Bull. Fac. Agric., Cairo Univ., 52
(2001) : 85-114.

MORPHOLOGICAL,HISTOLOGICAL AND CHEMICAL INVESTIGATIONS OF *Calendula officinalis* L.

(Received : 6. 6. 2000)

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ABSTRACT

Botanical information on Asteraceae species is little . Hence, this investigation was planned to undertake a careful study on the external and internal structure , as well as the chemical composition of *Calendula officinalis* L. throughout the consecutive ages of two seasons . Data included germination of seed and seedling growth, length of the main stem, plant height, number of internodes of the main stem, diameter of basal internode of the main stem, length of 7 lateral branches, dry weight of leafless shoot and leaves, the inflorescence, the fruit, structure of the main root and stem, trichomes, structure of the leaf , structure of ray and disc flowers, structure of the fruit and determination of the percent and qualitative analysis of the volatile oil . As far as authors are aware, such data on Pot marigold were not previously given in detail . Accordingly, this information might be of value for investigators dealing with various aspects of this plant.

Key words : *Calendula officinalis L., histology, morphology, volatile oil .*

1. INTRODUCTION

Asteraceae (Compositae) consist of more than 1100 genera and about 20000 species, the largest family of flowering plants. *Calendula officinalis* L. (Pot marigold) a species of this family, is a locally well known plant.

Bailey (1947) mentioned that heads of Pot marigold are used sometimes for flavouring in cookery . In addition, florets are used in medicine as a vulnerary and anti-emetic . The flowering plant was formerly used for removing warts.

Botanical characteristics of Pot marigold have been stated by Christopher (1958), Rendle (1959), Wallis (1960), Bailey (1969) and Cutter (1975). *Calendula* comprises annual or perennial herbs with simple alternate leaves and mostly large heads with yellow or orange rays; involucre broad, the usually scarious-margined bracts in one or two rows; receptacle naked, plane; ray-achenes glabrous, incurved; disc-florets infertile; pappus none.Pot marigold is more or less hairy annual plant, 30 to 60 cm high, leaves thickish, oblong to oblong-obovate 5 to 15 cm. , or more long, entire or minutely and remotely denticulate, more or less clasping; heads solitary on stout stalks, showy 3.8-5 or 10 cm across the flat spreading rays white -yellow to deep orange; closing at night, sometimes the plant is proliferous from the involucre, bearing several peduncled heads in a circle .

Wallis (1960) stated that Pot marigold contains traces of volatile oil, a bitter principle, and calendulin; the latter being a tasteless substance swelling in water. The plant is used chiefly in the form of the tincture diluted with water as an application to bruises to promote the absorption of effused blood.

Megahed (1985) identified different constituents of the volatile oil of Pot marigold heads.

Many of Asteraceae species are ill- defined. Accordingly, it seems desirable to undertake careful phytochemical studies on plants of this family. *Calendula officinalis* L . is chosen in this work to disclose its different morphological, histological and chemical characteristics.

2. MATERIALS AND METHODS

Seeds of *Calendula officinalis* L. were procured from the Experimental Station of Medicinal Plants, Faculty of Pharmacy, University of Cairo, Giza,where the field work was carried out. Test of germination was conducted in the Seed Testing Department, Agricultural Research Centre, Giza; according to the International Rules for Seed Testing (Anon., 1985).

Planting in the field was carried out at mid-October in both 1985 and 1986 . Seeding was conducted in warm seed beds. Plants were then transplanted to the field at mid-November. Planting was achieved according to a randomized complete block design with 4 replicates.Plot dimensions were 2.5 x 6 m, with 10 ridges 50 cm apart. Plants were disseminated in hills spaced 50 cm on the northern side of the ridge at the rate of one plant per hill . Cultural parctices were carried out as recommended. Vegetative characters were followed up fortnightly using 20 random plants , 5 plants per plot. Dry weight was performed using 20-plant- samples , dried in an oven at 70°C till a constant weight was reached (almost 48 hrs.). Various characters of inflorescence, fruit and seed were investigated.

A full microscopical study was carried out. Materials were taken fortnightly . Specimens represented different plant organs. Microtechnique procedures given by Willey (1971) were followed.

Duplicate water distillation of the volatile oil were performed (Anon. 1980) . Time of distillation was 3-4hrs. (Guenther, 1952).GLC technique was used to separate and detect the volatile oil constituents. GLC conditions were as follows : Detector : FID; PEGA glass column 1.5m x 4mm; Temperature : injector 220°C, oven 60-180°C increased by 4°C/min, detector 300°C; Flow rate ml/min: N_2 30, H_2 33, air 330; Chart speed: 10 mm/min .

Data were subjected to various conventional methods of statistical analysis, according to Snedecor and Cochran (1982).

3. RESULTS AND DISCUSSION

3.1. External morphology

3.1.1. Germination of seeds and seedling growth

Germination is epigeal as the two cotyledons are brought above the ground. At first , the pericarp of achene enclosing the seed together with seed testa imbibe water , softened and burst . This lasted 48 hrs. from sowing. Then , the tip of the radicle emerges from the basal end of the fruit coat. This takes 57 hrs. Thereafter, the radicle grows rapidly downward through the soil. This is followed by an elongation of the hypocotyl which raises the two cotyledons and the partially enveloping remains of the achene above the soil, 118 hrs. after sowing. The hypocotyl is bent in its growth before emergence above the soil, then becomes straight and the cotyledons take a horizontal position in a period of 166 hrs. The completely developed cotyledon averaged 5.6 cm in length and 0.9 cm in width. By now, the plumule is also upward. In the mean time, i.e., after 166 hrs. of sowing the secondary roots develop. The first differentiated true leaves are two foliage ones, developing 22 days after sowing.

Using the rules of ISTA (Anon., 1985) the speed of germination (after 7 days) was 56.29% and the capacity of germination (after 14 days) was 78.57% .

3.1.2. The stem : After completion of the seedling stage (3 week old) the plumule started its development to produce the shoot. At the age of 4 weeks , the first basal internode of the main stem was the only visible one forming the majority of plant height . The other developed internodes were too short and were surrounded with crowded leaves in the form of a rosette .When the plants were 6 weeks old, the first and the second internodes were nearly angular, while the upper internodes were still too short. In 8-week-old plants, the upper internodes elongated and became distinct. Axillary buds at the third to eighth nodes began to burst .

At flowering onset (The age of 10 and 12 weeks in the first and second seasons, respectively) the apex of the stem differentiated into a flower bud. At the same age, the two following axillary buds at the ninth and tenth nodes, also began to burst . The lower buds developed into lateral branches. Throughout the following ages , tertiary branches were formed on the elongated secondary branches . All lateral branches behaved in their growth in a manner similar to that of the main stem except the terminal branch which did not bear any lateral branches.

Stem is green in colour, herbaceous, bearing 5-6 branches developed on the fifth node and upward. The basal internodes are short and the branches are distributed on the main stem. The stem is erect, glabrous, more or less hairy, solid pentagonal. The epidermis of the stem gives rise at the corners and sides trichomes .

It is worthy to mention that the lateral branches are responsible of the actual height of plant since the growth of the main stem is determinated by an inflorescence produced at its apex. Moreover, the lateral branches play a vital role in yield production since each of them ended with an inflorescence .

3.1.2.1. Length of the main stem : The quadratic equations and lines of the main stem length and periods are shown in Fig. 1. Results of length of the main stem at the consecutive periods revealed that a significant increase in length was detected from sowing upto the age of 12 weeks in the first season where the average stem length was 21.4 cm . In the second season, however, the significant increments in length of the main stem continued till the age of 14 weeks , being 24.7 cm. There were no substantial increments in length of the main stem at further ages. This means that the growth of the stem is determinate and reaches its maximum length during the first half of the growing season .

3.1.2.2. Plant height : The equations and lines of periods and plant height during the two experimental seasons are presented in Fig.2. Data of plant height throughout successive ages of the first season indicate that significant increments were attained during the consecutive periods upto the end of the growing season where the plants reached an average height of 62.3 cm at the age of 24 weeks. Similar increments were also achieved in the second season although the increase after the age of 18 weeks was statistically insignificant . Plant height averaged 61.1 cm at the end of the second growing season . The aforementioned results are in agreement with those of Christopher (1958) who mentioned that the height of Pot marigold plants ranged from 45 to 60 cm. Bailey (1969) also stated that plants reached a height from 30 to 60 cm .

3.1.2.3. Number of internodes of the main stem : The quadratic equations and lines of periods and number of internodes of the main stem during the two experimental seasons are shown in Fig. 3. It is evident from values belonging to number of internodes of the main stem that a significant increase in numbers was recorded from sowing through the age of 14 weeks where numbers were 13.9 and 12.0 in the two consecutive seasons. No further substantial increments were observed in the number of internodes of the main stem till the end of the growing season .

It is worthy to note that growth in length of the main stem, mentioned earlier , and its number of internodes behave in a similar manner. It was found that neither stem length nor the number of internodes recorded considerable increments after the age of 14 weeks proving the determinate pattern of stem growth.

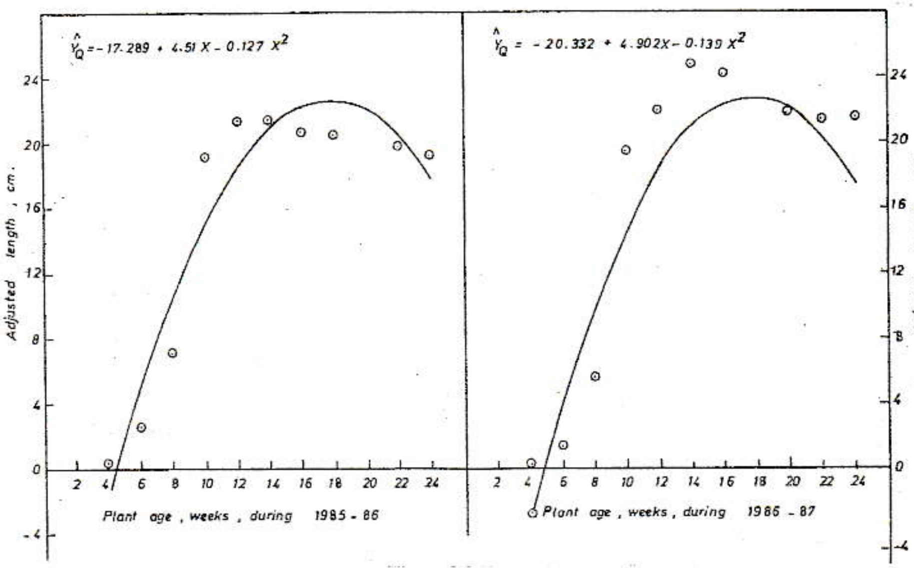

Fig.(1): Graph of regression of length of the main stem on plant age in *Calendula officinalis* L. in two seasons.

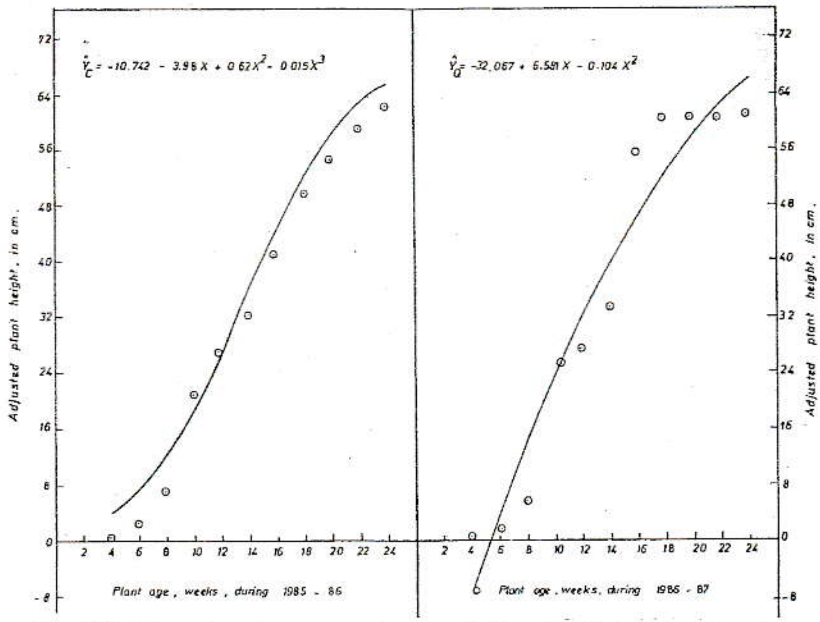

Fig.(2):Graph of regression of plant height on plant age in *Calendula officinalis* L.in two seasons.

3.1.2.4. Diameter of basal internode of the main stem : The quadratic equations and lines of plant ages and diameter of basal internode of the main stem throughout two growing seasons are presented in Fig.4. It is obvious that the diameter of basal internode of the main stem achieved significant increments upto 18 weeks of age in the first season (13.8 mm) . In the second season , however , this was attained at the age of 16 weeks being 14.4 mm .

It is important to note that no substantial increments were recorded at certain periods during the growing season such as the ages of 10, 14 and 16 weeks in the first season and the age of 14 weeks in the second one . This might be attributed to the fact that plants devoted their growth at these periods to develop more branches or inflorescences .

At the final sampling date, *i.e.*, (24 week old), the diameter of basal internode of the main stem significantly decreased being 10.8 and 12.7 mm in the two experimental seasons,respectively. This decrease might be a result of the normal dryness and shrinkage associated with senescence .

3.1.2.5.Length of lateral branches : The periodic growth in length of lateral branches was followed up fortnightly throughout the growing season. Seven secondary branches arose from the main stem. Branches started their development at the age of 8 weeks . At this age, 5 branches developed. When plants were 12 week old all secondry branches were formed being 7 in number . Data on length of the main seven lateral branches throughout two seasons are given in Table 1 .

3.1.2.5.1.The first secondary branch : This branch arose at the age of eight weeks . Its length was 0.3 and 0.4 cm in the first and the second season , respectively . Significant increments in length of this branch were achieved during the consecutive sampling dates up to the age of 16 weeks , being 35.0 and 35.6 cm in the two experimental seasons .

No considerable increase was observed during the following period (18 week old). However , the length reached a maximum when plants aged 20 weeks being 39.1 and 38.6 cm in the first and the second seasons , respectively.

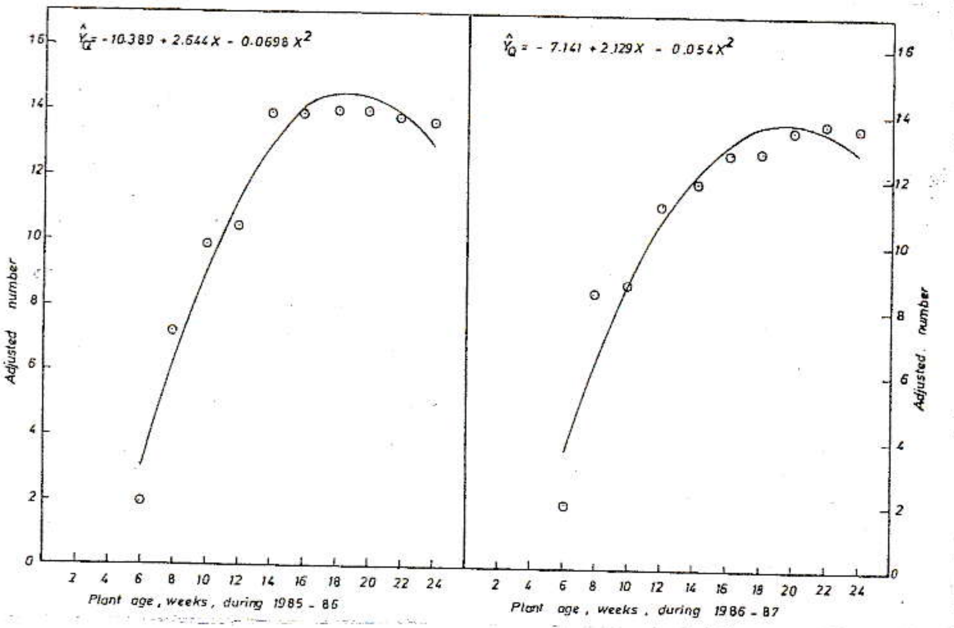

Fig.(3):Graph of regression of number of internodes of the main stem on plant age in *Calendula officinalis* L. in two seasons.

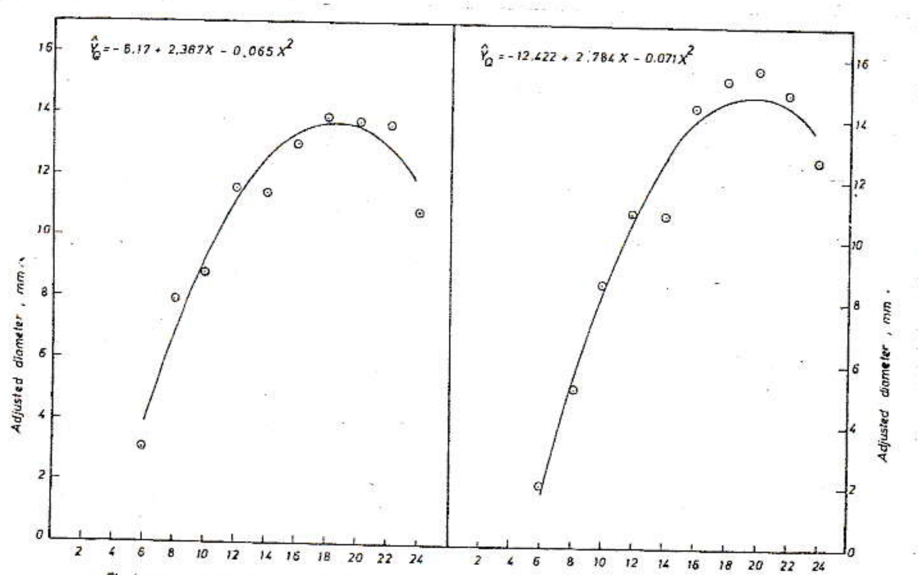

Fig.(4):Graph of regression of diameter of basal internode of the main stem on plant age in *Calendula officinalis* L. in two seasons .

Table (1): The periodic growth in length, in cm , of secondary branches of *Calendula officinalis* L. in two seasons .

Plant age in weeks	First branch		Second branch		Third branch		Fourth branch		Fifth branch		Sixth branch		Seventh branch	
	First season	Second season	First season	Second season	First season	Second season	First season	Second season	First season	Second season	First season	Second season	First season	Second season
8	0.3	0.4	0.2	0.3	0.3	0.2	0.2	0.2	0.1	---	---	---	---	---
10	4.9	4.0	4.9	4.9	6.6	3.6	7.0	4.6	4.4	4.9	---	4.8	---	---
12	8.5	10.6	5.4	4.9	6.8	5.5	8.7	7.0	9.3	9.5	3.3	7.2	1.7	4.3
14	31.5	31.0	23.7	23.7	24.4	27.6	24.1	21.8	21.2	21.2	17.8	19.2	7.8	8.7
16	35.0	35.6	30.7	30.7	27.9	29.7	27.2	26.5	22.6	24.3	18.9	21.9	10.2	11.4
18	35.7	37.7	34.4	34.4	31.9	31.6	27.1	26.8	29.5	24.4	24.4	20.3	13.2	16.4
20	38.2	39.1	30.6	31.4	30.2	30.2	28.7	29.2	28.2	23.8	21.3	18.8	18.7	17.1
22	39.1	38.6	21.3	29.6	30.0	29.9	28.3	26.8	27.7	22.0	19.0	18.7	18.3	16.2
24	38.8	38.6	28.1	28.4	28.9	28.2	25.4	25.4	25.2	22.4	19.0	18.1	17.0	15.8
New L.S.D. (0.05)	2.6	2.2	3.1	2.5	2.2	1.8	3.7	1.3	4.3	1.4	6.0	2.4	3.4	1.2

3.1.2.5.2. The second secondary branch : This branch developed after 8 weeks being 0.2 and 0.3 cm in the two experimental seasons, respectively . By elapse of time , progressive increments in length were attained reaching its maximum when plants aged 18 weeks being 34.4 cm in each of the two growing seasons . It was noticed that the increase in branch length at 12 week old was not statistically significant .

3.1.2.5.3. The third secondary branch : Length of this branch at the age of 8 weeks was 0.3 and 0.2 cm in the first and the second seasons, respectively. Apart from the period of 12 week old, the length achieved progressive increments throughout the consecutive sampling dates reaching a maximum length of 31.9 and 31.6 cm in the first and the second seasons, respectively when plants aged 18 week old .

3.1.2.5.4. The fourth secondary branch : At 8 week old , length of this branch was 0.2 cm in each of the two growing seasons. Substantial increments were almost added to the length upto the age of 20 weeks , being 28.7 and 29.2 cm in the first and the second seasons, respectively. The fourth secondary branch did not exhibit a consistent trend in the following periods , namely 22 and 24 week old where the increase was indifferent in the first season although in the second one it proved significant .

3.1.2.5.5. The fifth secondary branch : This branch arose in the first season at the age of 8 week, being very short (0.1 cm). In the second season, it was noticeable at 10 week old , being 4.9 cm. This branch progressively increased in length till the age of 18 week in the first season where it was 29.5 cm . In the second season, however , the maximum length was achieved at the age of 16 week, being 24.3 cm . Toward the end of the growing season , the length of this branch decreased where it was 25.2 and 22.4 cm at 24 week old in the first and the second seasons, respectively .

3.1.2.5.6. The sixth secondary branch : This branch developed at the age of 10 weeks in the second season . Its length was 4.8 cm . However , in the first season its development started two weeks later where the length was 3.3 cm . The branch attained its maximum length after 18 weeks of sowing in the first season (24.4 cm). But in the second season this occurred at the age of 16 weeks (21.9 cm) .

3.1.2.5.7. The seventh secondary branch: This branch occurred when plants aged 12 weeks. Its length was 1.7 and 4.3 cm in the first and the second seasons, respectively. The length increased significantly upto the age of 20 weeks reaching a maximum of 18.7 and 17.0 cm in the two successive seasons. No substantial increments took place after the age of 20 weeks .

3.1.2.6. Dry weight of leafless shoot : The cubic equations and lines of this trait as well as plant ages are given in Fig. 5. The dry weight of leafless shoot gained no considerable increments upto 12 week old . From the age of 14 weeks onwards, significant increments were achieved in the dry weight of leafless stem and lateral branches reaching a maximum at the end of the growing season (24 week old) being 128.5 and 119.2 g in first and second seasons, respectively .

3.1.3.The leaf : The first foliage leaf developed 3 weeks after sowing. All leaves were similar in shape. Even cotyledons were similar to the foliage leaves, apart from being smaller in size. Leaves are 5-15 cm or more long, simple, alternate, exstipulate, thickish , hairy, sessile, oblong to oblong- obovate in shape, with entire margin or minutely and remotely dinticulate, cuspidate apex and more or less clasping. Venation is reticulate-pinnate. The midrib of the leaf is slightly convex at the abaxial surface, and convex at the adaxial one (Fig. 6). The aformentioned description agreed with that stated by Rendle (1959) and Bailey (1969).

3.1.3.1. Dry weight of leaves : The linear equations as well as lines of this character and plant ages are presented in Fig . 7. It is obvious that the dry weight of leaves achieved a significant progressive increase throughout the growing season apart from the two sampling dates of 12 and 18 weeks old. This was true in the two growing seasons. The first drop in dry weight of leaves which took place at the age of 12 weeks coincided with onset of flowering . It would be naïve to expect that start of flowering would affect temporarily leaf production. Likewise, the second drop in the dry weight of leaves occurred at 18 week, parallel to leaf withering that happened at this period. However, the newly developed branches carrying leaves added once more to the dry weight recorded at the following dates. The dry weight of leaves at the end of the growing season was 26.8 and 33.0 g in the two experimental seasons.

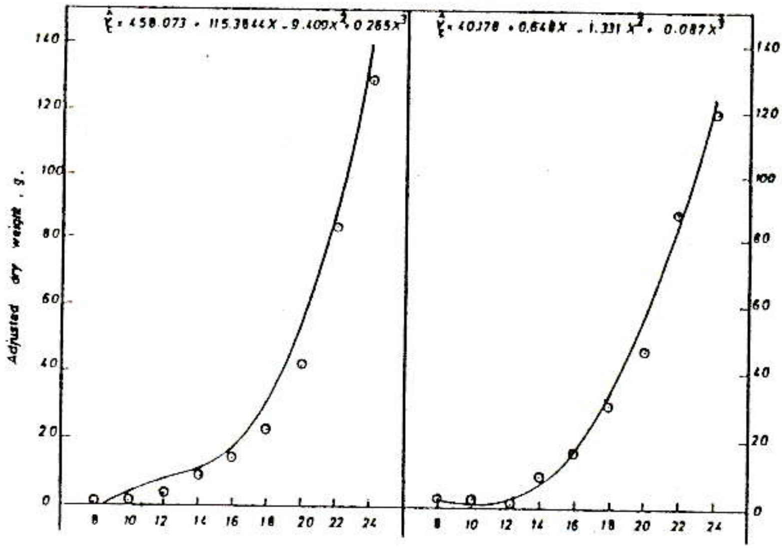

Fig. (5): Regression of dry weight of leafless shoot on plant age in *Calendula officinalis* L. in two seasons .

Fig.(6): A photograph of terminal portion of *Calendula officinalis* L. branch showing morphological features of the leaf .

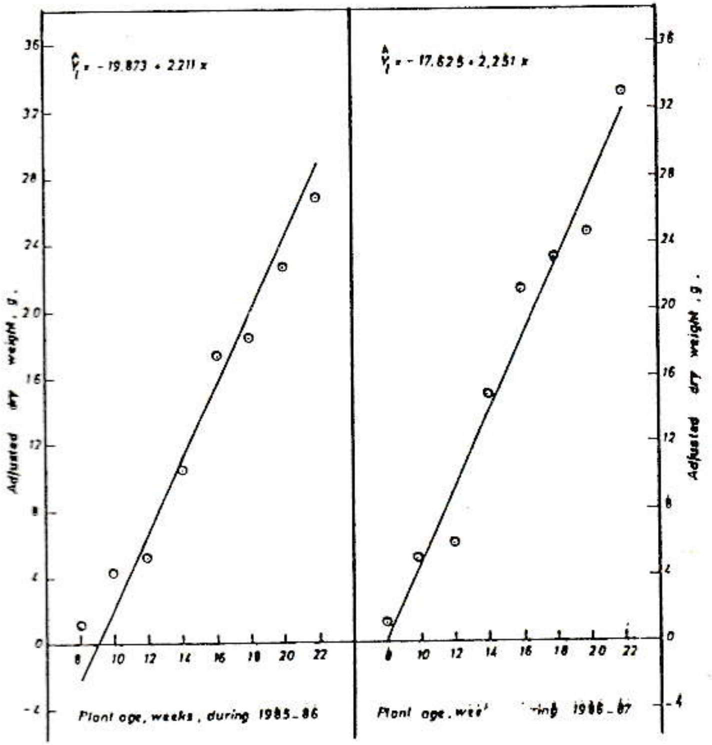

Fig.(7): Regression of dry weight of leaves on plant age in *Calendula officinalis* L. in two seasons.

3.1.4. The inflorescence : The inflorescence formation started toward the end of December and early in January when plants are 10 to 12 week old . The development of reproductive and vegetative organs continued parallel to each other . The inflorescence is a capitulum reaching to about 10 cm across . It is simple , terminal and racemose . Capitula are solitary on stout stalks . The average period from flower bud differentiation in the inflorescence till fruits are produced took about 25 days (Fig. 8). The inflorescence is consisting of ray and disc flowers. The flowers in the capitulum open in acropetal succession , the youngest being in the center . The two types surrounded by an involucre of subequal bracts arranged in two series . The bracts , known as phyllary, are covered with different types of trichomes which are similar to those of leaves. The description of both of the ray and the disc flowers is presented in what follows (Fig. 9).

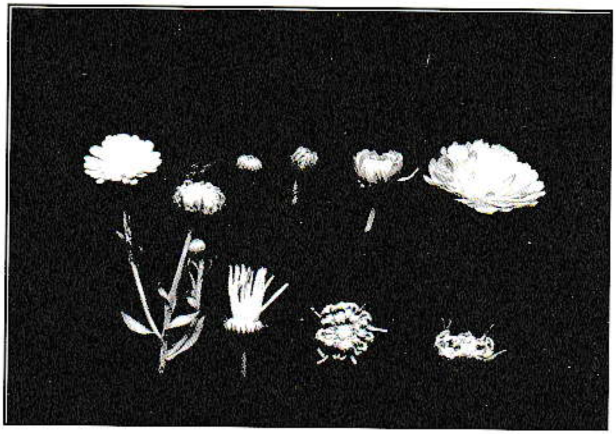

Fig .(8): A photograph of *Calendula officinalis* L. showing to the left a portion of flowering branch and to the right successive developmental stages of inflorescence starting at top left with flower bud, blooming, the ray flower in upright position then the ray flower in horizontal position . At lower left fertilization , then two views of mature fruits .

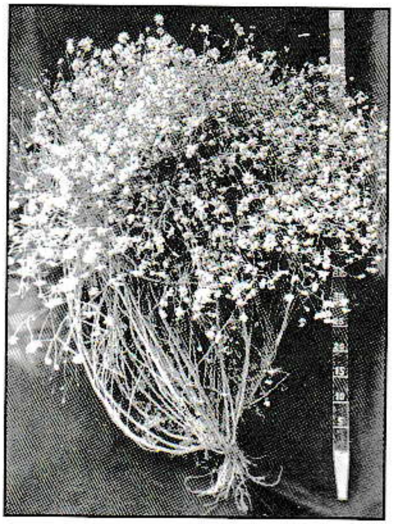

Fig. (9): A photograph showing habit of *Calendula officinalis* L. in flower (22 week old)

3.1.4.1.The ray flower is deep orange in colour, sessile and zygomorphic. The floral bracts are absent . Flowers are female. They are arranged in the two to three outermost rows of the receptacle . Calyx absent. Corolla is ligulate . The limb or strap of the corolla is broad at the widest part. It is oblanceolate and is terminated by 3 , sometimes 2 or 4 , acute teeth . It has 4 , sometimes 5 to 7 , principle veins which are joined by arches at the apex and bears characteristic trichomes externally at the base of corolla . The trichomes are similar to those found on the leaf being mentioned throughout the histological part of this study. Androecium absent. Gynoecium is compound pistil of two united carpels , with one locule , ovule one of basal placentation, ovary inferior , long style and bifid stigma.
Floral formula for the ray flowers is as follows:

$$\%, ♀, CA^{x}, CO^{(3\text{-}5)}, A^{x}, \overline{G}^{(2)}$$

3.1.4.2.The disc flower is deep-orange in colour, sessile, actinomorphic, hermaphrodite but sterile , tubular and epigynouse calyx absent corolla, consists of 5 united petals , 5 lobed and tubular. It bears trichomes at the base similar to those developed on the stem. Androecium consists of 5 stamens epipetalous alternating with the petals, with syngenesious stamen , anthers pointed at the base and filaments free. Gynoecium mainly consists of remains of the style and stigma. The stigma is slenderly conical and covered with papillae. Ovary is rudimentary .
Floral formula for the disc flowers is as follows :

$$\oplus, ⚥, CA^{x}, \overset{\frown}{CO^{(5)}, A^{(5)}}, G^{x}$$

It is worthy to mention that seeds are produced only in ray flowers. Disc flowers, however, are sterile due to its undeveloped ovary.

The aforementioned data recorded for the inflorescence including the ray and disc flowers are in accordance with those given by Rendle (1959) , Wallis (1960) and Bailey (1969) .

3.1.4.3.Cumulative number of inflorescences: Number of inflorescences per plant (Fig. 10) increased steadily reaching a maximum at mid-March being 41.1 and 41.0 in the two consecutive seasons, respectively. Thereafter, the average number of produced inflorescences decreased toward the end of flowering period being 5.7 in the first season and 3.5 in the second one . It is worthy to mention that fruit formation in developed inflorescences started at late March and proceeded parallel to formation of inflorescences .

3.1.5. The fruit: It is one - seeded inferior achene . The pericarp is free from the seed coat . The seed is filling the fruit . Endosperm is absent . Embryo is oily , curved , with - short inferior radicle and two expanded cotyledons . Fruits are polymorphic on the same head . The outermost are much elongated with small spines on the back and often beaked at the tip .The next set has broad wings inrolled at the margin and are without a beak . The innermost fruits are smaller , much incurved , often forming a ring and have also narrow involute wings .

The aforementioned description of fruit is in accordance with that given by Rendle (1959) , Wallis (1960) , Bailey (1969) and Cronquist (1981) .

3.1.5.1. Specific weight of fruit : Various types of fruits (Table 2) recorded a highly significant difference among their specific weight . The big incurvate fruits were on top among different fruit types. The weight of 100 big incurvate fruits was 1.456 g. The corresponding values for elongated and small incuravate fruit types were 1.277 and 0.699, g , respectively .

3.1.5.2. Size of fruit : The trend of results dealing with size of different fruit types (Table 2) was similar to that of specific weight. The big incurvate fruits gave the largest size (5.5 cm^3) followed by the enlongated(3.3 cm^3) then the small incurvate (1.5 cm^3) fruits .

Table (2) : Average specific weight , and size , of 100 fruits of *Calendula officinalis* L .

Fruit types	Specific weight, g	Size, cm^3
Elongated	1 . 277	3.3
Big incurvate	1 . 456	5.5
Small incurvate	0 . 699	1 .5
L .S .D . (0 .05)	0 . 118	0 .5

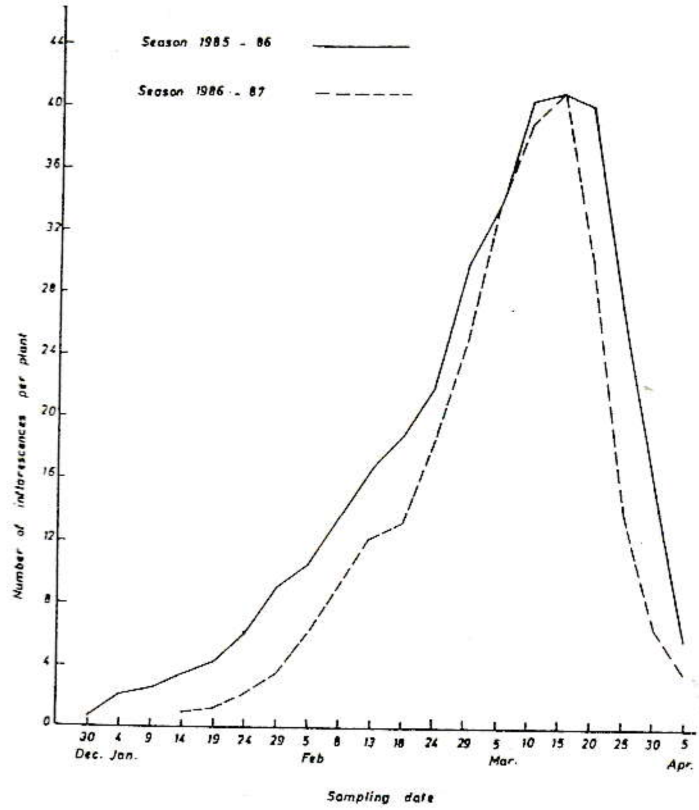

Fig. (10): Cumulative production of inflorescences at five- day intervals during two growing seasons in *Calendula officinalis* L .

3.2. Histological studies

3.2.1 Structure of the main root : The main root of 7 - day-old seedlings (Fig.11) has an uniseriate epidermis of tubular- shaped cells , followed by a cortex of 7 to 9 layers of thin- wal ed irregular parenchyma cells of obvious intercellular spaces, the innermost layer of the cortex is the endodermis . Its casparian strip is detected as dots in the transverse sections . The pericycle consists of one layer following the endodermis to the inside . The stele is composed of two phloem strands alternating with a similar number of exarch xylem ridges (Diarch protostele type) . Each xylem ridge comprised of 6 to 7 vessels. The phloem is characterized by a large amount of parenchyma cells . The pith is absent since the metaxylem vessels are advanced to the centre of the root .

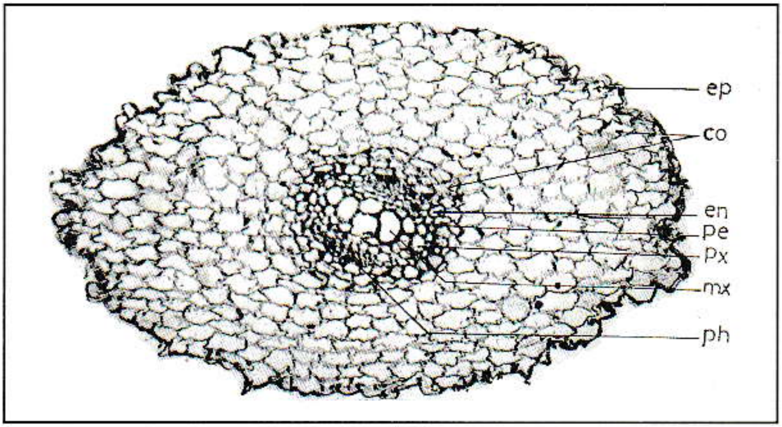

Fig.(11):Transverse section of 7-day-old main root of *Calendula officinalis* L., showing its primary structure . Details : co, cortex ; en , endodermis ; r , rays ; sph , secondary phloem ; sx , secondary xylem . (X 125) .

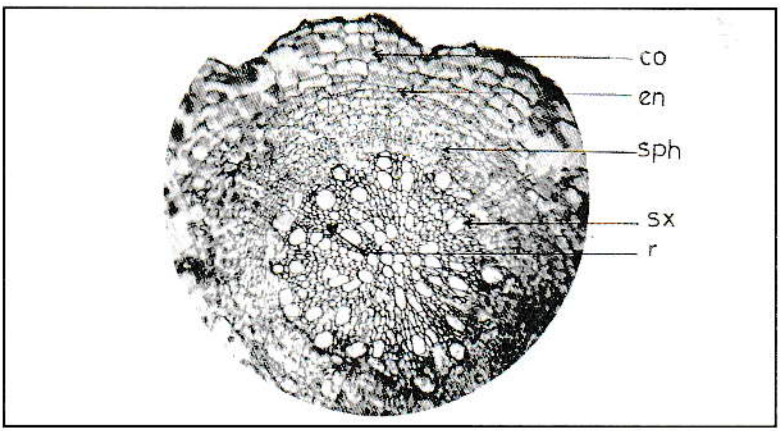

Fig. (12) : Transverse section of the main root of *Calendula officinalis* L., 6 week old, showing its secondary structure. Details : co, cortex; en , endodermis ; r, rays ; sph , secondary phloem ; sx , secondry xylem. (X 125) .

Secondary growth of the main root takes place when plants are 2 week old in the common form . At the age of 6 weeks (Fig.12) formation of secondary xylem and phloem proceeds and the cambium which is originated in the pericycle produces lignified parenchymatous rays each consists of 2-3 rows of radially elongated cells . The secondary xylem consists of tracheary elements mostly vessels and parenchyma cells . The fibres appear in groups adjacent to the phloem . The cortex is ruptured at many regions and the endodermis is still intact keeping pace with the increase in root girth through anticlinal divisions of its cells .The seondary thickening is more prominent as plant age proceeds .

3.2.2. Structure of the main stem : The shoot apex is dome - shaped consists of 2- layered tunica overlying the corpus . The median portion of the main stem at flowering onset shows in transverse section an epidermis composed of a single layer . The cortex consists of 10-15 layers, the outer 2-3 layers are collenchymatous underlying the epidermis (Fig.13) . The innermost layer of the cortex, the starch sheath , is hardly recognized. The bundles are endarch and collateral , forming a dictyostele . The fascicular cambium forms much secondary xylem and little secondary phloem . The interfascicular cambium produces new bundles .Thus , the stele consists of 35-40 bundles , each with about 10-15 rows of vessels . The fibrous caps are hardly recognized . The pith across its diameter consists of 40-45 polygonal cells , the diameter of pith averages 5100 μ . The medullary rays are 1-3 rows of cells .

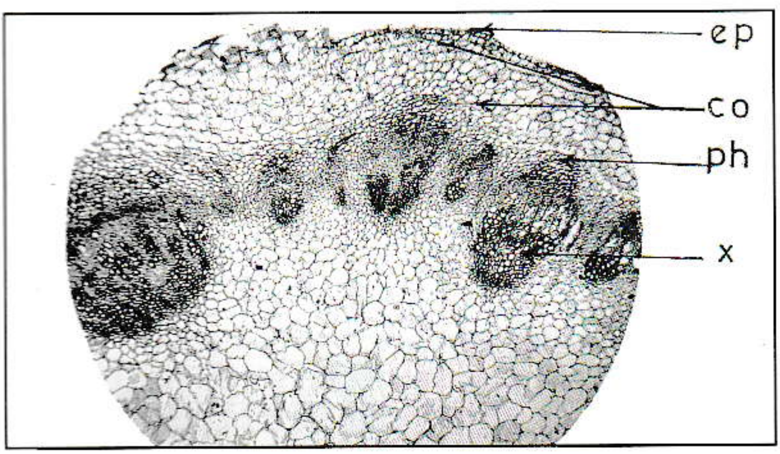

Fig .(13) : Transverse section of the median internode of the main stem of *Calendula officinalis* L., at flowering onset. Details : co, cortex ; ep , epidermis ; ph , phloem ; x , xylem .(X 32).

3.2.3. Trichomes : The leafless shoot of Pot marigold forms three types of trichomes (Fig .14) namely :

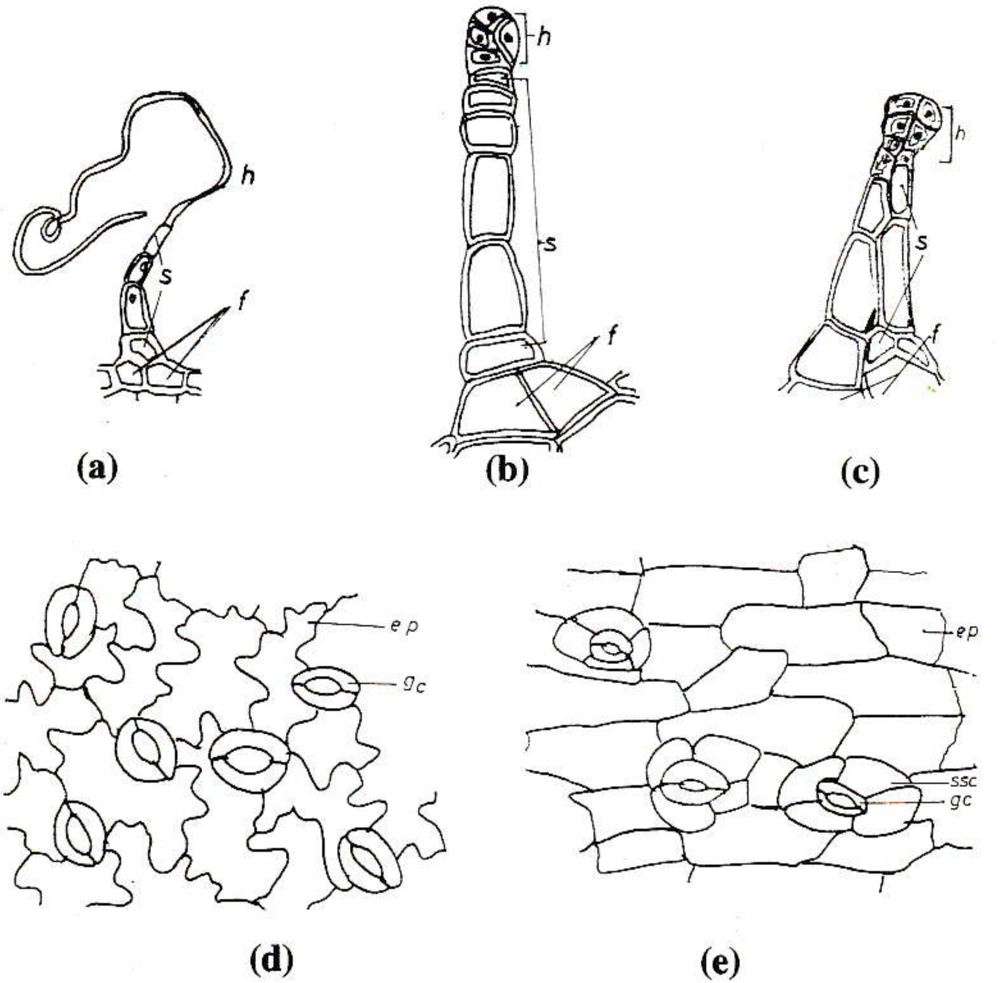

Fig. (14) : Type of trichomes and stomata developed by *Calendula officinalis* **L. (a) aseptate-flagellate hair. (X 300) (b) uniseriate glandular trichome. (X 400) . (c) biseriate vesicular capitate glandular hair (X 300) . (d) anomtetracytic stoma . (X 300)(e) anomcytic stoma (X 300) . Details : ep, epidermis; f , foot ; gc , guard cell ; h , head , s , stalk ; ssc , subsidiary cell .**

3.2.3.1. Aseptate-flagellate hair: This type is found on stem corners. It consists of a foot which is a part of the trichome lying in the epidermis, usually 2 cells in thickness, a stalk of 4 cells in length and a very long terminal one cell.

3.2.3.2. Uniseriate glandular trichome : It is spread on all sides of the stem . This trichome consists of a foot which is a part of the trichome lying in the epidermis, usually 2 cells in thickness and body of the trichome consists of 5 cells in one row . Head of the trichome consists of 4 or 5 cells .

3.2.3.3. Biseriate vesicular capitate glandular hair : Different developmental stages of this trichome are found spread on the stem sides . The mature trichome consists of a compound foot which consists of cells that are more in number than the cell rows of the immediately overlying part of the trichome . In addition to a differentiated body which consists of structurally and usually also functionally 2 different parts , *i.e.* (i) the stalk , representing the proximal and (ii) the head , representing the distal region .

The previously mentioned trichome types are in harmony with those mentioned by Ramayya (1969) .

The stomata are of anomotetracytic type . A stoma consists of two guard cells and four subsidiary cells .

3.2.4. Structure of the leaf : The blade of the mature leaf (Fig.15) is composed of 2 epidermal layers (abaxial and adaxial), a mesophyll (palisade and spongy tissues), and a midrib which is the principle vascular vein . The epidermal cells are thin -walled and covered with a thin cuticle . Both upper and lower epidermis cells appear tangentially elongated in shape .

Stomata are more numerous on the lower epidermis than on the upper one and it is of anomocytic type, *i.e.*, without subsidiary cells . At the midrib region , both upper and lower epidermis are convex, and the thickness at this region averages 1097 μ. While that of the mature blade averages 336 μ.The palisade tissue consists of one layer of slender cells full of plastids, occupying one-half of the whole thickness of the mesophyll. The spongy tissue is composed of 2-3 layers of chlorenchymatous loosely arranged cells, with many wide intercellular spaces. On the abaxial side at at the midrib region, there are 5-6 layers of cells. The 1-2 layers abutting the lower epidermis are angular collenchyma, whereas the remainder are chlorenchymatous cells. The same is observed for the adaxial side. The midrib bundle has relatively narrow phloem on the abaxial side, and a wider xylem on the adaxial one. The number of xylem rows in the midrib of the mature leaf averages 9, each of 3-7 vessels. The bundle is surrounded by a sheath of one layer of parenchymatous cells.

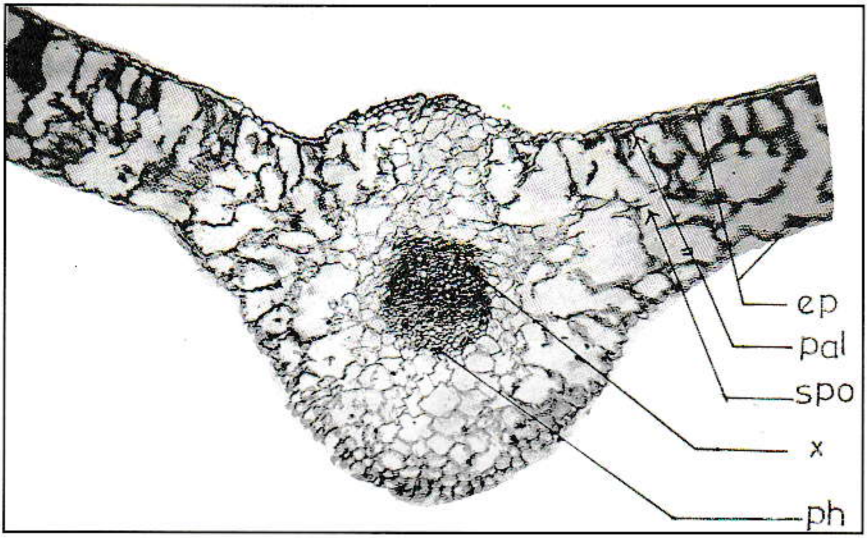

Fig .(15):Transverse section of mature leaf of *Calendula officinalis* L. crossing the midrib. Details: ep , epidermis ; pal , palisade tissue ; ph , phloem ; spo , spongy tissue ; x, xylem . (X 50) .

3.2.5. Structure of the ray and the disc flowers :

3.2.5.1.The ray flower : The ray flowers are arranged on the margin of the flattened receptacle . They are located around the periphery . The calyx is absent . The corolla is found at the abaxial side and consists of 2 epidermal layers with 8-10 layers of rounded parenchymatous cells in between. There are 6-7 bundles extending through it. Stamens are absent. The two united carpels are supplied by 2 bundles running through a mesophyll of 8-11 layers of polygonal parenchymatous cells . The vascular bundles in the different flower parts are collateral and closed . The xylem is composed of 1-2 rows each of 1-2 vessels. The phloem consists of few cells mostly parenchyma .

3.2.5.2. The disc flower : The disc flowers are arranged in the centre of the flattened receptacle . Transverse section of the flower bud shows that the sepals are absent (Fig.16) . The corolla tube consists of 2 epidermal layers with 4-5 layers of loosely arranged polygonal parenchymatous cells in between . There are 5 bundles extending through the tubular portion of the corolla. Filament of the stamen is more or less round in shape, bound by an uniseriate layer of square-shaped cells. Its ground tissue consists of 4-6 layers of round parenchymatous cells . There is a bundle located in the centre of the filament. Style of gynoecium is supplied by 2 bundles running through a mesophyll of 8-9 layers of polygonal parenchymatous cells. The vascular bundles are collateral and closed. The xylem is composed of 1-2 rows with 2-3 vessels each. The phloem consists of few cells mostly parenchyma. The mechanical tissue surrounding the bundles is almost absent in the flower parts .

3.2.6. Structure of the fruit : The achene fruit develops from an inferior ovary. The fruits are polymorphic on the same inflorescence, namely, small incurvate, big incurvate and elongated. It is irregular in outline. The transverse section presented in Fig.17 shows the structure of the fruit and the seed. The fruit coat consists of an outer layer (exocarp) of parenchyma cells varying in shape and size. The narrow winged region of the fruit consists of epidermis and subepidermis. The outer epidermis cells are nearly rounded in shape while cells of the inner layer are palisade-like. The subepidermal cells have an elongated or barrel shape. Some of the epidermal cells differentiate into trichomes, which are similar to those of other organs of the plant. The median region of the fruit coat (mesocarp) consists of three layers. The first layer laying below the epidermis is pistillate in shape. This layer collapses in most places of the fruit. The cells of the other two layers have thin wall, elongated in shape with intercellular spaces. The vascular bundles are present among these layers. The inner layers of the fruit coat (endocarp) are characterized

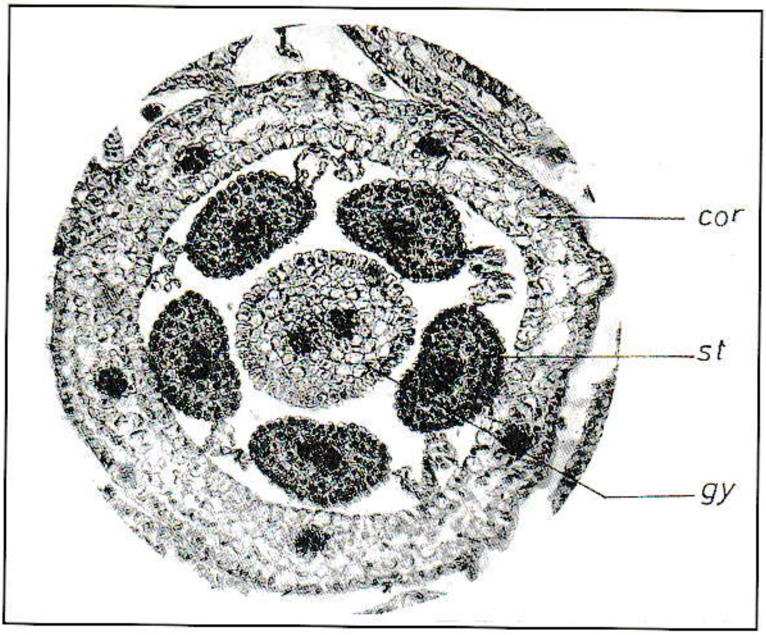

Fig. (16) :Transverse section of flower bud of *Calendula officinalis* L., showing the structure of the disc flower. Details : cor, corolla ; gy , gynoecium ; st , stamen . (X 200) .

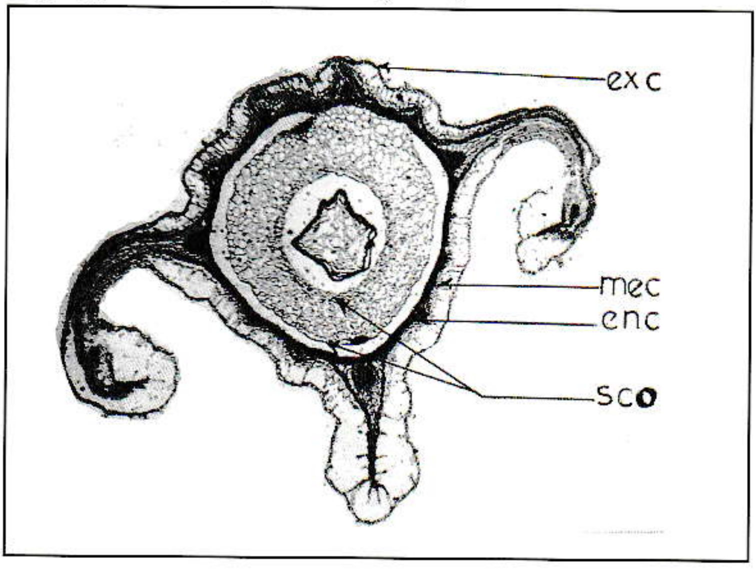

Fig. (17) : Transverse section of the mature fruit of *Calendula officinalis* L, showing the structure of the fruit and the seed. Details : enc , endocarp; exc , exocarp ; mec , mesocarp; sco, seed coat . (X 50).

by having cells with thick lignified walls. The seed coat consists of more or less spongy parenchymatous cells. The embryo which is comprised of two thick curved cotyledons, radicle and plumule forms the major part of the seed .

3.3. The volatile oil

3.3.1. Determination of the volatile oil percentage: Statistical differences were recorded in volatile oil percent among different stages of inflorescence development (Table 3). Concerning the developmental stages, the highest concentration of volatile oil (0.26%) was produced at the horizontal ray flowers stage. In direct contrast, the least concentration (0.06%) was found at budding stage. Values of volatile oil related to the interaction between seasons and stages also proved that the horizontal ray flowers stage was characterized by the highest volatile oil concentration, being 0.26 and 0.25% in the two respective seasons. The total yield of inflorescences per plant at horizontal ray flowers stage was 44.399 and 45.116 g in the two seasons. Hence, the volatile oil yield per plant at this stage was 0.113 and 0.115 ml, respectively .

Table (3) : Percentage of volatile oil in *Calendula officinalis* L., inflorescence at different developmental stages in two seasons.

Stage	First season	Second season	$\bar{X}$
Budding	0.06	0.06	0.06
Flowering onset	0.15	0.17	0.16
Upright ray flowers	0.15	0.17	0.16
Horizontal ray flowers	0.26	0.25	0.26
Mature fruit	0.06	0.08	0.07
$\bar{X}$	0.14	0.15	

L.S.D. (0.05) for : Stages = 0.01 %
Sn x St = 0.03 %

3.3.2. Qualitative analysis of the volatile oil : The volatile oil at budding stage is bright yellow in colour. At the following stages, however, it becomes yellowish cream in colour. It is mobile liquid with pleasant aromatic smell. The constituents of the volatile oil had been previously identified by Megahed (1985) . Components stated to be produced by this plant were spotted on the chromatograms according to their retention time to find out any quantitative changes that might take place at different developmental stages. Using GLC technique, it was detected that linalol was mostly found as the major constituent. Apart from the variation in proportional amounts of different constituents of the volatile oil, the chemical composition at five studied stages was identical to Fig. 18 .Linalol was the major constituent at horizontal ray flowers stage (34.36%) in the injected sample according to the surface area of its peak on the chromatogram. Hence, the retention times of other constituents were related to it. Under conditions of this investigation, linalol had a retention time of 11.3 minutes. The volatile oil is a complex mixture containig 26 components. The retention times, the relative retention times to linalol as well as the precentages of these components were recorded (Table 4) . Components which had been identified were as follows : Number 1 : α - pinene, 2 : β pinene, 9 : geraniol , 10 : nerol, 13 : linalol and 22 : Linalyl acetate.

Constituents of volatile oils obtained from inflorescences at different developmental stages differed in the ratio of their respective concentrations. Components 13 and 26 comprised the major compounds throughout the four stages of flowering. Components 13 comprised 20.43, 22.85, 19.00 and 34.36% of the volatile oil at the first four stages. The corresponding values for component 26 were 37.00, 26.44, 43.06 and 27.14%, respectively. Whilst the volatile oil obtained from mature fruit stage contained mainly component 3 (44.32%). The GLC behaviour of this component suggested that it was a monoterpene hydrocarbon. In the mean time, components 11, 12, 21 and 24 were relatively found to a considerable extent.

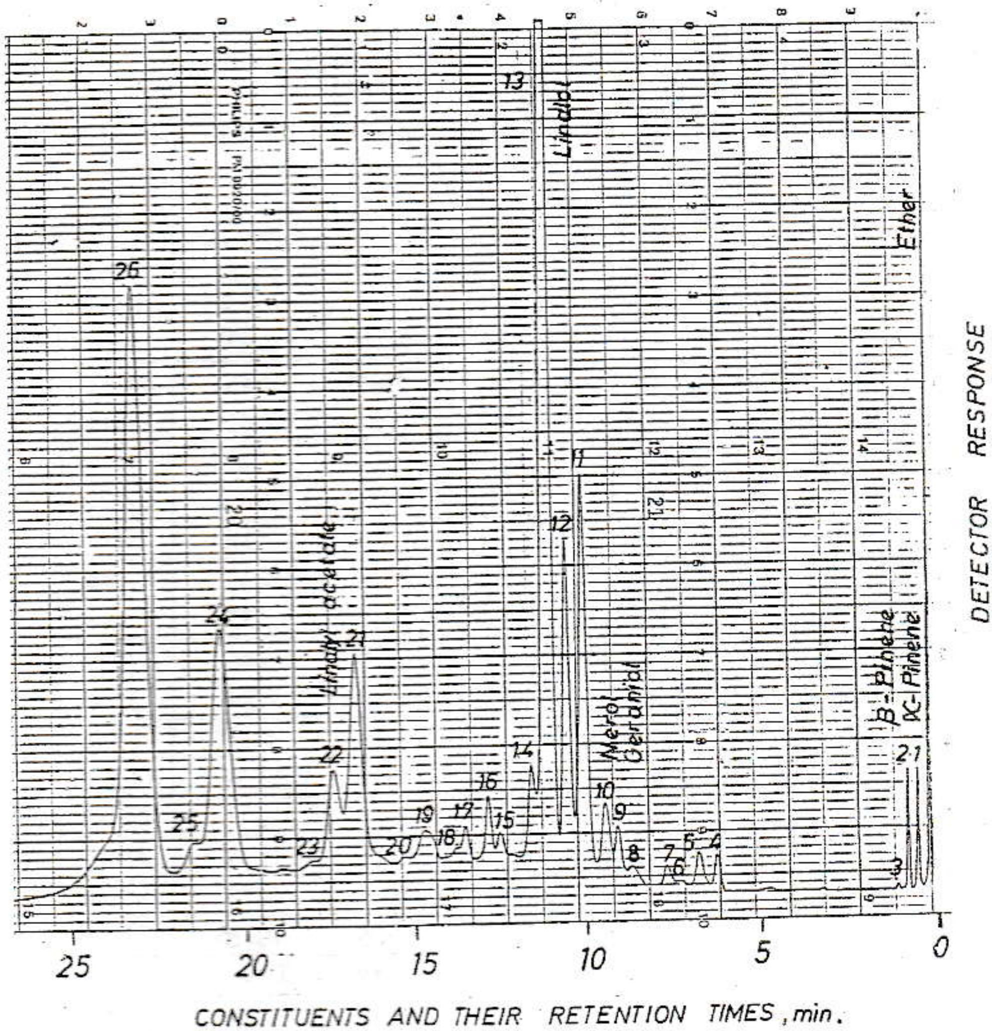

Fig. 18 : Gas chromatogram of volatile oil of *Calendula officinalis* L. at budding stage . (Instrument : Pye Unicam. Pye series 104 chromatograph) .

Table (4) : Retention times , relative retention times to linalol and percentages of volatile oil components in *Calendula officinalis* L. inflorescences at consecutive developmental stages

Comp-onent	r.t. min.	r.r.t. to Linalol	%				
			Budding	Flowering onest	Uprigh t ray fl.	Horizontal ray fl.	Mature fruit
1	0.8	0.07	1.21	1.32	1.31	1.23	0.83
2	1.0	0.10	0.95	2.77	0.10	2.63	4.40
3	1.3	0.12	0.03	7.61	Traces	0.16	44.32
4	6.7	0.59	0.52	0.44	0.44	0.41	Traces
5	7.2	0.64	0.52	0.31	0.42	0.49	Traces
6	7.7	0.68	0.17	Traces	Traces	Traces	Traces
7	8.1	0.72	0.23	0.12	0.23	0.16	Traces
8	9.0	0.80	0.26	0.09	0.08	0.33	Traces
9	9.4	0.83	0.61	0.47	0.47	0.33	Traces
10	9.7	0.86	1.01	0.88	1.30	0.25	Traces
11	10.3	0.91	9.28	9.22	6.58	7.24	2.14
12	10.8	0.96	4.80	3.78	4.18	4.11	2.08
13	11.3	1.00	20.43	22.85	19.00	34.36	11.53
14	11.8	1.04	0.92	0.66	0.94	0.90	0.12
15	12.7	1.12	0.29	0.35	0.37	0.25	Traces
16	13.1	1.16	0.86	2.30	1.51	0.74	Traces
17	13.8	1.22	0.43	0.35	0.26	0.15	Traces
18	14.0	1.24	0.06	0.03	0.08	Traces	Traces
19	14.9	1.32	0.92	0.50	0.94	1.73	Traces
20	15.9	1.41	0.09	Traces	0.11	0.16	Traces
21	16.7	1.48	6.17	7.93	5.56	4.90	3.75
22	17.4	1.54	1.82	1.75	1.88	2.30	2.26
23	18.1	1.60	0.09	0.09	0.18	0.25	0.30
24	20.7	1.83	11.09	9.06	11.74	9.13	3.92
25	21.5	1.90	0.24	0.28	0.26	0.74	Traces
26	23.1	2.04	37.00	26.94	43.06	27.14	24.36

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دراسات مورفولوجية وتشريحية وكيميائية على الأقحوان
Calendula officinalis **L.**

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ملخص

إن المعلومات النباتية عن الأنواع التابعة للفصيلة المركبة قليلة ، لذلك فقد تم إجراء هذا البحث لتقديم دراسة دقيقة عن التركيب الخارجي، و الداخلي ، و كذلك التحليل الكيميائي لنبات الأقحوان خلال الأعمار المتتالية في موسمين زراعيين . واشتملت الدراسة بيانات عن إنبات البذرة ، ونمو البادرة، وطول الساق الرئيسية ، وارتفاع النبات ، وعدد السلاميات بالساق الرئيسية ، وقطر السلامية القاعدية بالساق الرئيسية، وطول السبعة أفرع الجانبية ، والوزن الجاف للساق ، والأفرع ، والوزن الجاف للأوراق ، ووصف النورة ، والثمرة ، والتركيب التشريحي للجذر الرئيسي، والساق الرئيسية ، والزوائد ، والورقة ، والزهرة الشعاعية ، والزهرة القرصية ، والثمرة ، والنسبة المئوية والتحليل النوعي للزيت الطيار. وفى حدود معلومات الباحثين ، لم يسبق وجود مثل هذه المعلومات التفصيلية عن الأقحوان ، ولا شك أن مثل هذه المعارف ذات فائدة لكل باحث يهتم بالخصائص المختلفة لهذا النبات .

المجلة العلمية لكلية الزراعة – جامعة القاهرة – المجلد (52) العدد الأول (يناير 2001) : 85-114.