km to Bakır village, olive field
5. BI. Soma, Turgutalp village exit, road side.

6;BI Turgutlu; Avşar village, Alacalı environs, vineyards.

7. B2. Salihli, Taytan village, Bezirganlı environs, road side.

BALIKESİR

8. B1 Savaştepe: near Halkapınar, field
9. B1 Ayvalık: Altınova village exit, next to cemetery
10. B1 Burhaniye: Karaağaç village
11. A1 Manyas: Salurköy, north, pasture
12. A1 Bandırma: Erdek exit, field
13. B2 Susurluk: In city, around the bridge
14. B2 Bigadiç: City extrance, irrigation channel side
15. B2 Sındırgı: Kumluca environs, field

ÇANAKKALE

16. A1 Ayvacık: Süleymanköy, garden side
17. A1 Ezine: City centre
18. A1 Bayramiç: Old cemetery
19. A1 Çan: Hurmaköy, garden side
20. A1 Lapseki: City entrance, garden
21. A1 Biga: Hamdiköy mahal.

İZMİR

22. B1. Kınık: city entrance, road side, field
23. B1. Bergama: Bakırçay environs, garden
24. B1. Menemen: Türkeli village, Çanakkale road side, vineyard
25. B1. Aliağa: Çaltıdere village, Kalabak environs, sea side.
26. B1. Foça: Bağarası village entrance, field
27. B1. Urla: Çeşmealtı junction.

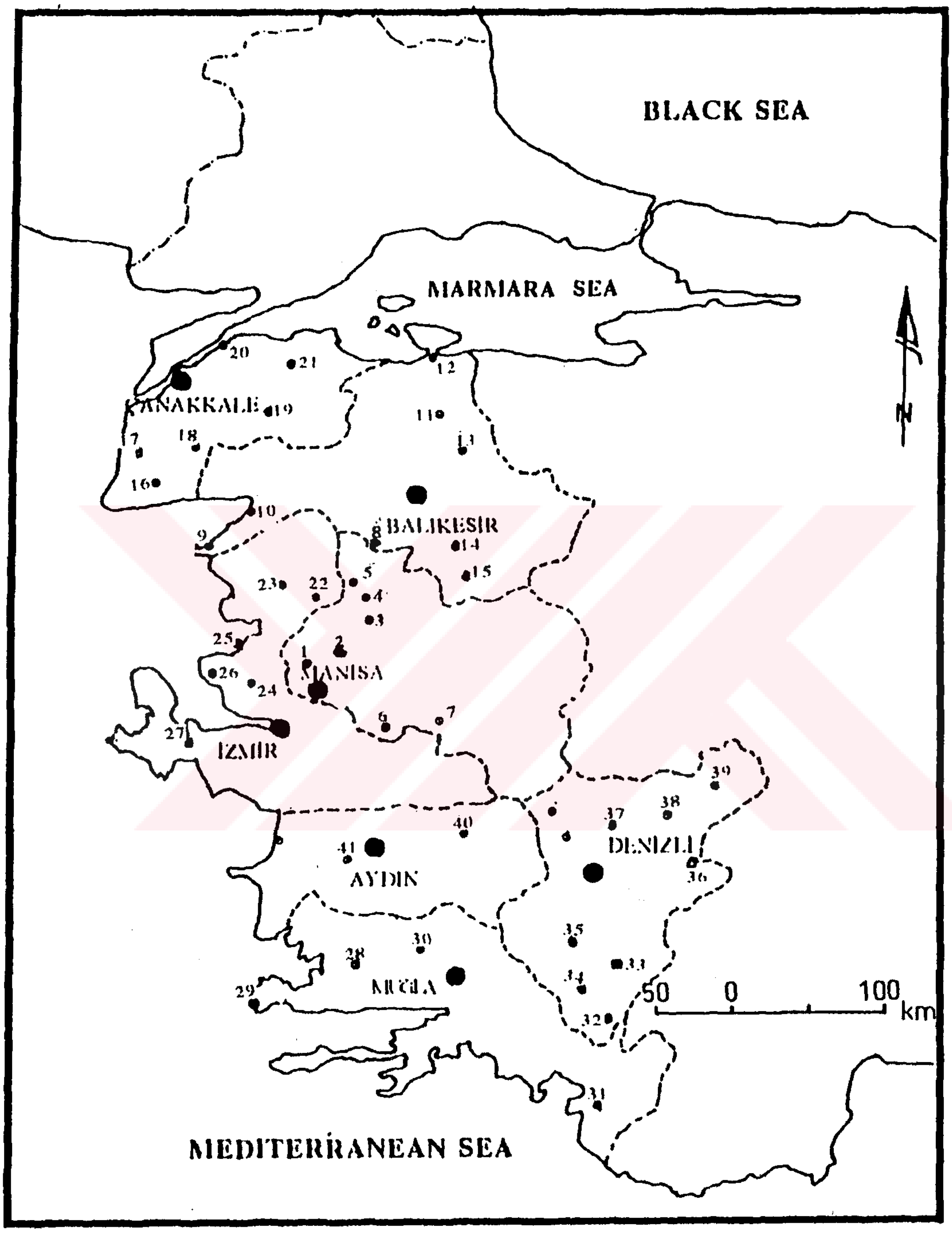

Figure 2.2: Study area of *R.tinctorum* in West Anatolia.

MUĞLA

28. C1. Milas: Ağaçlıhöyük village, Beyevi door.

29. C1. Bodrum: Turgutreis, Akyarlar, east, garden

30. C2. Yatağan: Bozarmut village, Patlıbükü environs, garden

31. C2. Fethiye: Seke, near forest

DENİZLİ

32. C2. Çameli: Kınıkyeri village, garden side

33. C2. Acıpayam: Oğuzköy, vineyard

34. C2. Kale: Kavakdede environs

35. C2. Tavas: Medet village entrance, cemetery

36. C2. Çardak: Beylerli village

37. B2. Çal: Denizler village exit, road side

38. B2. Baklan: city centre

39. B2. Çivril: Menteş village, old water well environs.

AYDIN

40. C2. Nazilli: Durasallı village

41. C1. Koçarlı: Cotton field.

2. 2 Morphology

41 and 42 samples were used in the biometrik measurements. Mean and standard deviation values of the measurements were calculated according to Rummel (1970).

2. 3 Anatomy

Plant materials of *C. tinctoria* and *R. tinctorum* were collected from different localities, were fixed in 70% alcohol and then the anatomical sections of root, rhizome, stem and leaf were taken. After staining with "sartur" reactive, photographs were taken by means of an optic microscope.

2. 4 Germination

Fresh and one year old seeds were used. Seeds were collected immediately after their maturation and germinated after subjected to shock treatment at low temperature (0°-5°C). Since seed coat was hard rubbing with a sandpaper facilitated the germination. Seeds were sterilized with 5% sodium hypochloride to prevent the formation of fungus during the experiment. Seeds were left for germination after following treatments:

1-Seeds with hard seed coat were thined at only one point by using emery.

2-Two different types of strelized seeds were placed in concentrated H_2SO_4 for 5, 10, 15, 20 and 30 minutes, washed with top water for 1 or 2 minutes and left in distilled water for 15 minutes (Özdemir, 1993).

3- 0-(-5)°C cold shock was given for 6, 12 and 24 hours (Başlar, 1990).

4- Both types of seeds were left in distilled water for 5 minutes at 70°C (Doğan, 1994).

5- After the above mentioned treatments the seeds were left in mud and sawdust for one day, three days, one week, two weeks, one month and three months in order to follow the germination behaviour.

After the five treatments given above seeds were placed for seed germination for 6, 12, 18 and 24 hours under light as well as continuous darkness using the temperatures 5°, 10°, 15°, °20, 25°, 30°, 35°, 40°, 45° and 50°C in preset avens.

2.5 Soil Analysis

Soil samples were collected from above mentioned localites between July-August. The soil samples were collected, after cleaning the litter on the soil, put into polyethylene bags and brought immediately to the laboratory. They were left under laboratory conditions to get air dried. The completely dried up soil samples were ground, passed through a 2 mm sieve and subjected to analysis.

pH, texture, water holding capacity, total soluble salts, calcium carbonate ($CaCO_3$) and organic matter, total phosphorus and potassium contents in soils were determined by the methods outlined in detail in Öztürk et al. (1996).

2. 6 Plant Analysis

Aboveground parts of the plants were collected from different localities in the flowering and fruiting periods (July-August), dried at 80°C in the air blown oven for 24 hours ground with blender and prepared for analysis.

Nitrogen(N), Phosphorus(p), Potassium (k), Calcium (Ca), Sodium (Na), Manganese (Mn), Zinc (Zn), and Copper (cu) were determined according to the methods given in detail by Kacar (1962).

2.7 Statistical Evaluation of the Soil and Plant Analysis Results

Positive or negative correlation between organic matter pH, P, K, total soluble salts, $CaCO_3$ in soils and N, P, K, Ca, Na, Mn, Zn and Cu in plants were investigated. Regression curves and correlation coefficients were obtained from the analysis results statistically by means of Jmp software in the computer according to Akkaya and Hasgür (1989).

2.8 Growth of Plants in Different Media

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

Seeds were left for, germination in the pots containing sand. After reaching 15 cm size seedlings were transplanted to five different mixtures given above with 3 in each. These were allowed to grow under two different conditions, light and shade. Pots were irrigated as once a day, once in two days, once in four days and once in six days and these were replicated. *C. tinctoria* grows in 132 days and *R. tinctorum* grows in 902 days. Measurments of plants were taken, standard deviation calculated and figures given in tables (Rummel 1970).

2.9 Vegetative Propogation

The cuttings taken from roots, shoots, parts between roots and stem, and stem were used for this purpose. The lenghts of 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 used as treatments before growing cuttings. Hormonal treatment was given under four different times as 1, 4, 7 and 10 hours. IBA was applied with hormonal concentrating like 10, 20, 30, 40 and 50 ppm.

2.10 Dye Extraction and Colour Density

All parts of *C. tinctoria* and roots of *R. tinctorum* were collected from each locality and left under room conditions for 30 days for drying. These wara then ground by Rotar Beater blender. From each plant 100 gr were taken and 1 kg water added, these were boiled for one hour and filtered. The extract was obtained and boiled again for one hour at 70°C with a ratio of 1/30 flotte (for 1 gr wool, 30 cc extraction) (Öztüreli, 1992). Wools were washed until the clear water flows, and remisions (minumum reflection) taken with spectral photometer DC 3881 colour measurment device and evaluation made by Kubella-Munk equality (Duran, 1983).

CHAPTER THREE

RESULTS

3. Results

3. 1 Morphology

C. tinctoria, the only species of the *Chrozophora* genus in *Euphorbiaceae* family in Turkey is an annual plant (Fig. 3.1). Our phenological observations showed that flowering season is between the months of June and September.

C. tinctoria is an ascending herb with a mean length/breadth as 38.59 cm × 48.81 cm, often becoming woody at base (6.14 mm × 4.75 cm) mean root length being 5.14 mm and breadth 17.33 cm. ; leaves are alternat, rombic-ovate to ovate-lanceolate, mean length being 2.75 cm breadth 4.45 cm, apex is acute or obtuse, base cuneate to shallowly cordate, shallowly repond-dentate; mean petiole length is 4.99 cm and pedicel is 14.14 cm. long, stipules 2-5 mm. Inflorescences paniculat, (3 × 6 cm.) male flowers have 5 which are linear-lanceolate (0.98 mm × 3.11 mm), stellat pubuscent outside, glabrous inside; petals are 5, yellowish, eliptic-lanecolate, mean (1.25 mm × 3.66 mm), lepidote outside, pubescent inside, hairs simple; stamens 3-12, (0.73 mm × 1.22 mm), filaments, have a mean lenght of 0.98 mm, female flowers have sepals and petals both resembling male sepals with a (0.96 mm × 3. 12 mm) petals have a length/breadth of 1.19 mm × 3.88 mm; ovary is densely lepidote, mean 3.04 mm; style is stellat-pubescent outside, papillose inside, homostylous, mean length 1.06 mm; stigma is bifulcat, mean length 1.30 mm; thecal arrangement is parallel, anthers open lenghtwise, anther base obtuse, anther basifixed, mean length 1.22 mm; stamens are antipetalus, filament has a mean length 0.98 mm; fruit is schizocarp, sparingly to evenly lepidote, mean length 0.58 cm × 0.55 cm. ; purple, seeds have a mean length/breadth 3.72 mm × 4.51 mm, pale grey. Placental position is free-central (Table 3.1).

Figure 3.1 General appearance of *C.tinctoria*.

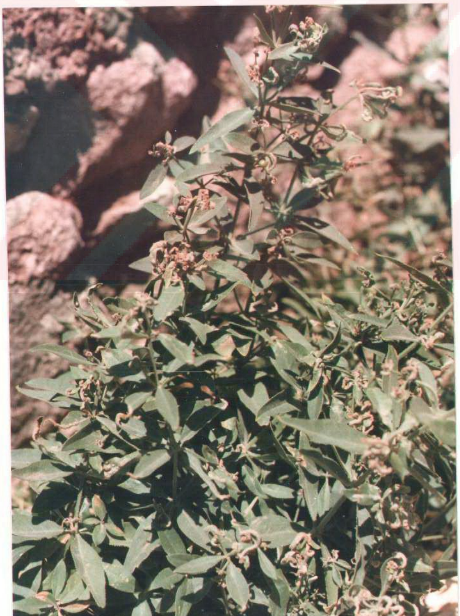

Figure 3.2 General appearance of *R.tinctorum*.

Table 3.1 Biometric measurements of *C.tinctoria*.

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

Table 3.2 Biometric measurements of *R.tinctorum*.

Plant parts	No of measurements	Width			Length		
		Min	Max	mean ±.s.d.	Min	Max	mean±.s.d.
Rhizome	42	2.00mm	7.00mm	3.90±1.32	20.40cm	51.30cm	32.23±9.54
Stem	42	1.00mm	5.00mm	2.97 ±1.04	60.20cm	172.40cm	118.08 ±34.15
Leaf	42	1.10cm	3.30cm	2.15 ±0.63	1.30cm	5.90cm	3.93 ±1.23
Pedicel	42	-	-	-	0.75cm	8.50cm	4.56 ±2.71
Corolla	42	2.10mm	3.10mm	2.53 ±0.23	3.20mm	5.40mm	4.31± 0.68
Stamen	43	-	-	-	0.50mm	0.90mm	0.65 ±0.13
Flower	42	-	-	-	1.00mm	16.00mm	6.50 ±4.70
Style	42	-	-	-	0.10mm	0.97mm	0.40± 0.34
Stigma	42	-	-	-	0.10mm	0.95mm	0.52± 0.33
Ovary	42	-	-	-	0.40mm	0.95mm	0.68 ±1.17
Fruit	43	-	-	-	3.00mm	8.00mm	5.27 ±1.24

Rubia genus belonging to *Rubiaceae* family has 5 species in Turkey. *R. tinctorum* is one of these 5 species, being perennial, herbaceous and hermofrodit plant. Our phenological observations show that flowering occurs between Jine and August (Fig. 3.2).

R. tinctorum is a trailing or scrambling plant, length/breadth being 60-172 cm; rhizome is red (3.90 mm × 51.30 cm.), branched, without runners. Stems are herbaceous (112.78 cm × 2.97 mm.), quadrangular to subulate, ± sparingly retrorsely scabrid on angles to almost smooth, glabrous, or sometimes pubuscent on nodes. Leaves subcoriaceous, in whorls (2.15 × 3.93 cm.), lanceolate or oblong-elliptic to broadly ovate, cuspidate or ± shortly acuminate, usually distinctly petiolate, petiole being 15(-20) mm long, midrib and margins retrorsely aculeolate, the latter often antrorsely aculeolate towards apex, lateral veins distinct beneath. Inflorescence lax, much-branched, many-flowered, pyramidal to broadly pyramidal, partial inflorescences terminal and lateral, up to 30 cm. subtended by elliptic to broadly ovate bracts. Pedicels show a mean length of 4.56 mm. Perianth actinomorph. Corolla pale greenish-yellow, infundibular (2.53 mm × 4.31 mm.), lobes are triangular-lanceolate, gradually to rather abruptly aristate (awns up to 0.7 mm.).

Stamen are epipetalus, number of stamens is 6-7(5), mean length 0.65 mm. Anthers are oblong, dorsifixed, base obtuse, opening lenghtwise, thecal arrangement being parallel. Stigma is types capitate, mean length 0.52 mm, style homostylous, mean length 0.40 mm, ovary is hypogynous, mean length 0.68 mm. Fruits are mericarp, mean length 5.27 mm, black. Placental position is basal (Table 3.2).

3. 2 Anatomy

3. 2. 1 Root

Then cross-section of *C. tinctoria* root shows epidermis, cortex with schleranchymatic as a groups of few cells, phloem and xylem tissue which occupies a large area. Endodermis and pericycle is not visible (Fig. 3.3).

The cross-section of the root of *R. tinctorum* shows disintegrated epidermis and cortex. Endodermis and pericyle are not visible. There is a vascular system and a large pith area with big cells take place (Fig. 3.4).

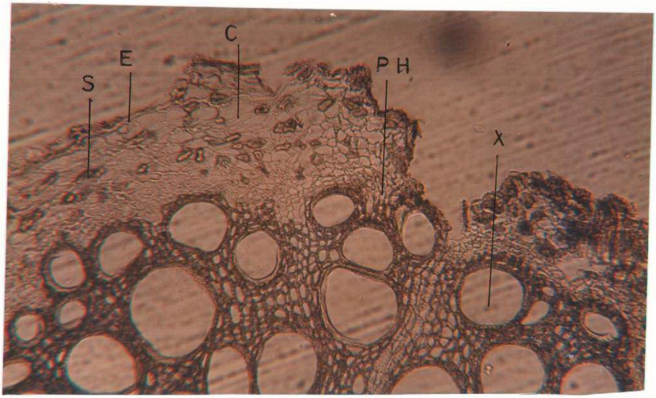

Figure 3.3 The cross section of the root of *C.tinctoria*. (10x6.3). E-Epidermis, C-Cortex, S-Sclerenchyma, PH-Phloem, X-Xylem

3. 2. 2 Rhizome

R. tinctorum rhizome shows disintegrated epidermis followed by periderm, cortex and vascular system. In the center a pith occupies a large area and has big cells (Fig. 3.5).

3. 2. 3 Stem

The non-glandular unicellular epidermal hairs cover the epidermis of *C. tinctoria*. Epidermis is followed by cuticle, collenchyma with five rows, cortex with schleranchymatic tissue in the form of groups, vascular system and pith, later occupies a large area (Fig. 3.6).

The cross-section of the stem of *R. tinctorum* shows an epidermis, covered with thick-walled cuticle, with a single row of round cells. Below the epidermis, chlorenchyma lies as a thin layer followed by cortex, vascular system and pith(Fig. 3.7). Stomata occur on some parts of the stem (Fig. 3.8).

3. 3. 4 Leaf

The cross-section of the leaf of *C. tinctoria* shows an upper and lower epidermis covered with cuticle and unicellular and non-glandular hairs. Hairs are stellate type. The leaves are bifacial different upper and lower surfaces. Amerryllis type of stomata are found at the same level with epidermis on the upper and lower surface of the leaves. They are of paracytic type. A cross-section of the leaf of *C. tinctoria* shows epidermis, palisade and spongy paranchyma. The palisade parenchyma cells are arranged tightly and form one row, with small intercellular area. These cells are rich in chlorophyll. Spongy paranchyma cells contain less chlorophyll, possess bigger intercellular area but occupy less area. Leaves are amphistomatic with many stomata on the upper and lower epidermis (Figs. 3. 9, 10, 11).

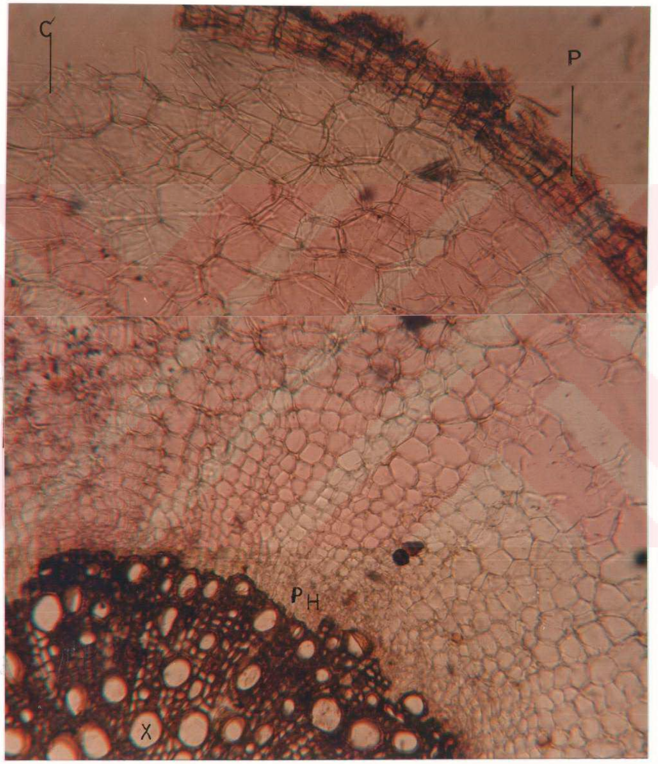

Figure 3.4 The cross section of the root of *R.tinctorum*. (10x6.3). P-Periderm, C-Cortex, PH-Phloem, X-Xylem

The leaves of *R. tinctorum* too are bifacial. Lower and upper epidermis are covered with a thin cuticle consisting of big and small one row of cells. Palisade parenchyma cells are in a single row and their thickness is half of the leaves. Spongy parenchyma cells lie beneath the palisade parenchyma and are more rounded with bigger intercellular spaces. Stomata lie only on the lower epidermis the species is hypostomatal. Stomata of *R. tinctorum* are of ameryllis type. A wrinkled cuticle is found on the lower surface, no cuticle on the upper surface. It has paracytic type of stomata (Figs. 3.12, 13, 14).

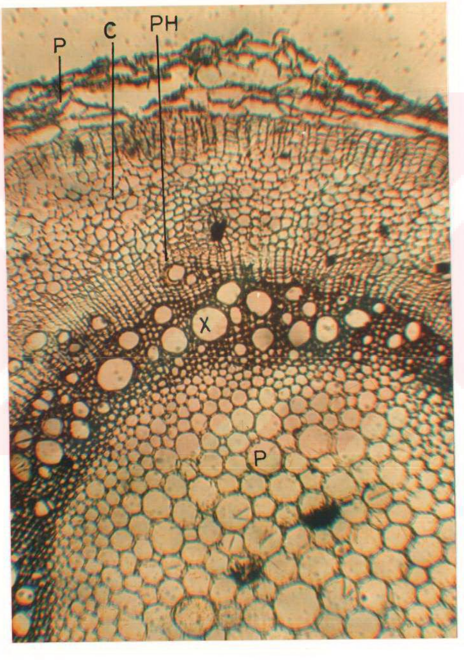

Figure 3.5 The cross section of the rhizome of *R.tinctorum* (3.2x6.3). (10x6.3). P-Periderm, C-Cortex, PH-Phloem, X-Xylem, p-Pith

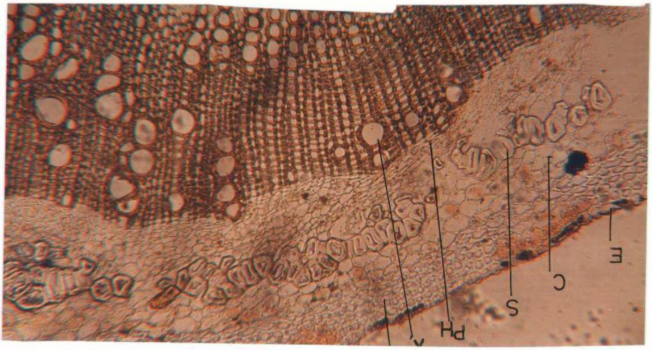

Figure 3.6 The cross section of the stem of *C.tinctoria* (10x6.3). E-Epidermis, C-Cortex, PH-Phloem, X-Xylem, CO-Collenchyma, S-Scleranchyma

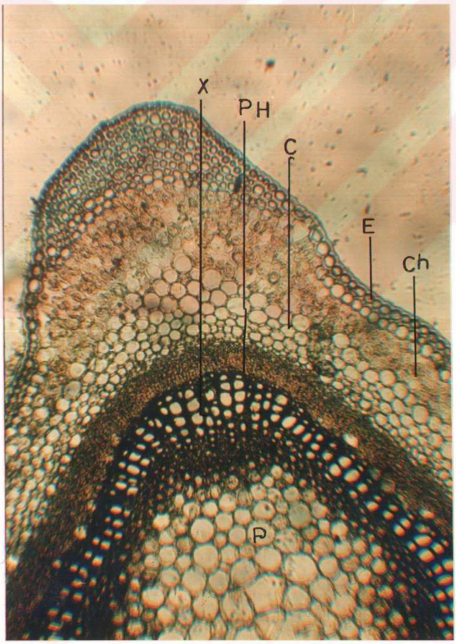

Figure 3.7 The cross section of the stem of *R.tinctorum*. (3.2x6.3). E-Epidermis, C-Cortex, PH-Phloem, X-Xylem, Ch-Chlorenchyma

Figure 3.8 The cross section of the stem of *R.tinctorum* (40x6.3). St-Stomata

Figure 3.9 The cross section of the leaf of *C.tinctoria* (40x6.3). NH-Non-glandular hair. PP-Palisade paranchyma, Sp-Spongy paranchyma, UE-Upper Epidermis, LE- Lower Epidermis

Figure 3.10 Upper epidermis with stomata in the transverse section of the leaf of *C.tinctoria* (40x6.3). Ps-Paracytic stomata

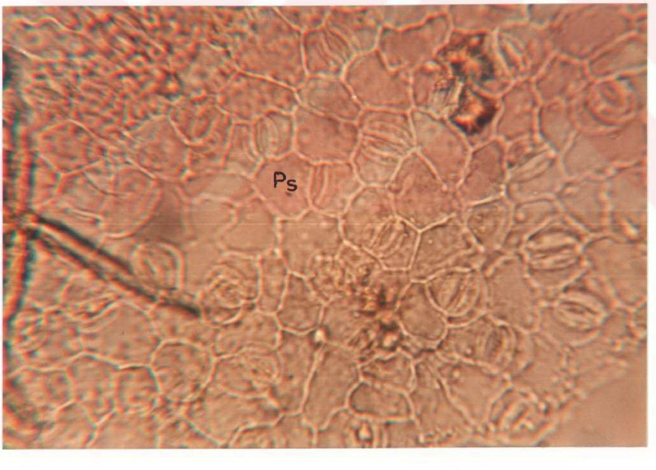

Figure 3.11 Lower epidermis with stomata in the transverse section the leaf of *C.tinctoria* (40x6.3). Ps-Paracytic stomata

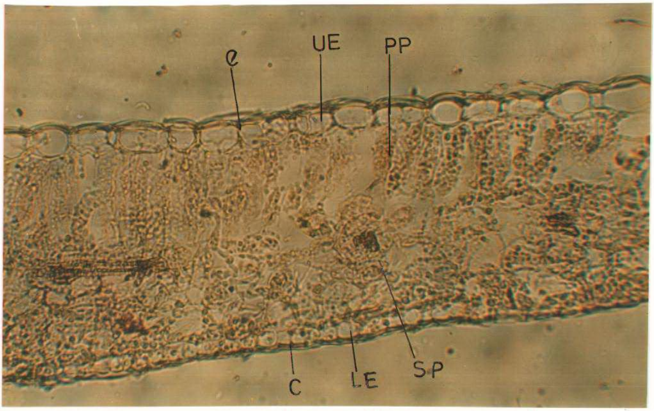

Figure 3.12 The cross section of the leaf of *R.tinctorum* (6.3x10).

PP-Palisade paranchyma, Sp-Spongy paranchyma, UE-Upper Epidermis,

LE- Lower Epidermis, C-Cuticile

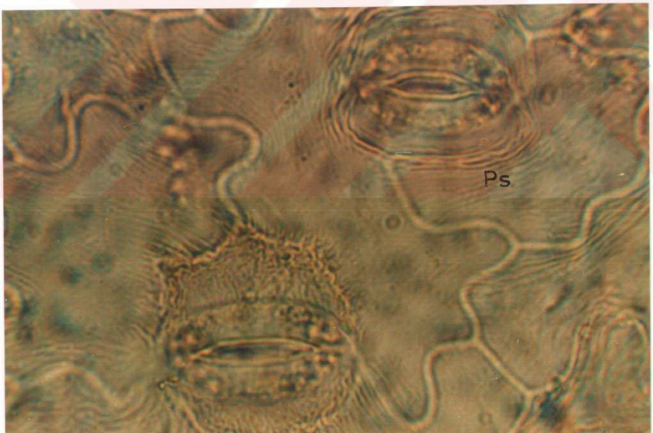

Figure 3.13 Lower epidermis with stomata in the transverse section of the leaf of *R.tinctorum*. (40x6.3). Ps-Paracytic Stomata

15°, 20°, 25°, 30°, 35°, 40°, 45° and 50°C in preset avens. These operations were followed every year and continued for three years, but germination of *C. tinctoria* seeds could not be observed.

Table 3.3 Germination behaviors of *R.tinctorum* under different conditions.

Temperature (°C)	Germination(%)	Light (hours)	Germination(%)
10	53	Dark	52
15	60	3	60
20	62	6	62
25	72	12	84
30	20	18	24
-	-	24	12

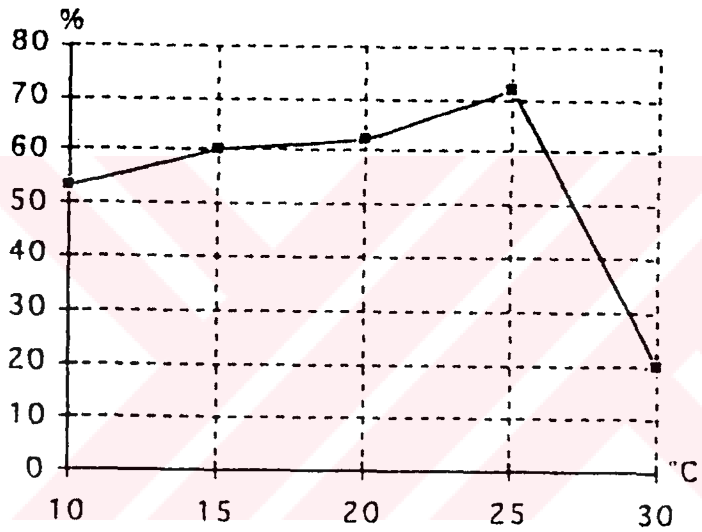

Figure 3.15 Germination rates of *R.tinctorum* seeds at different temperatures.

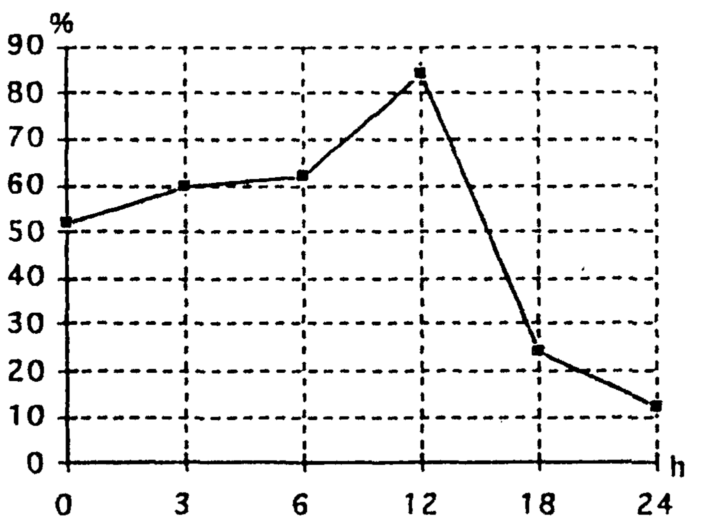

Figure 3.16 Germination rates of *R.tinctorum* seeds under different photoperiods.

R.tinctorum seeds were treated as above and left in hot water for three hours. After second day germination was observed. At different temperatures, germination ratios and time was recorded. At 10°C and 30°C temperatures, there was no germination. At 10 °C germination began after 23 days. Optimum germination was seen at 25°C being 72% (Table 3.3, Fig. 3.15).

Investigations on the effects of different photoperiods on the seeds placed under light for 0, 3, 6, 12, 18 and 24 hours were followed. Seeds germinate at the end of second day with a 8 percent germination. 12 hours photoperiod shows the highest germination percentage (84%) (Table 3.3, Fig. 3.16).

3. 4 Physical Analysis Results of the Soils

It is seen that the pH of the 42 soil samples in which *C. tinctoria* grows lies between 6. 28-7.90 (Table 3.4), 2.38% of the soils are weakly acidic, 33.33% neutral, 61.90 % slightly alkaline and 2.38% moderately alkaline (Fig. 3.17).

Table 3.4 shows that *C. tinctoria* grows on loamy (45.23%), clayey-loam (42.85%) and turfy(11.90%) soils (Fig. 3.18). $CaCO_3$ content of the soils varies between 0-30.33% (Table 3.4). It is seen that 40. 47% of these soils are poor in $CaCO_3$, 19. 04% are calcareous, 16. 16% are rich in $CaCO_3$ and 23. 80% are very rich in $CaCO_3$ content (Fig. 3.19). Maximum water holding capacity values of these soils show that 54.76% are above 50% and 45, 23% lie between 35. 56-50% (Fig. 3.20). In table 3.4, the salinity values of the soils in which *C. tinctoria* grows are presented. Soil salinity vries between 0.017-0.206%. 95.23% of these soils are non-saline and 4.76% slightly saline (Fig. 3.21).

pH of the 41 soil samples in which *R. tinctorum* grows lies between 6.22-7.98 (Table 3.5). 2.44% of the soils are weakly acidic, 31.72% neutral, 63.44% slightly alkaline and 2.4% are moderately alkaline (Fig. 3.22).

The soils in which *R. tinctorum* grows (Table 3.5) are loamy (29.28%) clayey-loam (43.88%) and turfy (26.84%) (Fig. 3.23) $CaCO_3$ content of the soils in which *R. tinctorum* grows varies between 0-29.78%, 31.76% very calceareous, 19.52% rich in $CaCO_3$, 10.51%

being calcerous in $CaCO_3$ and 29.28% poor in calcerous (Fig. 3.24). Maxsimum water holding capacity rates of 41 soil samples are given in Table 3.5. Water holding capacity of 75.60% of these soils are above 50% and in 24.39% it lies between 36.88-50% (Fig. 3.25). Salinity values of the soils vary between 0.017-0.406%, 78.08% being non-saline, 19.52% slightly saline and 2.39% moderately saline (Fig. 3.26).

3. 5 Chemical Analysis Results of the Soils

Results of the chemical analysis of the *C. tinctoria* soils collected from the study areas during flowering and fruiting period are given in Table 3.6. The organic matter content of the soils varies between 0.31-6.88%, 11.90% are very poor, 23.80% poor 11.90% moderately rich, 28.60% rich and 23.80% very rich in organic matter (Fig. 3.27).

The results of phosphorous contents of our soil samples are given in table 3.6. 21.44% of the soils are poor, 33.33% moderate and 45.23% rich in phosphorus (Fig. 3.28). Potassium values are presented in table 3.6, these vary between 0.002-0.187%. 7.17% of the soils are deficient, 45. 23% low, 11. 90% sufficient, 19. 04% high and 16. 66% very high in potassium content (Fig. 3.29).

The results of chemical analysis of the soils of *R. tinctorum* collected from study area during flowering and fruiting period are given in table 3.7. Organic matter of soils varies between 0.17-8.04%. 12.2% of these soils are very poor, 14.60% poor 24.4% moderataly rich, 29.28% rich and 19.52% very rich in organic matter (Fig. 3.30).

Phosphorus contents of the soil samples are shown in table 3.7. 4.84% of the soils are poor, 14.64% moderate and 80.52% rich in phosphorus (Fig. 3.31). Potassium contents of the soils too are shown on table 3.7. Soil potassium varies between 0.005-0.093%. 7.32% of the soils are deficient, 4. 88% low, 9. 76% sufficient, 17. 08% high and 61% very high in potassium content (Fig. 3.32).

Table 3.4 Physical analysis of the soils of *C. tinctoria*.

Locality	Saturation Values	MWHC	Textural Classification	pH	Total soluble salts(%)	$CaCO_3$(%)
1	60	60.18	loamy	7.83	0.141	9.80
2	55	56.240	clayey-loamy	7.70	0.104	20.54
3	47	48.25	loamy	7.90	0.067	26.39
4	44	46.68	turbier	7.46	0.053	23.38
5	47	48.101	clayey-loamy	7.52	0.087	1.05
6	38	36.89	loamy	7.13	0.050	0.97
7	33	35.56	loamy	7.59	0.017	30.33
8	60	64.73	clayey-loamy	7.52	0.157	23.20
9	55	57.15	clayey-loamy	7.77	0.102	5.94
10	40	38.19	loamy	7.72	0.033	4.83
11	52	54.73	clayey-loamy	7.49	0.097	3.57
12	57	59.03	clayey-loamy	7.65	0.111	23.97
13	56	58.95	clayey-loamy	7.35	0.120	21.04
14	52	52.41	clayey-loamy	6.70	0.113	-
15	56	58.24	turbier	7.28	0.133	9.29
16	34	36.67	loamy	6.75	0.020	-
17	38	40.95	turbier	7.70	0.028	6.22
18	55	54.90	clayey-loamy	7.77	0.044	7.03
19	57	54.22	clayey-loamy	7.30	0.074	0.97
20	45	48.30	loamy	7.01	0.073	1.37
21	50	51.49	loamy	7.30	0.082	1.53
22	46	43.68	loamy	6.28	0.079	-
23	52	53.06	clayey-loamy	7.50	0.089	2.34
24	63	60.11	clayey-loamy	6.90	0.206	-
25	39	42.90	loamy	7.47	0.067	26.09
26	53	56.39	clayey-loamy	7.55	0.119	4.69
27	63	60.09	clayey-loamy	7.42	0.053	2.87
28	37	37.43	loamy	7.22	0.105	3.19
29	46	48.967	loamy	7.58	0.108	2.07
30	57	61.13	clayey-loamy	7.50	0.114	22.58
31	38	41.99	loamy	7.07	0.063	1.20
32	67	71.13	turbier	7.46	0.147	6.14
33	45	48.07	loamy	7.88	0.068	4.95
34	55	59.34	clayey-loamy	7.42	0.089	1.60
35	55	58.38	clayey-loamy	7.28	0.085	11.09
36	62	58.99	clayey-loamy	7.27	0.070	4.79
37	44	41.33	loamy	7.60	0.019	2.00
38	56	55.70	turbier	7.45	0.068	1.94
39	44	47.30	loamy	7.23	0.060	2.15
40	39	35.76	loamy	7.48	0.057	0.64
41	44	41.555	loamy	7.47	0.070	4.71
42	47	51.478	loamy	7.24	0.080	0.96

Table 3.5 Physical analysis of the soils of *R.tinctorum*.

Locality	Saturation Value	M.W.H.C.	Textural Classification	pH	Tot.soluble salts(%)	$CaCO_3$(%)
1	55	58.04	clayey-loamy	7.60	0.179	2.93
2	69	69.54	clayey-loamy	7.73	0.203	5.45
3	50	52.54	loamy	7.83	0.141	9.80
4	50	52.15	loamy	7.77	0.093	29.78
5	49	47.74	turbier	7.58	0.065	23.70
6	69	68.48	clayey-loamy	7.73	0.406	3.07
7	59	62.69	clayey-loamy	7.51	0.127	6.71
8	62	60.42	turbier	7.30	0.121	23.66
9	60	63.20	clayey-loamy	7.52	0.157	23.20
10	55	59.48	clayey-loamy	7.77	0.102	5.94
11	77	69.43	turbier	7.13	0.237	2.62
12	57	60.04	clayey-loamy	7.65	0.111	23.97
13	35	38.15	loamy	7.68	0.025	2.22
14	68	71.73	clayey-loamy	7.72	0.219	1.99
15	49	52.33	loamy	7.05	0.045	0.97
16	60	64.03	clayey-loamy	7.40	0.187	2.14
17	56	41.51	clayey-loamy	7.58	0.109	4.51
18	60	60.30	turbier	7.53	0.122	0.71
19	58	61.05	turbier	6.22	0.102	-
20	52	51.48	clayey-loamy	6.70	0.113	-
21	39	43.80	loamy	7.55	0.032	21.50
22	56	59.00	turbier	7.28	0.133	9.29
23	34	36.88	loamy	6.75	0.020	-
24	50	54.80	loamy	7.30	0.082	1.53
25	48	53.61	clayey-loamy	7.50	0.089	2.34
26	39	42.77	loamy	7.52	0.142	0.48
27	64	62.24	loamy	7.47	0.067	26.09
28	64	62.22	clayey-loamy	7.46	0.218	18.91
29	55	57.03	turbier	7.17	0.048	1.24
30	52	53.06	turbier	7.38	0.017	8.78
31	79	75.21	turbier	7.52	0.075	4.39
32	62	60.13	turbier	7.50	0.115	18.03
33	59	62.31	clayey-loamy	7.31	0.074	4.79
34	62	65.13	clayey-loamy	7.27	0.070	4.79
35	62	65.20	clayey-loamy	7.40	0.055	12.76
36	47	49.85	turbier	7.62	0.028	4.83
37	58	60.00	clayey-loamy	7.46	0.053	17.56
38	61	59.94	clayey-loamy	7.63	0.085	14.34
39	44	41.92	loamy	7.69	0.046	7.99
40	41	41.53	loamy	7.98	0.045	0.48
41	44	45.40	loamy	7.34	0.085	13.57

Table 3.6 Chemical analysis of the soils of *C. tinctoria*.

Locality	Organic matter(%)	P (%)	K (%)
1	3.56	0.0099	0.086
2	1.27	0.0013	0.014
3	2.28	0.0043	0.017
4	5.80	0.0038	0.018
5	3.00	0.0069	0.077
6	1.50	0.0074	0.012
7	2.40	0.00003	0.034
8	2.28	0.0013	0.031
9	1.10	0.0015	0.035
10	0.31	0.0037	0.010
11	2.76	0.00003	0.025
12	1.48	0.00003	0.014
13	3.24	0.0001	0.040
14	2.20	0.0036	0.032
15	5.80	0.0032	0.093
16	0.57	0.0014	0.017
17	5.52	0.0005	0.010
18	1.72	0.0001	0.013
19	3.44	0.0033	0.025
20	0.80	0.0017	0.018
21	1.84	0.0033	0.069
22	3.92	0.0021	0.049
23	3.00	0.0043	0.082
24	2.72	0.0029	0.086
25	1.96	0.0031	0.031
26	1.90	0.0012	0.055
27	1.66	0.0020	0.012
28	1.12	0.0037	0.002
29	1.36	0.0014	0.009
30	3.00	0.0009	0.039
31	2.74	0.0017	0.013
32	6.88	0.0023	0.008
33	2.88	0.0004	0.003
34	1.04	0.0005	0.011
35	1.21	0.0011	0.028
36	1.92	0.0023	0.022
37	0.98	0.0005	0.004
38	4.28	0.0027	0.031
39	3.24	0.0020	0.007
40	0.63	0.0011	0.007
41	1.24	0.0019	0.013
42	1.04	0.0035	0.020

Table 3.7 Chemical analysis of the soils of *R.tinctorum*.

Locality	Organic matter(%)	P (%)	K (%)
1	3.68	0.0035	0.091
2	1.58	0.0029	0.041
3	3.56	0.0099	0.086
4	1.45	0.0025	0.031
5	5.52	0.0036	0.017
6	1.44	0.0047	0.023
7	3.12	0.0054	0.075
8	4.00	0.0015	0.034
9	2.28	0.0013	0.031
10	1.10	0.0015	0.035
11	6.44	0.0094	0.083
12	1.48	0.0000	0.014
13	0.17	0.0000	0.021
14	1.36	0.0015	0.072
15	2.08	0.0029	0.077
16	1.84	0.0088	0.074
17	2.64	0.0055	0.058
18	5.80	0.0033	0.065
19	4.00	0.0102	0.074
20	2.20	0.0036	0.032
21	0.68	0.0021	0.005
22	5.80	0.0032	0.093
23	0.57	0.0014	0.017
24	1.84	0.0033	0.069
25	3.00	0.0043	0.082
26	3.92	0.0050	0.069
27	1.96	0.0031	0.031
28	2.40	0.0041	0.058
29	5.44	0.0033	0.036
30	4.28	0.0043	0.042
31	8.04	0.0078	0.069
32	6.32	0.0044	0.065
33	2.51	0.0051	0.069
34	1.92	0.0023	0.022
35	2.52	0.0074	0.069
36	7.84	0.0033	0.011
37	2.59	0.0060	0.060
38	2.60	0.0060	0.069
39	1.49	0.0150	0.045
40	0.31	0.0065	0.044
41	0.72	0.0017	0.021

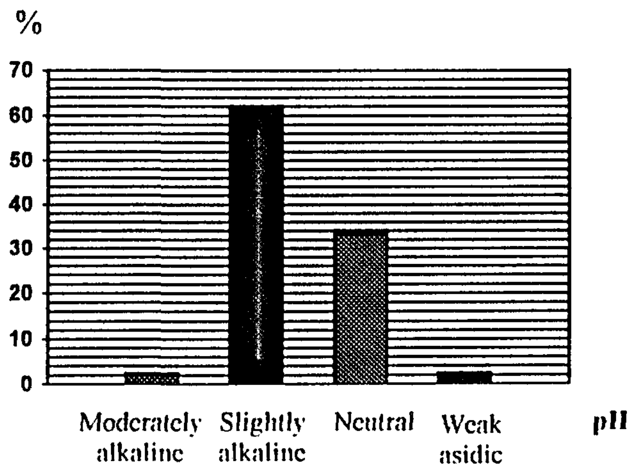

Figure 3.17 pH in soils of *C.tinctoria*.

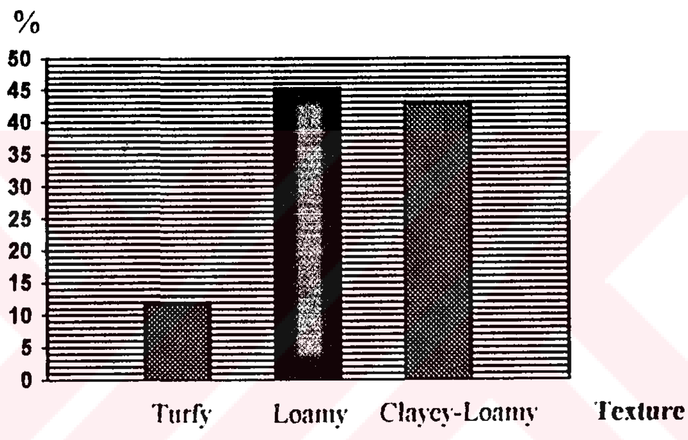

Figure 3.18 Texture in soils of *C.tinctoria*.

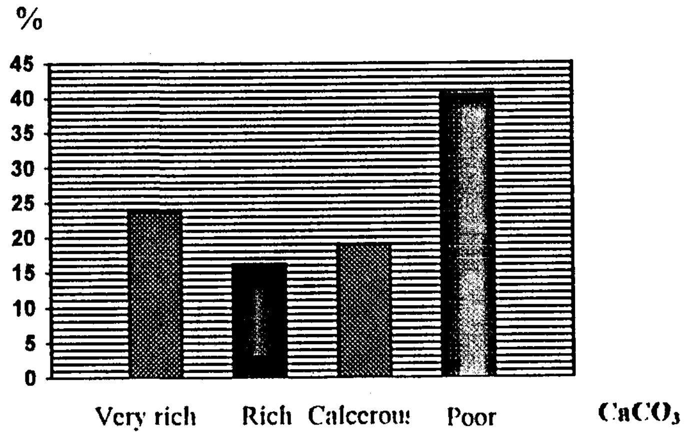

Figure 3.19 $CaCO_3$ in soils of *C.tinctoria*.

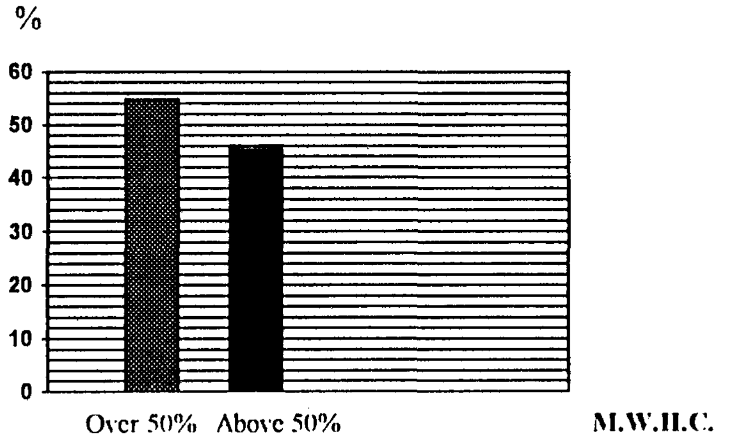

Figure 3.20 Maximum water holding capacity in soils of *C.tinctoria*.

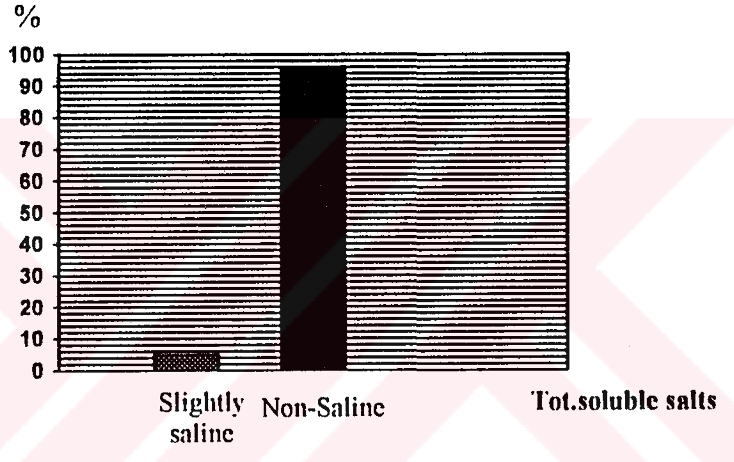

Figure 3.21 Total soluble salts in soils of *C.tinctoria*.

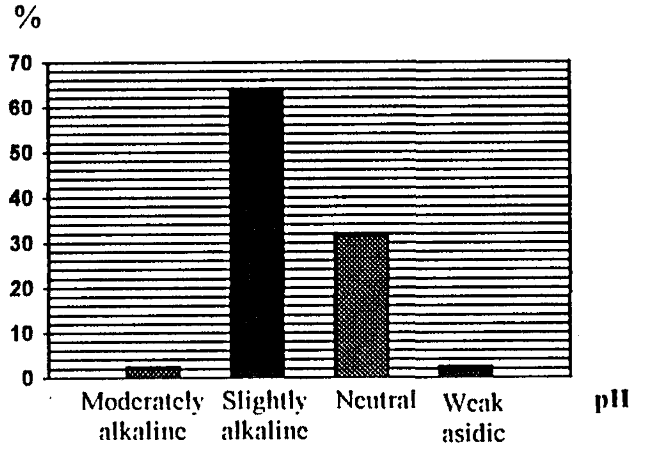

Figure 3.22 pH of soils of *R.tinctorum*.

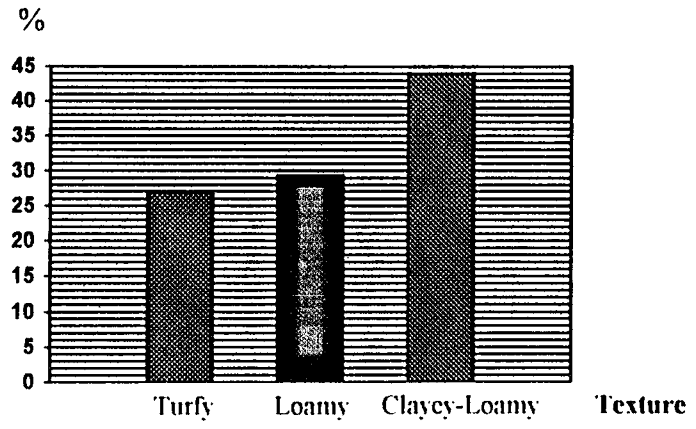

Figure 3.23 Texture in soils of *R.tinctorum*.

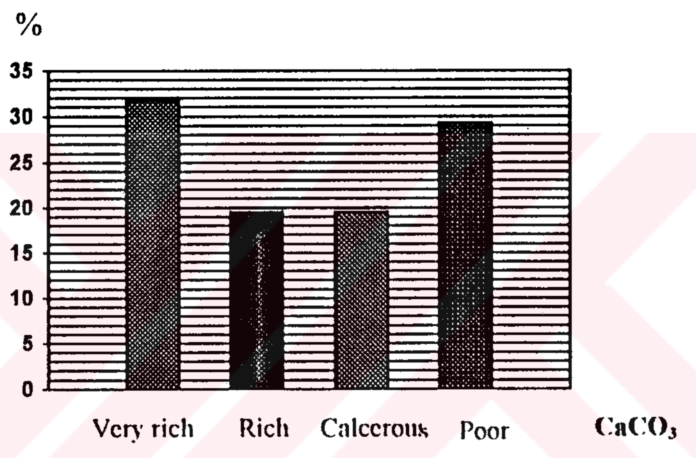

Figure 3.24 $CaCO_3$ in soils of *R.tinctorum*.

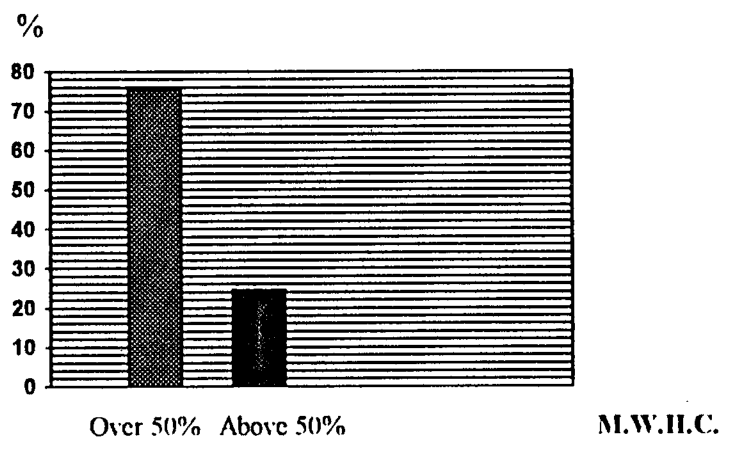

Figure 3.25 Maximum water holding capacity in soils of *R.tinctorum*.

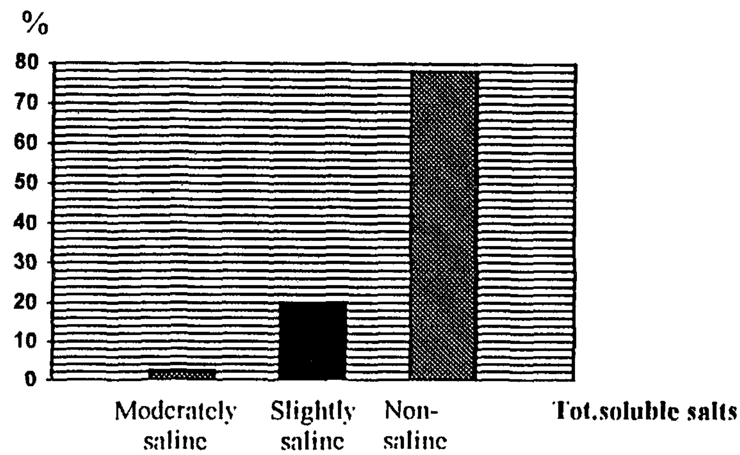

Figure 3.26 Total soluble salt content of *R.tinctorum* soils.

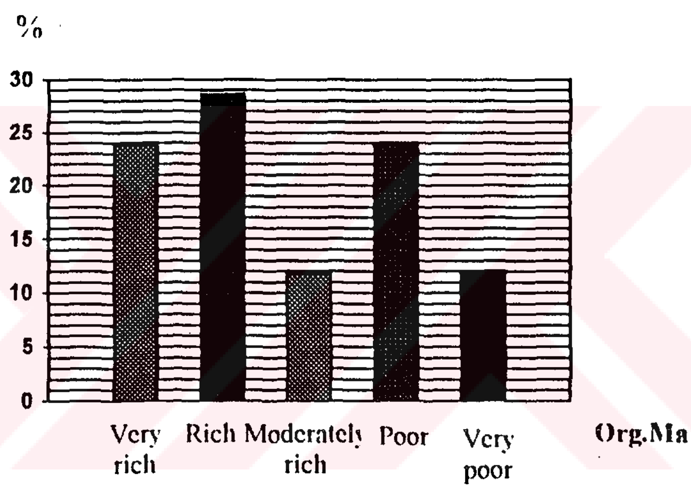

Figure 3.27 Organic matter content of *C.tinctoria* soils.

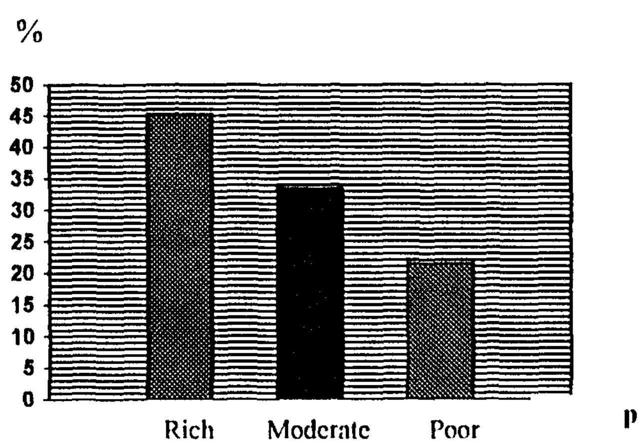

Figure 3.28 Phosphorus content of *C.tinctoria* soils.

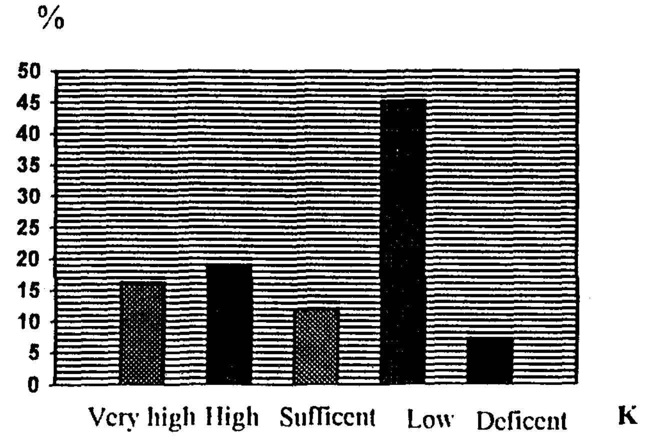

Figure 3.29 Potassium content of C.tinctoria soils.

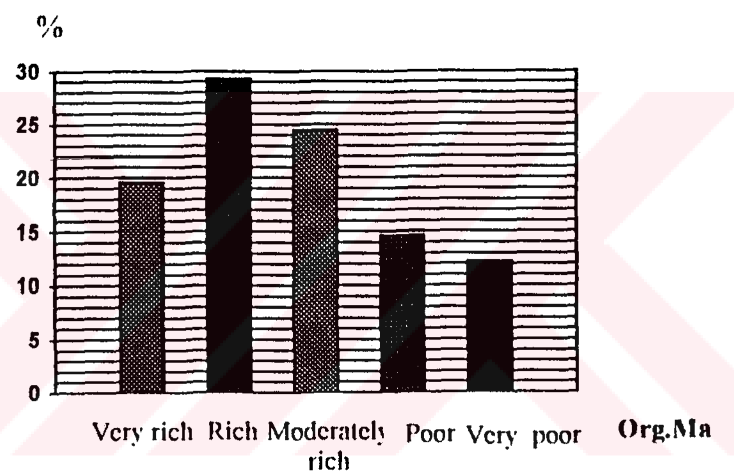

Figure 3.30 Organic matter content of *R.tinctorum* soils.

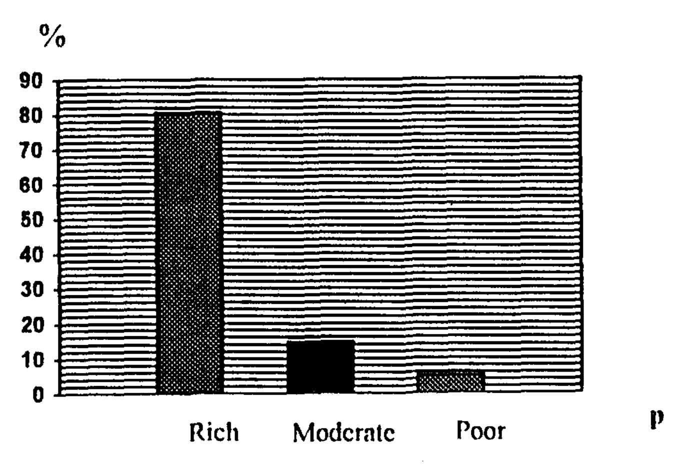

Figure 3.31 Phosphorus content of *R.tinctorum* soils.

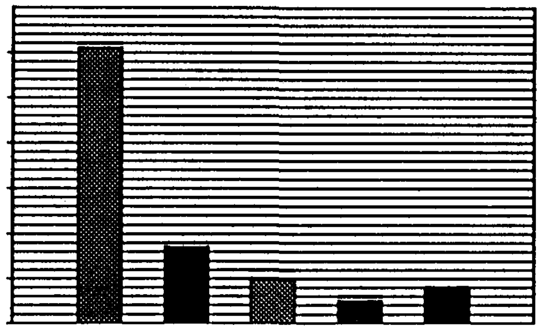

Figure 3.32 Potassium content of *R.tinctorum* soils.

3. 6 Chemical Analysis Results of the Plants

The chemical analysis results of the *C. tinctoria* plants collected in flowering season from the study area are given in table 3.8. Total nitrogen, phosphorus, potassium, calcium, sodium, manganese, zinc and copper contents vary between 1.610-3.094%, 0.100-0.300%, 1.040-2.620%, 1.620-2.920%, 0.06-0.24%, 35-125 ppm., 26.4-85.8 ppm. and 8-20 ppm. respectively on dry weight basis.

Table 3.9 shows the chemical analysis results of *R.. tinctorum* plants collected in flowering season from the study area. According to the dry weight of the plant amounts are between for Total nitrogen, phosphorus, potassium, calcium, sodium, manganese, zinc and copper values lie between 1.078-2.898%, 0.032-0.282%, 1.78-3.36%, 1.18-2.80, 0.06-0.36%, 50-200 ppm., 34.3-102.9 ppm and 8-20 ppm. respectively on dry weight basis. Since data evaluation changes from plant to plant no inter pretations are given here.

3.7 Statistical Evaluation of the Soil and Plant Analysis Results.

An attempt was made to determine relations between organic matter, P, K, pH, total soluble salts, $CaCO_3$ content of the soils and N, P, K, Ca, Na, Mn, Zn and Cu content of the *C. tinctoria* and *R. tinctorum* plants. Regression curves and correlation coefficients showed that a negative relation exists between soil organic matter and plant manganese content of *C. tinctoria* (Table 3.10 ; Fig. 3.33) . In *R. tinctorum* a positive relation between soil pH and

Table 3.8 Chemical analysis of the plants of *C.tinctoria*.

Locality	N (%)	P (%)	K (%)	Ca (%)	Na (%)	Mn (ppm)	Zn (ppm)	Cu(ppm)
1	2.142	0.232	2.160	2.040	0.12	50	50.2	12
2	2.156	0.252	2.040	2.340	0.06	60	46.2	8
3	2.148	0.212	2.120	2.680	0.12	65	48.4	12
4	2.536	0.256	2.340	2.410	0.08	40	55.3	20
5	1.838	0.268	2.620	2.680	0.10	80	66.8	16
6	2.118	0.252	1.420	2.410	0.012	45	62.4	12
7	2.460	0.196	2.110	2.920	0.06	60	53.4	8
8	1.806	0.180	1.760	2.640	0.08	45	59.4	12
9	1.720	0.220	2.120	2.310	0.14	80	63.4	20
10	1.934	0.268	2.320	2.480	0.06	85	76.8	16
11	1.726	0.300	1.840	2.400	0.24	90	46.2	12
12	2.716	0.160	1.060	2.120	0.08	70	48.4	8
13	2.860	0.224	1.320	1.860	0.10	70	57.6	8
14	3.002	0.212	2.520	2.000	0.14	60	60.4	16
15	1.820	0.220	1.700	2.460	0.08	50	52.8	12
16	2.128	0.264	1.820	2.520	0.08	80	56.8	12
17	2.506	0.256	2.060	2.620	0.24	55	70.6	8
18	2.002	0.100	2.180	2.240	0.06	85	82.3	20
19	1.610	0.180	1.640	2.860	0.12	55	42.8	16
20	2.450	0.268	1.260	2.460	0.08	75	60.7	12
21	1.890	0.300	1.860	2.400	0.06	60	39.6	12
22	1.932	0.224	1.600	2.940	0.06	65	59.4	12
23	2.320	0.220	1.080	2.420	0.12	50	66.3	8
24	2.700	0.268	1.540	1.860	0.24	45	55.6	20
25	1.792	0.160	1.560	2.340	0.06	40	63.4	16
26	2.716	0.204	1.380	3.000	0.12	60	46.2	12
27	1.604	0.252	2.020	2.080	0.08	75	46.4	8
28	2.340	0.252	1.240	2.580	0.10	100	46.2	16
29	2.280	0.212	1.700	2.580	0.24	75	60.7	16
30	1.804	0.244	2.160	2.460	0.06	70	83.2	12
31	2.800	0.252	1.760	1.920	0.08	100	68.6	20
32	3.094	0.252	1.040	2.460	0.10	35	59.4	16
33	2.520	0.252	1.360	1.620	0.14	70	34.3	16
34	2.534	0.256	1.380	1.680	0.10	90	85.8	20
35	2.506	0.256	1.620	2.220	0.06	55	39.6	20
36	2.002	0.196	1.820	2.760	0.06	75	50.2	12
37	1.610	0.100	2.700	2.510	0.06	35	26.4	8
38	2.198	0.192	1.680	2.280	0.06	40	60.7	16
39	2.144	0.204	1.720	1.740	0.08	65	41.4	12
40	2.380	0.224	1.680	2.760	0.06	125	68.6	20
41	2.310	0.244	1.520	1.800	0.10	70	55.4	16
42	2.002	0.244	1.420	2.830	0.06	70	44.9	8

Table 3.9 Chemical analysis of the plants of *R.tinctorum*.

Locality	N (%)	P (%)	K (%)	Ca (%)	Na (%)	Mn (ppm)	Zn (ppm)	Cu(ppm)
1	1.078	0.032	2.00	2.70	0.06	50	34.3	16
2	2.898	0.264	3.24	1.17	0.12	80	55.4	20
3	2.310	0.180	3.36	2.58	0.22	70	72.6	20
4	1.610	0.148	2.64	1.92	0.06	50	44.9	12
5	1.680	0.224	2.46	2.16	0.06	60	52.8	16
6	2.400	0.084	3.22	2.78	0.34	65	38.8	16
7	2.110	0.110	2.54	2.66	0.10	80	94.9	20
8	1.640	0.106	2.68	1.34	0.08	60	83.2	20
9	1.302	0.050	3.02	1.92	0.20	55	76.4	12
10	1.804	0.074	2.92	2.44	0.28	75	70.8	12
11	2.400	0.194	1.98	2.64	0.14	115	62.2	16
12	2.780	0.158	3.00	1.88	0.32	85	44.2	12
13	1.090	0.202	2.16	1.28	0.26	195	66.9	20
14	2.310	0.206	3.12	1.20	0.18	110	72.4	20
15	1.720	0.242	1.78	2.08	0.06	135	84.9	12
16	1.220	0.272	2.94	2.54	0.36	190	45.5	16
17	2.440	0.094	2.74	1.78	0.24	140	46.8	12
18	1.098	0.154	3.22	1.90	0.10	165	78.4	16
19	2.114	0.264	1.84	2.46	0.12	120	80.6	8
20	2.660	0.220	2.34	1.24	0.26	180	48.2	12
21	2.402	0.232	2.58	2.04	0.32	175	62.6	16
22	2.492	0.208	2.88	2.22	0.12	50	47.5	12
23	1.778	0.156	1.80	2.64	0.30	80	46.2	16
24	2.226	0.224	2.34	1.92	0.22	100	76.6	16
25	1.638	0.104	3.06	2.10	0.16	85	39.6	16
26	2.562	0.188	2.04	2.36	0.34	105	68.9	20
27	1.652	0.204	2.40	1.68	0.06	50	39.6	12
28	1.736	0.192	2.88	1.68	0.10	90	59.4	16
29	2.100	0.276	2.00	2.28	0.26	60	95	12
30	1.176	0.056	2.28	2.04	0.06	80	76.6	20
31	1.582	0.282	3.30	2.66	0.06	145	70.4	16
32	2.688	0.072	2.50	1.84	0.18	170	90.8	12
33	2.142	0.268	2.04	2.10	0.10	75	55.4	20
34	1.540	0.060	3.18	1.50	0.06	60	58.1	20
35	1.834	0.228	2.46	1.98	0.06	60	102.9	12
36	2.366	0.264	2.70	1.14	0.08	50	34.3	12
37	2.310	0.160	2.54	2.76	0.06	75	34.3	16
38	1.330	0.060	2.22	2.80	0.10	200	73.9	20
39	2.310	0.212	2.52	1.86	0.26	100	63.4	16
40	2.002	0.208	3.36	1.38	0.36	60	44.9	12
41	1.986	0.096	2.72	1.18	0.32	70	77.3	8

Table 3.10 Regression analysis of soil organic matter and plant manganase content of *C.tinctoria* (linear model).

Linear Fit

Summary of Fit

Rsquare	0.245518
Root Mean Square Error	16.96675
Mean of Response	65.95238
Observations (or Sum Wgts)	42

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	3747.074	3747.07	13.0165
Error	40	11514.831	287.87	Prob>F
C Total	41	15261.905		0.0008

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	81.09974	4.94784	16.39	0.0000
Organic Matter	-6.26232	1.73575	-3.61	0.0008

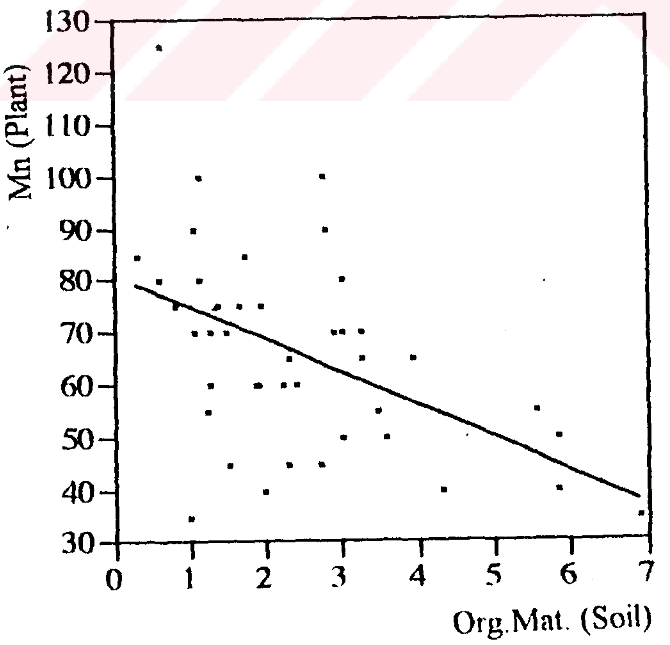

Figure 3.33 Regression analysis of soil organic matter and plant manganase content in *C.tinctoria*.

Table 3.11 Regression analysis of soil pH and plant potassium content of *R.tinctorum* (linear model).

Linear Fit

Summary of Fit

Rsquare	0.327137
Root Mean Square Error	0.387465
Mean of Response	2.609756
Observations (or Sum Wgts)	41

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	2.8466485	2.84665	18.9613
Error	39	5.8550491	0.15013	Prob>F
C Total	40	8.7016976		0.0001

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-3.49193	1.40256	-2.49	0.0172
pH (Toprak)	0.8196892	0.18824	4.35	0.0001

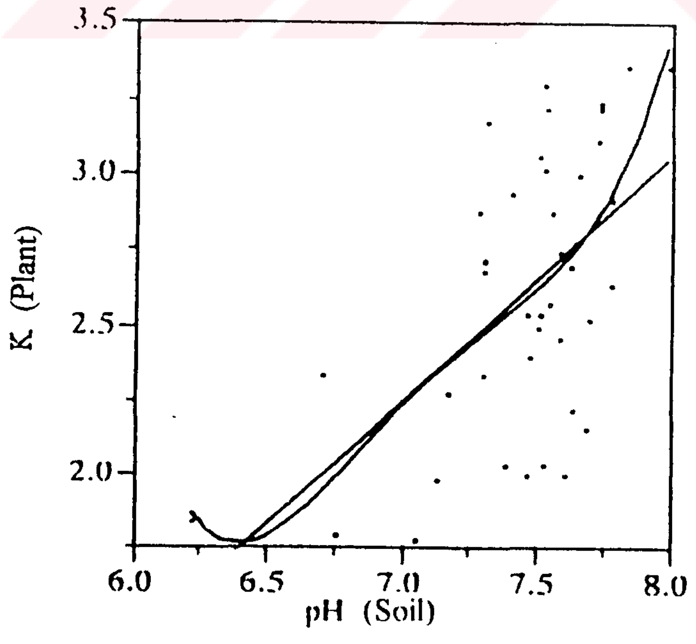

Figure 3.34 Regression analysis of soil pH and plant potassium content in *R.tinctorum*.

Table 3.12 Regression analysis of soil potassium and plant calcium content of *R.tinctorum* (linear model).

Linear Fit

Summary of Fit

Rsquare	0.238439
Root Mean Square Error	0.454464
Mean of Response	2.020341
Observations (or Sum Wgts)	41

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	2.521943	2.52194	12.2106
Error	39	8.054968	0.20654	Prob>F
C Total	40	10.576911		0.0012

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	1.6535125	0.12672	13.05	0.0000
K (Toprak)	6.6844389	1.91292	3.49	0.0012

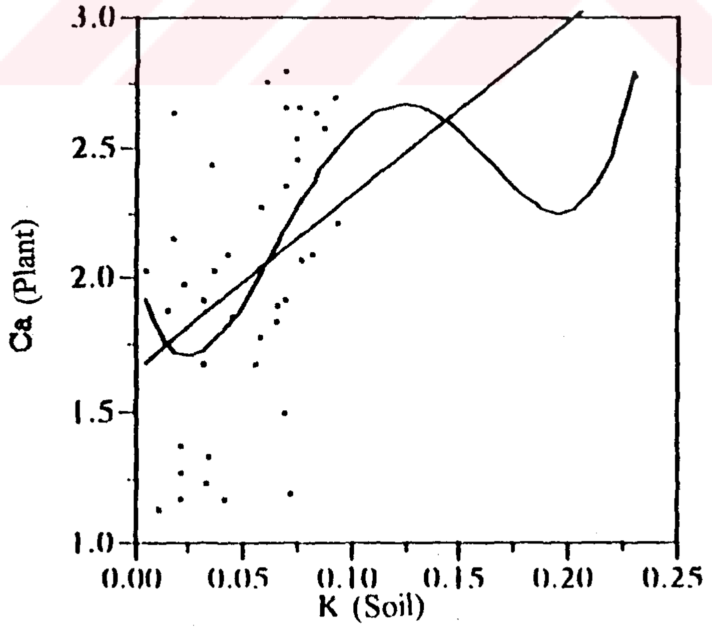

Figure 3.35 Regression analysis of potassium and plant calcium content in *R.tinctorum*.

Table 3.13 Regression analysis of soil organic matter and plant sodium content of *R.tinctorum*.

Linear Fit

Summary of Fit

Rsquare	0.264792
Root Mean Square Error	0.091012
Mean of Response	0.174146
Observations (or Sum Wgts)	41

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	0.11634826	0.116348	14.0462
Error	39	0.32304686	0.008283	Prob>F
C Total	40	0.43939512		0.0006

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.2534158	0.02548	9.94	0.0000
Organik madde (Toprak)	-0.026668	0.00712	-3.75	0.0006

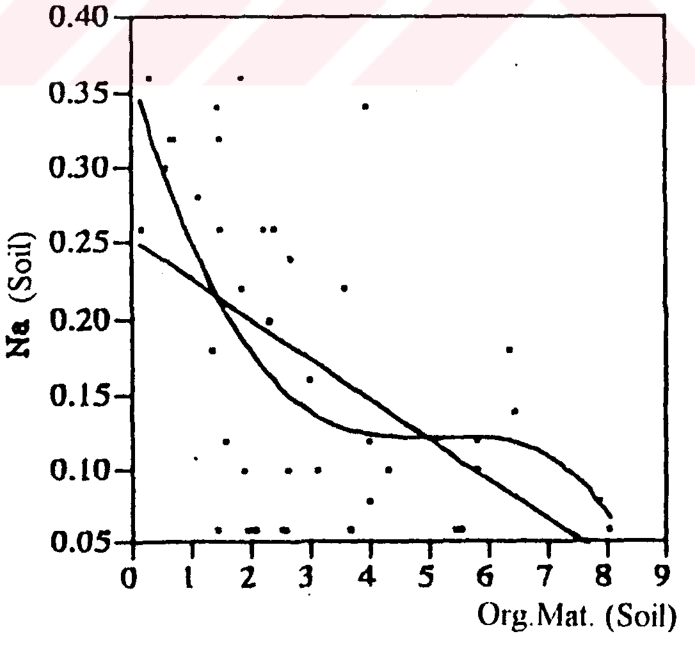

Figure 3.36 Regression analysis of organic matter and plant sodium content in *R.tinctorum*.

plant potassium (Table 3.11; Fig. 3.34), and soil potassium and plant calcium (Table 3.12; Fig. 3.35) were obtained. However a negative correlation between soil organic matter and plant sodium (Table 3.13; Fig. 3.36) was obtained. Other results gave neither positive nor negative correlations.

3. 8 Culture Experiments on Plant Growth and Development.

10 cm long *C. tinctoria* seedlings were collected from nature and transplanted under different conditions (Table 3.14, Fig. 3.37). The mean values of the biometric measurements of *C. tinctoria* grown in a mixture of 75% fitiliser + 25% soil under daylight conditions and watered every day are as follows: root length was 7.3 cm, root width 2.66 mm, stem length 55 cm, stem width 5 mm, leaf length 5.14 cm and leaf width 3.14 cm. In plants grown under similar conditions but, watered once in two days, mean root length was 11 cm, mean root width 3.33 mm, stem length 47.2 cm, stem width 4.66 mm, leaf lenght 5.25 cm and leaf width 3.63 cm. Plants watered once in four days showed mean root lenght as 7.7 cm, root width 3 mm, stem length 44.3 cm, stem width 4 mm, leaf length 5.2 cm and leaf width as 2.69 cm. Those watered once in six days had mean root length as 10 cm, root width 3.3 mm, stem length 39.5 cm, stem width 2.83 mm, leaf length 5.55 cm and leaf width 2.20 cm. Plants sown in a mixture of 75% fertilizer + 25% soil, kept under shade and watered differently got dried.

In 50% fertilizer+50% soil under day light, *C. tinctoria* plants watered every day, showed the mean, values as root length 9.4 cm, root width 3.66 mm, stem length 53.4 cm, stem width 4.6 mm, leaf length 3.7 cm and leaf width 1.80 cm. Under the same conditions plants watered once in two days had root length 8.46 cm, root width 3 mm, leaf length 3.62 cm, leaf width 1.98 cm , stem length 46.5 cm and stem width as 4 mm. Plants watered once in four days showed root length of 9. 46 cm, root width 3.33 mm, stem length 41.06 cm, stem width 3.16 mm, leaf length 3. 80 cm and leaf width of 2.12 cm, those watered once in six days had root length as 10.66 cm, root width 4 mm, stem length 34.6 cm, stem width 2.83 mm, leaf length 4.83 cm and leaf width as 1.91 cm. Plants grown under shade and watered differently dried completely.

Figure 3.37 *C. tinctoria* grown under various conditions.

In the 25% fertilizer+75% soil under day light, *C. tinctoria* watered every day showed mean root length of 11.1 cm, root width 4 mm, stem length 55.13 cm, stem width 3.6 mm, leaf length 2.32 cm and leaf width as 5.52 cm. Under the same conditions plants watered once in two days had root length as 11.9 cm, root width 5 mm, leaf length 1.87 cm, leaf width 1.68 cm., stem length 50 cm and stem width as 3.3 mm. Those watered once in four days reached a root length of 8.13 cm, root width 3.66 mm, stem length 42 cm, stem width 2.5 mm, leaf length 2.55 cm and leaf width of 1.39 cm. Plants watered once in six days had a root length of 8.93 cm, root width 3.33 mm, stem length 36.9 cm, stem width 1.83 mm, leaf length 3.93 cm and leaf width of 1.44 cm. (Figs. 3. 38, 39, 40). Plants grown in shade died soon.

C. tinctoria plants grown in a mixture of 25% lime+75% soil and 50% lime+50 soil, under daylight coditions and watered differently too did not behave well and got dried.

In the case of *R. tinctorum* seeds were placed in sand for germination and when seedling length was 20 cm, these were transplanted under different conditions (Table 3.15; Figs. 3.41, 42)

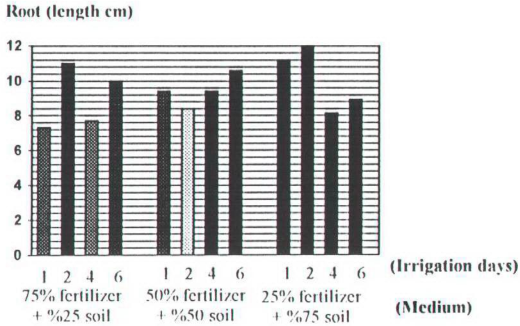

Figure 3.38 Root development of *C.tinctoria* grown under various conditions.

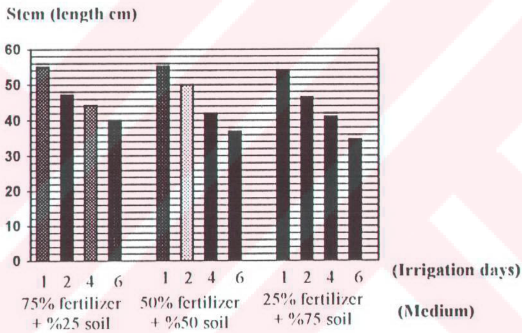

Figure 3.39 Stem development of *C.tinctoria* grown under various conditions.

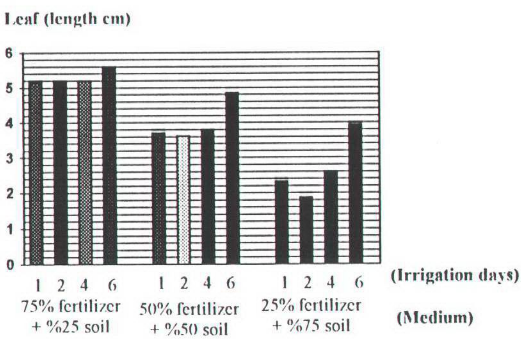

Figure 3.40 Leaf development of *C.tinctoria* grown under various conditions.

Figure 3.41 Germination of *R.tinctorum*.

Figure 3.42 *R.tinctorum* grown under various conditions.

The mean values of the biometric measurements of *R. tinctorum* in a mixture of 75% fertilizer + 25% soil, under to daylight and watered every day were as fallows: root length 13.5 cm, root width 7.17 mm, stem length 10.1 cm, stem width 4.5 mm, leaf length 4.9 cm and leaf width 1.63 cm. Plants watered once in two days showed mean root hength as 12.2 cm, root width 4.4 mm, stem length 83.8 cm, stem width 3.65 mm, leaf length 4.61 cm and leaf width as 1.59 cm. The plants watered once in four days had mean root length of 15.7 cm, root width 5.25 mm, stem length 68.6 cm, stem width 2.08 mm, leaf length 4.46 cm and leaf width of 1.30 cm. Those watered once in six days showed mean root length of 15. 6 cm, root width 5.39 mm, stem length 48.5 cm, stem width 1.76 mm, leaf length 2.47 cm and leaf width of 0.6 cm.

R. tinctorum plants grown under shade and watered every day grew up to 38 cm and dried at the and of 168 days, when watered once in two days, it grew up to 40 cm and dried at the end of 85 days, in watering once in four days plants grew up to 38 cm and dried in 75 days. If watering was done once in six days length was 34 cm but plants dried in 53 days, the year after these plants did not show any development.

In 50% fertilizer+50% soil under daylight, *C. tinctoria*, watered every day, had the following mean values, root length 22.25 cm, root width 6.6 mm, stem length 91.9 cm, stem width 2.77 mm, leaf length 6.07 cm and leaf width 1.89 cm. Under the same conditions when watering was done once in two days root length was 21.4 cm, root width 6 mm, leaf length 5.75 cm, leaf width 1.72 cm, stem length 71.8 cm and stem width 2.26 mm. Plants watered once in four days had root length of 11.6 cm, root width 4.33 mm, stem length 45.1 cm, stem width 1.78 mm, leaf length 4.20 cm and leaf width as 1. 55 cm. Those watered once in six days showed root length of 7.3 cm, root width 4.26 mm, stem length 35.1 cm, stem width 1.47 mm, leaf length 3.5 cm and leaf width as 1.37 cm.

R. tinctorum sown under the same conditions and left in shade watered every day grew up to 40 cm but dried at the and of 159 days. Plants watered once in two days grew up to 27 cm and dried at the and of 150 days, those watered once in four days grew up to 25 cm and dried in 146 days. When watering was done once six days, it became 24 cm long put dried in 125 days. The year after, these plants too did not show any development.

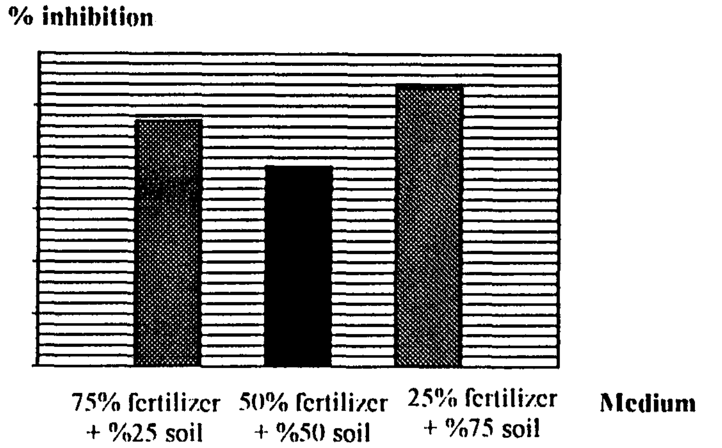

Figure 3.43 Stem development of *R.tinctorum* grown under various conditions.

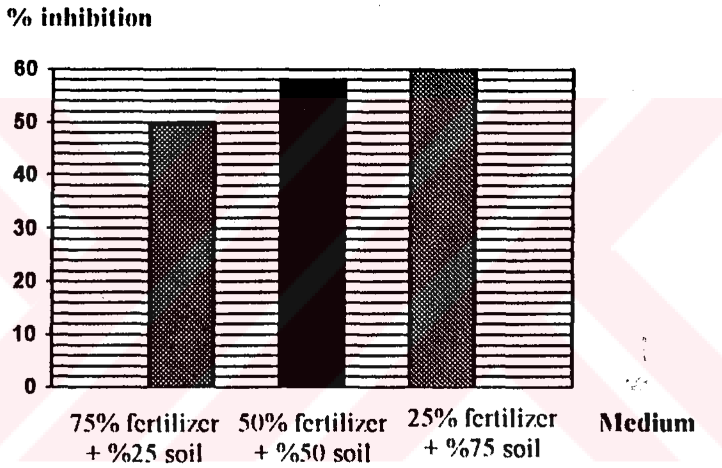

Figure 3.44 Leaf development of *R.tinctorum* grown under various conditions.

In 25% fertilizer+75% soil under daylight, *C. tinctoria*, watered everyday, showed the following mean values: root length 6 cm, root width 8.79 mm, stem length 95.7 cm, stem width 2.65 mm, leaf length 4.96 cm and leaf width 17.9 cm. Under the same conditions when watering was done once in two days root length was 10.32 cm, root width 6.6 mm, leaf length 4.61 cm, leaf width 1.42 cm., stem length 10.32 cm and stem width 6.6 mm. Plants watered once in four days had root length 7.66 cm, root width 4.36 mm, stem length 50.26 cm, stem width 1.94 mm, leaf length 4.44 cm and leaf width 1.33 cm, those watered once in six days showed root length as 9.48 cm, root width 6.45 mm, stem length 5.1 cm, stem width 1.68 mm, leaf length 2.96 cm and leaf width of 0.62 cm. (Figs. 3.43, 44).

R. tinctorum grown under the same conditions but left under shade and watered every day grew up to 40 cm but dried at the and of 109 days. When watered once in two days, it grew up to 35 cm and dried at the and of 108 days. If watering was done once in four days plant grew up to 30 cm and dried in 98 days. Plants watered once in six days became 30 cm long and dried in 94 days. The year after, these plants did not show any development.

R. tinctorum plants grown in a mixture of 25% lime+75% soil and 50% lime+50 soil, under daylight coditions and watered differently too did not behave well and got dried.

3.9 Vegetative Propogation

The materials taken from the stem, shoot and parts between the root and stem of *C. tinctoria* were used for propogation studies under greenhouse conditions materials were sown in sand. Soil fertilizer and distillled water observations were made on materials with nodes. There was no pasitive response. In second set different sizes of materials were taken and IBA hormone applied. This called by farmers as "pokon hormone" and is used much. This application also proved of no used.

R. tinctorum propogation experiments too did not give positive results. But depending upon our field observation propogation experiments were done using 2-3cm long subsoil root and rhizome parts. These were left in pots with soil, sand and dung. Under greenhouse conditions *R. tinctorum* roots in all pots.

3.10 Dye Production and Determination of Colour Produced Intensity

Dye obtained from *C. tinctoria* due to high solubility in water produced dark red colour, but it did not show reaction with wool fibre, as such, dyeing of wool fibres was of low grade. Wool fibres is thus give as light coloured appearance. The lowest colour intensity was 3.213 and the highest 6.408 (Table 3.16).

In the case of *R. tinctorum*, dye obtained reacts with wool fibres as such the colour intensity was very high. It is reported that highest colour intensity was 28.070 and the lowest 11.011 (Table 3.17).

Table 3.16 Color intensity of *C.tinctoria* collected from various localities.

Locality	Color Intensity	Locality	Color Intensity
1	3.213	22	4.115
2	4.823	23	6.040
3	5.322	24	5.315
4	4.210	25	5.565
5	3.412	26	3.962
6	4.610	27	4.806
7	4.372	28	4.945
8	4.218	29	3.584
9	4.745	30	3.174
10	4.813	31	4.203
11	5.322	32	4.980
12	5.710	33	4.758
13	4.893	34	3.938
14	4.508	35	4.625
15	5.599	36	5.704
16	5.305	37	4.732
17	4.974	38	4.668
18	5.806	39	4.410
19	6.204	40	4.332
20	6.408	41	4.274
21	6.228	42	4.576

Table 3.17 Color intensity of *R.tinctorum* collected from various localities.

Locality	Color Intensity	Locality	Color Intensity
1	11.305	22	21.635
2	11.011	23	10.258
3	11.972	24	13.102
4	18.024	25	25.051
5	27.899	26	14.689
6	13.842	27	17.395
7	19.932	28	23.28
8	26.035	29	16.315
9	27.058	30	19.475
10	16.042	31	24.916
11	18.416	32	28.070
12	22.568	33	25.270
13	14.788	34	16.710
14	25.325	35	14.076
15	24.046	36	22.933
16	20.820	37	18.410
17	19.710	38	12.042
18	17.950	39	13.592
19	17.640	40	14.216
20	18.097	41	18.224
21	18.420		

3.11 Ecological Distribution

C. tinctoria grows on ecologically different habitats. It was observed to grow in macchias, phrygana, *Pinus brutia* forests, stomy sites saline steppes, plonghed stony fields, path sides, on rubble siles and on grown or fallow soils. In Turkey it grows between 0-1650 m attitudes.

R. tinctorum too can grow ecologically in different habitats like; field sedes, vineyards gardens, rever sides, fallow fields, and road sides. In Turkey it shows distribution between the altitudes of 0-1250 m.

CHAPTER FOUR
DISCUSSION AND CONCLUSION

4. Discussion and Conclusion

Natural sources are a cornerstone of the wealth of a country. Our country has got this richeness of natural sources, but unfortunately, these richnesses are destoryed unconsciously. Thousands of tons of roots, leaves and bulbs of our plant cover are exported without any control. We have to known that firstly we have to protect our richness that forms our vegetation and natural sourves and also we have to raise the economical value of these sources.

Up to the second part of 19th century, plants used in dye manufacturing were taken mainly from our country. The underground parts of *R. tinctorum* had been used for obtaining "Turkish scarlet" dye which was famous all over the world. Turkey fullfilled the need of world madder root production up to two third. We selected the underground parts of *R. tinctorum* and above ground parts of *C. tinctoria* due to their dye characteristics. These two plants are present in West Anatolian region and are used for obtaining red colour and its tones, for this reason we carried out autecological studeis on these two species.

C. tinctoria belonging to the family *Euphorbiaceae*, is an annual plant and is the only species of *Chrozophora* genus which is seen in our country. *R. tinctorum* belonging to the family *Rubiaceae*, is a prennial plant, being one of the species out of five of *Rubia* genus which can be seen in country. In the morphological studies on these two species measurments on the parts of flower, seed and fruit and plancentation type as well as position of leaves and on the other parts on the plant were noted and the average of these measurements and standart deviations of these measurements calculated (Table 3.1,2). All the values are in full confirmity with Davis (1987).

The anatomical investigations have been done generally on the other species of the family *Euphorbiaceae* (Metcalfe & Chalk, 1957). In many species of this family, it is reported that there are laticiferaus cells and laticiferaus vessals (Fahn, 1967; Esau, 1960). In our anatomical works, we did not see any structures like these in *C. tinctoria*. In *R. tinctorum* there is epidermis, cortex, phloem and a wide area of xylem. These reasults show conformity with other members of *Euphorbiaceae* family (Metcalfe & Chalk, 1957). Also, schlerenchyma cellbands can be seen (Fig 3.3). This is not reported in the results of Metcalfe & Chalk (1957).

Anatomy of only other species of *Rubiaceae* has been studied by Metcalfe & Chalk (1957) and Fhan (1967), but Algan (1976) and Başlar & Oflas (1996) have done some studies on *R. tinctorum*. In transverse section of the root of *R. tinctorum* it was seen that there is a disinlegrated epidermis, but cortex,vescular sytem and pith area have big cells (Fig. 3.4). These results agree with those of Metcalfe & Chalk (1957) and Algan (1976). Algan in his work emphasised about the structure of endodermis and pericycle but in our work these structures were not visible.

In the sections of rhizome of *R. tinctorum*, we find epidermis, periderm, cortex and vascular system. Our findings agree with the works of Algan (1976). The difference is that in our findings, in the centre there is a wide pith area with big cells (Fig.3.5).

An examination of *C. tinctoria* stem (anatomical structure examination) shows there are non-glandular unicellular hairs originating from epidermis, followed by epidermal chlorenchyma of five lines, cortex, vascular system and a wide pith area. These findings concide with those of Metcalfe & Chalk (1957). Differently, chlorenchyma bands can be seen in our findings (Fig. 3.6). When *R. tinctorum*'s stem anatomy is examined, we find cuticle, thick walled epidermis with single layer and rounded cells, cortex, vascular system and pith (Fig. 3.7). Although, chlorenchyma is observed in our findings, this is not reported by Metacalfe & Chalk (1957) and Algan (1976). In addition, because *R. tinctorum*'s above ground part dies every year secondery thickening is impossible and cambiyum tissue can not exist here as mentioned in Algan's work (1976). In our work, although stomata could be seen in the stem, there is no such record in the findings of Metcalfe & Chalk (1957) and Algan (1976) (Fig. 3.8).

Leaf anatomy of *C. tinctoria* shows cuticle, epidermis covered with stellat type of hairs; single layer of palisade with small intercelluler spaces but rich in chlorophyle and spongy paranchyma with wide intercelluler spaces. These results agree with those of Metcalfe & Chalk (1957) (Fig. 3.9). Stomata of anfistomatic and bifacial leaves are of amaryllis and parasitic type and is reported first time here (Figs. 3.10, 11). In *R. tinctorum* leaf section shows wrinkled cuticle is found on lower side but in the upper surface there is no such structure. Upper epidermis is covered with cuticle and has single layer of cells followed by single layer of palisad paranchyma and then spongy paranchyma (Fig. 3.12). In the works of Metcalfe & Chalk (1957) and Algan (1967) wrinkled cuticle is not mentioned. Stomata of the hypostomatic and bifacial leaves are of amaryllis and parasytic type (Fig. 3.13).

In the upper epidermis no stomata were observed in our work on the leaves of hypostamatic *R. tinctorum* but stomatal existence is mentioned in the upper epidermis by Algan (1976). These results do not agree weth our findings (Fig. 3.14). In Fahn's report (1967) stomata type is reported as paracytic as in findings. Although raphid crystals are reported in spongy paranchyma cells by Algan (1976), these structures were not observed by us.

In general seeds subyected to one year strafication show highest germination however, in 4-5 years old seeds germination percentage is lower as reported by Heeger (1956). *C. tinctoria* fresh seeds and one year old seeds were used in the germination experiments and methods used are mentioned above; but there was no germination. There is no report published in this connection before. According to Mall (1956) no germination occurs in *C. rottleri* belonging to *Chrozophora* genus under normal conditions in four months, but seeds left under different conditions and treated with H_2SO_4 heat and in mud at 10°C germinated well. Similar studies were performed on *C. tinctoria* but no germination was observed. According to Crocker (1906) and Thornton (1935) lack of germination in som seeds is due to their non-permeability to gases although they are permeable to water . It is well known that a close relation-ship exists between the non-germinating seed and prevention of diffusion of O_2. Bewley (1982) reports that non-germination of some seeds is due to the chemical inhibitors present in the seeds.

Unsuccessfullness in the germination of seeds of *C. tinctoria* under different processes may be due to the same factors reported by Thornton (1935), Crocker (1906) and Bewley (1982).

Germination of *R. tinctorum* under different photoperiods are show in figure 3. 16 and table 3.3. The highest germination is seen in 12 hours light period. It is seen that up to 12 hours the germination increases gradually, after this period it decreasses. similarly *Myrtus communis*, shows highest germination at 9-15 hours (Öztürk, 1970). Our findings coincide with this data. In *Asphodelus aestivus* optimal germination occurs at 3-9 hours which too is similar to our findings (Pirdal, 1986). This shows that optimum germination varies with the species. In *R. tinctorum* germination rate depends on temperature (Table 3.3, Fig. 3.15). Highest germinations percentage is observed 25°C being 72%. Generally up to 25°C germination increasses, after this it decreases. It is reported that with an increase in the temperature up to optimum germination increases (Vardar & Ahmet, 1969).

Table 3.4 shows that the soils on which *C. tinctoria* grows show a 6.28-7.90 pH . Figure 3.17 shows that out of soils taken from 42 different sites 33.33% are neutral and 61.90% slightly alkaline, according to the scale given by Öztürk (1975). In table 3.5 pH of the soils where *R. tinctorum* grows are given and values vary between 6.22-7.98. Using the scale given by Öztürk (1975) out of soils from 41 different places 24.39% are neutral and 68.29% slightly alkaline. *Myrtus communis*, *Inula graveolens*, *I. viscosa*, *Pistacia lentiscus*, *Asphodelus aestivus*, *Vicia sativa*, *Cappris ovata*, *Vitex agnus-castus* that grow under similar conditions too prefer the neutral and slightly alkaline soils (Pirdal, 1980; Öztürk & Ataç, 1982; Pirdal, 1986; Kanısanlı, 1990; Özdemir, 1993; Doğan, 1994). Smilarly the soils of sugar-cane, onion and sunflower also grow on neutral and slightly alkaline ones. *C. tinctoria* chooses these soils because of their neutral and slightly alkaline character (Hidalgo at al., 1990). *Chrozophora* genus, generally prefers soils which are neutral and slightly alkaline (Mall, 1956). Both the plants choose loamy and clayey-loamy soils as mentioned by tüzüner (1990) too. (Figs. 3.18, 23). *Ceratonia siliqua*, *Inula graveolens*, *Asphodelus aestivus*, *Vicia sativa* and *Vitex agnus-castus* also choose loamy and clayey-loamy soils

(Seçmen, 1972; Öztürk, 1975; Pirdal, 1986; Kanısanlı, 1990; Doğan, 1994). Other works on *C. tinctoria* report that this plant chooses loamy and clayey-loam soils (Hidalgo et al. 1990). *C. tinctoria* soil texture is reported to be loamy (Mall, 1956). As such, our results agree with this data. Tables 3.4 and 5 show that $CaCO_3$ values of the soils of *C. tinctoria* and *R. tinctorum* change between 0-30% 0-29.78%. An examination of figures 3.19, 24 show that *C. tinctoria* and *R. tinctorum* grow on soils rich and poor in $CaCO_3$ (Hidalgo et al., 1990; Mall, 1956; Başlar & Oflas, 1996). We obtained the similar results. Maximum water holding capacity of 41-42 solis of *R. tinctorum* and *C. tinctoria* is given in figures 3.20, 25 It is seen that the water holding capacities of 54.76% of soils of *C. tinctoria* and 75.60% of soils of *R. tinctorum* are higher than 50%. Water holding capacities of *Vicia sativa, Vitex agnus-castus* and *Rubia tinctorum* coincide with our findings (Kanısanlı, 1990; Doğan, 1994; Başlar & Oflas, 1996). In general both plants like wet solis. The total salt content of soils where *C. tinctoria* and *R. tinctorum* grow show that *C. tinctoria* 95.23% of the soils are non-saline. The solis where *R. tinctorum* grows too are non-soline mainly (80.48%) (Figs. 3.21,26). It is reported that *Asphodelus aestivus, Vicia sativa, Capparis ovata, C. spinosa, Vitex agnus-castus* and *R. tinctorum* plants prefer non-soline soils (Pirdal, 1986; Kanısanlı, 1990; Özdemir, 1993; Doğan, 1994; Başlar & Oflas, 1996). Organic matter content of *C. tinctoria* varies between 0.31-6.88% and that of *R. tinctorum* between 0.17-8.04% (Figs. 3.27, 30). Figures 3.27 and 30 that *C. tinctoria* and *R. tinctorum* moderately rich and very rich soils according to the scala of organic matter given by Öztürk and Görk (1989). It is reported that *Pictacia lentiscus, Inula viscosa, Capparis ovata, Cistus laurifolius, Rumex obtusifolius* subsp, *subalpinus* and *R. tinctorum* like *C. tinctoria* choose mederate, rich and very rich soils (Pirdal, 1980; Öztürk & Ataç, 1982; Özdemir, 1993; Başlar & Oflas, 1990,1996).

C. tinctoria chooses the soils which are moderate to rich in phosphorus and *R. tinctorum* chooses the soils with rich phosphorus (Figs. 3.28, 31). It is reported that *Pictacia lentiscus, Capparis ovata* and *C. spinosa* plants too choose solis rich in phosphorus (Öztürk & Ataç, 1982; Özdemir, 1993). It is seen that *C. tinctoria* and *R. tinctorum* choose very rich potassium soils (Figs 3.29, 32). It is reported that *Myrtus communis, Vicia sativa, Chrozophora rottleri* like our species choose soils rich in potassium (Öztürk & Görk, 1979; Kanısanlı, 1990; Mall, 1956).

A correlation study on the soil and plant analysis was undertaken using statistical methods. It was observed that there is a negative correlation between plant manganase and soil organic matter in *C. tinctoria* (r : 0.24) (Table 3.10 ; Fig. 3.33) but positive are between soil pH and plant potassium in *R. tinctorum* (r: 0.32) (Table 3.11; Fig. 3.34), and between soil potassium and plant calcium (r: 0.23) (Table 12; Fig. 3.35), and negative correlation between soil organic matter and plant sodium (r: 0.26) (Table 3.13; Fig. 3.36). Other analysis results show no pasitive or negative correlations.

Growth and development of root, stem and leaf of *C. tinctoria* under various soil, light and watering conditions was fallowed in cultural experiments(Table 3.14). Studies showed that root growth was minimum in 75% fertilizer+25% soil as compared to 75% soil+25% fertilizer and 50% fertilizer+50% soil (Fig.3.38). Growth of stem and leaf on the other hand was optimum in 75% fertilizer+25% soil (Figs.3.39, 40). This depitics that there is an in root growth but stimulation of stem and leaf growth in 75% fertilizer+25% soil. In our opinion, *C. tinctoria* suffers from osmotic pressure change due to high fertilizer ratio which contains organic and inorganic substances. This osmotic pressure hinders these water intake and nutrients dissolved in water, consequently, the root growth is inhibited.

Growth and development of root, stem and leaf of *R. tinctorum* too was followed under various soil, light and watering conditions (Table 3. 15). It was determined that there were some impartant differences in the behaviour of stem and leaf between watering once a day and once in six days. (Figs. 43, 44).

Length of stem of *R. tinctorum* in the experiments watered once a day and once in six days, showed an inhibition of up to 47.04% in the case of 75% fertilizer+25% soil, 38.19%, in the case of 50% fertilizer+50% soil, 53.29% in the case of 25% fertilizer+75% soil.

Leaf length in the same watering frequencie, suffered inhibition up to 49.69% in the case 75% fertilizer+25% soil, 57.66% in the case of 50% fertilizer+50% soil, 59.67% in the case of 25% fertilizer+75% soil.

Root length of these plants showed no parallelity. In the light of these results, it is understood that water is very important for growth and development of stem and leaf of *R.*

tinctorum. In fact, this plant is generally observed in nature at places with high ground water level. Similar results have been reported by Algan, (1976) and Baykara,(1992) *C. tinctoria* and *R. tinctorum* did not show any growth in 25% lime+75% soil and 50% lime+50% soil even of watered once a day, once in two, four and six days. Soil samples taken from nature on which these plants grow too had less $CaC0_3$ content (Tables 3. 4, 5) This infarmation supports our findings. Studies on the effects of light on growth and development showed that *C. tinctoria* did not grow in shade(Table 3. 14), however, *R. tinctorum* a perennial plant; grew to some extent in shade (Table 3. 15). Table 3. 15 show that watering effects the growth of our plants. But, *R. tinctorum* under shade did not show any growth in second year.These studies prove that these plants are of photophillous nature.

Vegetative propogation studies on *C. tinctoria* proved of no use. In *R. tinctorum* some succes was gained with cuttings taken from roots and rhizomes. These result prove that for vegatative propogation of *R. tinctorum* underground parts should be used.

Aboveground parts of *C. tinctoria* and underground parts of *R. tinctorum* wereused for determining colour intensity on wool. In Table 3. 16 and 17 it is seen that the color intensity of *C. tinctoria* (purplish-brown) is between 3.213 and 6.408; that of underground parts of *R. tinctorum* (red color) is between 11. 011 and 28. 070. Lowest color intensity of *R. tinctorum* is a much more than the highest color intensity of *C. tinctoria*. Although the solubility of dye of both species in water is very good, but due to dissolved dying substance in water it can not react with wool fibres as such the dying of wool is lower. It is reported that because some of the dyes can not react with wool fibres, as such some mordan substances are used in wool fibres for dyeing (Harmancıoğlu, 1973). Our results reveal that dye substances taken from *C. tinctoria* need mordon addition, but dye substances taken from *R. tinctorum* does not need any helping substances.

Autecological studies on the plant species which grow in Turkey and are used for dyeing are quite limited. Consequently, autecological studies were done on *Chrozophora tinctoria* and *Rubia tinctorum* which are used as a source of dyeing material carpets, kilims and in other crafts in Western Anatolia. These plants have been used as drugs in medicine in addition to their dyeing value which augments further the importance of our research.

We expect that our autecological findings well help during the plantation in of *C. tinctoria* and *R. tinctorum* in future and this well prove an asselt to the economy of Turkey.

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Table 3.14: Biometric measurements of *C.tinctoria* grown under different conditions.

				Root								Stem								Leaf					
Medium	Light condition	Sample No	Irrigation day	Number of measurements	Number of roots	Length (cm)			Width (mm)			Number of measurements	Number of steams	Length (cm)			Width (mm)			Length (cm)			Width (mm)		
						min	max	mean±S.D.	min	max	mean±S.D.			min	max	mean±S.D	min	max	mean±S.D	min	max	mean±S.D	min	max	mean±S.D
		1	1	3		6.2	8.4	7.3±0.8	2	3	2.66±0.4	3		46.8	63.8	55±6.9	4	6	5±0.8	5.0	5.3	5.14±0.11	2.7	3.9	3.14±0.6
Fertilizer	Light	2	2	3		7.4	15.8	11.0±3.5	3	4	3.33±0.4	3		41.2	53	47.2±4.8	3	6	4.66±1.24	5.1	5.3	5.2±0.08	3.6	4.3	3.63±0.81
		3	4	3		6.3	9.7	7.7±1.4	2	4	3±0.8	3		38.7	50.6	44.3±4.87	3	5	4±0.8	5.2	5.8	5.2±0.08	2.5	3.1	2.69±0.61
75 % +		4	6	3		7.4	13.2	10±2.4	3	4	3.33±0.4	3		33.2	46	39.5±5.2	2.0	3.5	2.83±0.6	5.1	6.0	5.55±0.27	2.0	2.7	2.20±0.66
	Shade	5	1			*Plants	shifted	to	shade																
Soil 25 %		6	2																						
		7	4																						
		8	6																						
		9	1	3		7.2	12.2	9.4±2.0	3	4	3.66±0.47	3		47.2	65	55.13±7.3	3.5	6	4.6±1.02	3.6	4.1	3.71±0.25	1.6	2.2	1.80±0.41
Fertilizer	Light	10	2	3		6.5	10.1	8.46±1.48	2	4	3±0.8	3		43.4	57.4	50±5.7	3.0	5	4±0.8	3.4	3.9	3.62±0.15	1.7	2.4	1.98±0.21
		11	4	3		6.8	11.2	9.46±2.05	3	4	3.33±0.47	3		37.4	47.3	42±4.07	2.5	4	3.16±0.62	3.6	4.0	3.80±0.13	1.7	2.6	2.12±0.33
50 % +		12	6	3		7.5	14.3	10.66±2.79	3	5	4±0.8	3		32.7	40.0	36.9±3.09	2.5	3	2.83±0.23	4.2	5.3	4.83±0.33	1.5	2.3	1.91±0.22
		13	1			*Plants	shifted	to	shade																
Soil 50 %	Shade	14	2																						
		15	4																						
		16	6																						
		17	1	3		7.3	14.8	11.1±3.06	3	5	4±0.8	3		44.4	61	53.4±6.85	2.5	5	3.6±1.02	2.0	2.6	2.32±0.17	1.3	1.7	1.52±0.13
Fertilizer	Light	18	2	3		7.4	15.3	11.9±3.3	4	6	5±0.8	3		41.5	50.3	46.5±3.7	2.5	4	3.3±0.6	1.6	2.1	1.87±0.16	1.4	1.8	1.68±0.11
		19	4	3		6.6	9.4	8.13±1.15	3	4	3.66±0.47	3		38.6	43.8	41.06±2.13	2.0	3	2.5±0.4	2.0	3.4	2.55±0.42	1.0	1.5	1.39±0.12
25 % +		20	6	3		6.3	11.2	8.93±2.0	3	4	3.33±0.47	3		30.3	38.6	34.6±3.3	1.5	2	1.83±0.2	3.7	4.7	3.93±0.29	1.1	1.6	1.44±0.12
		21	1			*Plants	shifted	to	shade																
Soil 75 %	Shade	22	2																						
		23	4																						
		24	6																						
		25	1																						
Lime	Light	26	2																						
		27	4																						
25 % +		28	6																						
		29	1																						
Soil 75%	Shade	30	2																						
		31	4																						
		32	6																						
		33	1																						
Lime	Light	34	2																						
		35	4																						
50 % +		36	6																						
		37	1																						
Soil 50 %	Shade	38	2																						
		39	4																						
		40	6																						

Table 3.15: Biometric measurements of *R.tinctorium* grown under different conditions.

				Underground shoot								Stem								Leaf					
Medium	Light condition	Sample No	Irrigation day	Number of measurements	Number of roots	Length (cm)			Width (mm)			Number of measurements	Number of stems	Length (cm)			Width (mm)			Length (cm)			Width (mm)		
						min	max	mean±S.D.	min	max	mean±S.D.			min	max	mean±S.D	min	max	mean±S.D	min	max	mean±S.D	min	max	mean±S.D
		1	1	23	23	2.5	26	13.5±7.6	3	12	7.17±2.82	28	28	36	156	103.1±38.9	1	6	4.5±1.63	4.3	5.4	4.97±0.32	1.5	1.7	1.63±0.07
Fertilizer	Light	2	2	15	15	3.5	22.5	12.2±6.08	3	6	4.4±1.08	26	26	40	120	83.8±24.7	1	5	3.65±1.32	4.0	5.2	4.61±0.39	1.3	1.8	1.59±0.13
		3	4	16	16	4.0	29	15.3±8.26	3	8	5.25±1.63	22	22	30	100	68.6±24 44	1	3	2.08±0.71	4.0	5.0	4.46±0.30	1.2	1.6	1.30±0.09
75 % +		4	6	27	27	3.0	27	15.6±7.71	5	6	5.39±0.48	17	17	18	78	48.5±23.8	1	3	1.76±0.8	1.6	4.0	2.47±0.75	0.5	0.7	0.6±0.08
		5	1	168 th	day	(dried)	38 cm	tall																	
Soil 25 %	Shade	6	2	83 rd	day	(dried)	40 cm.	tall																	
		7	4	75 th	day	(dried)	38 cm.	tall																	
		8	6	53 rd	day	(dried)	34 cm	tall																	
		9	1	12	12	4 0	33	22.25±11.5	2	10	6.6±2.52	22	22	30	130	91.9±35.5	2	3	2.77±0.44	5.8	6.2	6.07±0.12	1.5	2.1	1.89±0.24
Fertilizer	Light	10	2	10	10	4 0	30	21 4±10.3	3	7	6±1.2	19	19	32	96	71 8±29.5	1	3	2.26±0.7	4.5	6.2	5.75±0.32	1.4	1.9	1.72±0.13
		11	4	23	23	4 0	27	11.6±6.9	2	7	4.43±1.7	18	18	30	64	45.1±14.4	1	3	1.78±0.8	3.5	6.0	4.20±0.69	1.3	1.8	1.55±0.15
50 % +		12	6	26	26	3.5	21.5	7.3±5.6	4	6	4.26±0.5	18	18	18	68	35.1±13.5	1	2	1.47±0.49	2.7	5.0	3.5±0.74	1.3	1.7	1.37±0.12
		13	1	159 th	day	(dried)	40cm	tall																	
Soil 50 %	Shade	14	2	150 th	day	(dried)	27cm	tall																	
		15	4	146 th	day	(dried)	25cm	tall																	
		16	6	125 th	day	(dried)	24cm	tall																	
		17	1	23	23	2 5	8 5	6±1.5	5	12	8.79±3.3	26	26	36	128	95.7±35.2	2	3	2.65±0.47	4.3	5.4	4.96±0.34	1.7	1.9	1.79±0.09
Fertilizer	Light	18	2	18	18	5 0	13 7	10.32±3 2	4	9	6.6±1.45	22	22	32	116	80 48±27.3	2	3	2.55±0 49	4.0	5.2	4.61±0.35	1.6	1.8	1.42±0.22
		19	4	11	11	3 5	12 0	7 66±2 9	3	6	4.36±1.0	18	18	25	80	50.26±19	1	3	1 94±0 62	4.0	5.0	4.44±1.03	1.2	1.7	1.33±0.14
25 % +		20	6	11	11	4.5	14.0	9.48±3 0	4	9	6.45±1.72	16	16	42	64	51±7.23	1	3	1 68±0.84	1.6	4 0	2.96±0.79	0.5	0.7	0.62±0.08
		21	1	109 rd	day	(dried)	40cm	tall																	
Soil 75 %	Shade	22	2	108 th	day	(dried)	35cm	tall																	
		23	4	98 th	day	(dried)	30cm	tall																	
		24	6	94 th	day	(dried)	30cm	tall																	
		25	1																						
Lime	Light	26	2																						
		27	4																						
25 % +		28	6																						
		29	1																						
Soil 75%	Shade	30	2																						
		31	4																						
		32	6																						
		33	1																						
Lime	Light	34	2																						
		35	4																						
50 % +		36	6																						
		37	1																						
Soil 50 %	Shade	38	2																						
		39	4																						
		40	6																						