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MORPHOLOGICAL, HISTOLOGICAL AND CHEMICAL INVESTIGATIONS OF *Tagetes patula L.*

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ABSTRACT

Available botanical information on species of Asteraceae is little . Hence, this study aimed to present detailed information about the morphology ,histology and composition of the volatile oil of *Tagetes patula* L. throughout consecutive stages of plant growth in two years. Data obtained could be summarized as follows :

Seeds of French marigold are disperesed in form of achene fruits, oblong 9 mm long and 1 mm width having a persistent calyx . Germination is epigeal with percentage of 36.75% .The seedling stage came to an end after 3-4 wks of sowing .

The apex of the main stem differentiated into an inflorescence. Lateral branches (4-5) were responsible of the majority of shoot growth. Stem and lateral branches were erect, glabrous, more or less hairy, solid and greenish purple in colour . The main stem length reached 15 cm. Lateral branches are responsible of the actual height of plant, 45 cm.The average number of internodes of the main stem was 7 and the average diameter of basal internode was 12 mm. The dry weight of leafless shoot gained no considerable increments upto 14 wks old . At the following ages significant increments in the dry weight were achieved till the end of the growing season .

The first foliage leaf develops 3 wks after sowing. Leaves are compound, opposite, decussate and imparipinnate . The active period in growth as indicated by dry weight of leaves occurred from 16 to 22 wks old.

Flowering starts when plants aged 10 weeks. Both reproductive and vegetative growth continued parallel to each other till the end of the growing season. Lateral branches played a main role in flowering. The inflorescence is a capitulum developing at terminals of main stem and lateral branches and consists of ray and disc flowers arranged on a receptacle which is surrounded by an involucre arranged in one series .As to colour , ray flowers are golden yellow with red marking . Disc flowers are yellow with red colour at terminal of its internal surface. Seeds are produced in both ray and disc flowers.Number of consecutive pickings of inflorescences carried out fortnightly was 8-9 .The highest yield of inflorescences was obtained at the seventh picking .Fruit is ebony in colour with creamy persistent calyx atop the fruit , 1-5 hairs . Its base is also creamy in colour .

From histological point of view, the main root is comprised of an epidermal layer followed by a cortex of 4-10 layers of cells ending with the endodermis . The pericycle surrounds a diarch vascular cylinder. The xylem
occupies the centre and the phloem parenchyma cells have relatively thick walls. At the following ages these cells serve as supporting fibres .The secondary growth starts after 4 wks, and continued in the common manner.

The shoot apex has 2- layered tunica surrounding the corpus. The xylem is the first vascular tissue to differentiate followed by the phloem, The stem directly below the apex is more or less pentagonal.The median internode of the main stem at flowering onset consists of an epidermis and cortex of 6-9 layers of cells. The first 2-3 layers of it are collenchymatous . The fascicular cambium forms more secondary xylem and less secondary phloem . The interfascicular cambium forms lignified parenchyma to the inside and thin walled parenchyma to the outside.

The leaflet has 2 epidermal surfaces . The upper epidermis is concave in shape, while the lower one is convex. Stomata are of anomocytic type.The palisade layer is formed of 1 row of slender loosely arranged cells .The spongy tissue is consisted of 4-5 layers of chlorenchymatous cells , occupying 1/2 the whole thickness of the mesophyll. The midrib bundle has relatively narrow phloem, and a wider xylem area. The number of xylem rows in the midrib is 5-7 each of 3-5 vessels.

The histology of ray and disc flowers was investigated. Also the fruit, the exocarp is consisted of epidermis and subepidermis . Some of the epidermal cells differentiate into a trichome of twinned hairs in addition to other glandular trichomes . The mesocarp is consisted of 2 layers which are palisade- like cells. The upper layer has cells which are more lignified than the lower one. The endocarp is consisted of 1-2 layers of thin- walled cells and prominent intercellular spaces. The seed coat is consisted of more or less spongy parenchymatous tissue .

The inflorescences and the shoot produced volatile oil reaching 0.5%. The volatile oil of the inflorescences comprised of 31 components , tagetone being the main component . The volatile oil of the shoot comprised of 33 components with γ - terpinene as the main component .

Key words*:* *Tagetes patula* L., morphology, histology, volatile oil.

INTRODUCTION

The family Asteraceae (Compositae) is one of the largest families , if not the largest family of flowering plants , consisting of more than 1100 genera and about 20000 species (Cronquist , 1981). Many of Asteraceae species are ill-defined. Thus , any new information about Asteraceae plants are urgently to be welcomed. It is aimed in this work to carry out a phytography study of *Tagetes patula* L. (French marigold) a valuable ornamental plant (*Tagetes* has 50 species) . Such knowledge may fulfill information acquisition in this concern and would be useful to specialists in various aspects of biology of this plant .

According to Christopher (1958), Bailey (1969), Nikiforova (1969), Anon . (1982) and El-Leithy (1987) there are two types of *Tagetes* , dwarf being 12 to 15 cm . and tall which is 60 to 90 cm . Plants are a widely used annual for edging (dwarf) and general bed planting . They make good cut flowers and bloom continuously over a long period . Its leaves are alternate or opposite and imparipinnate with sessile, lanceolate , and dentate leaflets. The flowers are borne in capitula and may be single or double, according to the variety, and carried on a long peduncle . The colour ranges from golden - yellow with striations to a velvety reddish - brown . Size of capitula is 3.5-5 cm . Place of origin is Mexico . Flowering time is summer to autumn. Plants need for cultivation a sunny position and temperate climate . Grows in any type of well-worked garden soil that has been enriched with plenty of organic material. Requires generous watering interspersed with a few applications of liquid fertilizer after planting out .

Structure of *T.erecta* and *T.patula* differed . The anatomical studies in the very early (2 - to 5- day - old) and later stages of seedling development showed that the rate of tissue differentiation was higher initially in the early flowering *T.patula* and subsequently in the later flowering *T.erecta* . The stem structure in the reproductive stage of growth was similar in the two species .

The xylem tissues were well developed , particularly in *T.erecta*, and the roots in both were diarchic .

Tagetes has an apparently well justified reputation for killing nematodes in the surrounding soil (Cronquist , 1981) . Little information was found on the chemistry of *T.patula* (Kaloshina and Mazulin , 1983 and Sutfeld *et al* ., 1985) . Some investigations , however, were carried out on other species of the genus such as *T.minuta* (Handa *et al* ., 1963) *T. signata, T.tenuifolia* and *T. minuta* (Kapelev , 1971) and *T.erecta* (Baslas and Singh , 1980).

Hethelyi *et al* . (1987) mentioned that the characteristic components of essential oils from flowering *T.minuta* and *T.tenuifolia* plants were β - ocimene. dihydrotagetone , tagetone and Z. and E. ocimenones . *T.patula* oil contained Z- and E- ocimenones in equal proportion . *T.patula* oil differed markedly from the other two species ; in addition to myrcene, limonene and β- caryophyllene it contained 15% piperitone and 15% of an unidentified component .

MATERIALS AND METHODS

Seeds of French marigold were obtained from Kato Aromatic Co., Al-Harraneia, Giza. Test of germination was conducted in the Seed Testing Department , A.R.C., Giza, according to the International Rules for Seed Testing (Anon., 1985).The field work was carried out in the Experimental Station of the Faculty of Pharmacy, Cairo University , Giza. Seeds were sown on March 10 $^{th.}$. during the two consecutive years 1986 and 1987, in warm seed beds, transplanted to the field 30- 45 days later .

Cultivation was achieved according to a randomized complete block design at the rate of 4 replicates . Plot dimensions were 2.5 x6m, with 10 ridges ,50 cm apart . Plants were disseminated on the northern side of the ridge at the rate of one plant per hill.

Cultural practices were carried out as recommended .Vegetative characters were followed up fortnightly using 20 randomized plants, 5 plants per plot . Dry weight was performed using 20 - plant - samples, dried in an oven at $70C^0$ till a constant weight was reached , almost 48 hrs. Various characters of flower, 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 3-4 hrs (Geunther, 1952). GLC technique was used to separate and detect the volatile oil constituents. GLC conditions were as follows : Detector : FID ; PEG stainless steel column 10feet x $^1/8$ inch; Temperature : injector $250C^0$, oven $70\text{-}210C^0$ increased by $4C^0$ /min, detector 300 C^0; Flow rate ml/min: N_2 30, H_2 30, air 60; Chart speed: 5 mm/min; Sample volume 0.2 µ l.

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

RESULTS AND DISCUSSION

1. External morphology :

1.1. Germination of seeds and seedling growth :

Seeds of French marigold are disseminated in form of achene fruits so that the fruit and seed are handled together as the seed since the fruit and seed coverings are contiguous . The one to five sepals of the calyx remain attached atop the fruit ; i.e., calyx is persistent . Fruit is oblong averaged 9 mm in length and 1 mm in width . It tapers towards the base at its attachment point to the receptacle . The fruit is ebony in colour , while both the persistent calyx and fruit base are creamy in colour . During germination the seed swells , fruit coats become more darker in colour and rupturs at side of its basal end . This takes 48 hrs from sowing . This is followed by the emergence of the radicle through the fruit covering , which denotes the onset of germination and occurs 72 hrs after sowing . The hypocotyl develops 12 hrs later , elongates curved inside the soil then straightens and after 96 hrs from sowing raises the cotyledons above the ground ; i.e., germination is epigeal . The hypocotyl is purple in colour .

As germination proceeds , the fruit coats shed , the two cotyledons separate from each other 118 hrs from sowing , expand 24 hrs later and structure of the seedling soon becomes evident . The embryo consists of a hypocotyl - root axis bearing two cotyledons with oil glands on their lower surface . The completely developed cotyledon averages 1.6 cm in length and 0.3 cm in width . The growing point of the root , the radicle , emerges from the lower end of the hypocotyl - root axis . The growing point of the shoot , the plumule , is at the other end of the hypocotyl - root axis above the cotyledons . The secondary roots develop 96 hrs after sowing . The first differentiated true leaves are two foliage ones developing 24 days after sowing . The germination speed (after 5 days) was 26% and the germination percentage (after 14 days) was 36.75% (Anon., 1985) . These values indicate that French marigold seeds are naturally of low viability .

1.2. The stem :

As the seedling stage came to an end , the apical meristem was seen protected by the developing young leaves which extended up and around it from below . A pair of foliage leaves expanded at the shoot tip and spreaded away from the stem with increasing maturity . New young leaves were progressively formed by the apical meristem . Nodes , with a pair of leaves attached to each , and internodes were designated .

At the age of 4 wks ., plants already developed the hypocotyl and the first basal internode of the main stem forming the majority of stem . Other internodes were still progressively formed and were too small to be distinguished easily . Length of stem averaged 1 cm .

Two weeks later , basal internodes of the stem were more distinguishable and elongated . They were purple in colour , more or less angular .

When plants aged 8 wks, the internodes elongated progressively . Axillary buds began to burst . Lateral branches followed in their growth the same pattern of behaviour shown by the main stem .

Stem (Fig. 1) is herbaceous bearing 4 to 5 branches developed at the basel nodes. Stem is erect , glabrous , solid , angular and tapering toward the tip . Stem is usually purple in colour with some spreaded green areas . Leaves are arranged on stem in opposite decussate pattern .

It is important to note that lateral branches are responsible of the actual height of plant, since growth of the main stem is determined by an inflorescence produced at its apex. Moreover, the lateral branches play a vital role in production of inflorescence yield since each branch in turn is terminated by an inflorescence.

Fig. 1: A photograph of mature *Tagetes patula* L. plant, 22 wks old , showing its habit of growth .

1.2.1. Length of the main stem :

The quadratic equations and lines of length of the main stem and periods are exhibited in Fig.2 .Results revealed that growth in length was slow till the age of 6 wks. Afterwards ,an enhancement in growth was achieved and a significant increase in length of the main stem was recorded when plants were 8 wks old.

A further increment was attained by 10- wk-old plants where average length of the main stem was 13.6 cm in the first season and 13.7 cm in the second one . No substantial increase was added to length of the main stem thereafter due to development of its apical meristem into an inflorescence .

It was difficult to distinguish precisely between the main stem and lateral branches after plant age of 16 weeks.

These findings are in harmony with those given by Christopher (1958) who stated that French marigold is dwarf being 12 to 15 cm in length having a low-spreading habit of growth .

Fig.2: Graph of regression of length of the main stem on plant age in *Tagetes patula* L. in the first (left) and the second (right) season.

Fig.3: Graph of regression of number of internodes of the main stem on plant age in *Tagetes patula* L . in the first (left) and the second (right) season.

1.2.2. Number of internodes of the main stem :

The quadratic equations and lines of periods as well as number of internodes of the main stem during the two years are illustrated in Fig. 3 . An actual increase in number was achieved when plants aged 6 to 8 wks . Main stem of 8-week-old plants had an average number of internodes of 6.8 in the first year and 6.7 in the second year . It is worthy to note that the average number of internodes of the main stem was almost 7, of which 6 were developed by plant during the period of 6-8 wks old .

1.2.3. Plant height :

The significant effect of periods on plant height was in the first year quadratic and in the second year linear . The equations , lines of periods and plant height in both seasons are exhibited in Fig.4. Values revealed that plant height increased significantly throughout consecutive sampling dates which started at 4 wks old upto the age of 24 wks, apart from the period of 10-12 wks old where no substantial increase was recorded . This period was associated with onset of flowering which might justify such behaviour . Maximum plant height reached at 24 wks old , being 44.8 cm in the first year and 45.4 cm in the second one . No further increase was recorded . Bailey (1969) stated that the height of French marigold plants averaged 30-45 cm .

Noteworthy that vegetative growth continued throughout the whole growing season indicating that French marigold plants developed vegetative and reproductive organs parallel to each other . The active vegetative growth continued to almost the end of the growing season and the reproductive growth started at 10 wks old and continued to the end of the growing season as well .

1.2.4. Diameter of the basal internode of the main stem :

The effect of periods on this character was cubic in the first year and linear in the second one . The equations and lines are illustrated in Fig.5. Average diameter of the basal internode of the main stem was 2.1 mm when plants were 4 wks old in both seasons . No substantial increments were recorded till plants were 10 wks old in the first year and 12 wks old in the second one being 3.3 mm . Afterwards , the average diameter increased steadily reaching a maximum of 12.2 mm in the first year and 9.7 mm in the second one when plants aged 18 wks . Thereafter, the average diameter decreased slightly due to senescence and shrinkage of tissues . As a result of branching of plants at the base of main stem , it was difficult to determine the diameters of the basal internode at the last two sampling dates .

1.2.5. Length of lateral branches :

The main stem developed five distinct secondary branches . At the age of 8 wks , three branches were developed . The other two , however , arised when plants were 14 wks old (Table 1).

1.2.5.1 The first secondary branch :

Significant increment in average length of this branch were achieved at successive ages . The maximum length was attained at the age of 22 wks, being 23.8 cm in the first year and 28.5 cm in the second one . No substantial changes in average length of this branch was observed after that age .

Fig.4: Graph of regression of plant height on plant age in *Tagetes patula* L . in the first (left) and the second (right) season

Fig.5: Graph of regression of diameter of basal internode of the main stem on plant age in *Tagetes patula* L . in the first (left) and the second (right) season .

1.2.5.2. The second secondary branch :

This branch increased steadily in length as plants advanced in age . The maximum length was recorded by plants 24 wks old , being 24.3 and 24.8 cm in the first and the second year , respectively .

1.2.5.3. The third secondary branch :

A significant increase in average length of this branch was achieved in most of sampling dates till plants were 22 wks old, when they reached their maximum length of 22.6 cm in both seasons .

1.2.5.4. The fourth secondary branch :

The first significant increase in average length of this branch took place at 18 wks old. The maximum length of this branch averaged 19.2 cm at 22 wks old in the first year and16.3 cm at 24 wks old in the second year .

1.2.5.5. The fifth secondary branch :

Average length of this branch increased steadily till plants were 22 wks old to record a maximum length of 16.1 in the first year and 11.4 in the second one .

In summary , French marigold developed at 8 wks old 3 secondary branches . When plants aged 14 wks another 2 branches arised . The total was 5 secondary branches . All branches increased steadily in their length till they recorded their maximum length at 22 wks old . Lateral banches played an important role in yield since each of them terminated into an inflorescence.

Similarly, Bailey (1969) described French marigold as a bushy annual, branched from near base .

Table (1): The periodic growth in length, in cm , of secondary branches of *Tagetes patula* L. at successive ages in two seasons .

Plant age In weeks	First branch		Second branch		Third branch		Fourth branch		Fifth Branch	
	First season	Second season	First season	Second season	First season	Second season	First season	Second season	First season	Second season
8	3.2	4.0	1.0	1.8	0.4	1.6	--	--	--	--
10	4.9	4.2	2.3	2.2	3.0	2.5	--	--	--	--
12	7.9	7.3	4.9	4.8	3.7	2.3	--	--	--	--
14	8.9	10.0	7.3	7.3	5.8	6.6	3.3	5.4	2.3	1.7
16	11.5	12.9	7.9	8.3	6.4	7.5	5.1	5.6	4.2	4.6
18	15.6	14.5	19.6	14.8	14.4	9.7	8.7	7.4	6.7	6.5
20	21.3	22.5	20.4	17.6	18.4	14.9	13.6	7.5	9.0	7.5
22	23.8	28.5	23.4	23.7	22.6	22.6	19.2	14.0	16.1	11.4
24	23.5	28.8	24.3	24.8	22.1	22.4	17.5	16.3	15.4	10.9
26	22.7	27.8	23.7	24.8	21.9	21.5	17.4	15.7	14.9	10.3
New L.S.D(0.05)	1.5	1.8	2.0	1.8	1.8	2.0	2.3	1.9	1.4	1.9

1.2.6. Dry weight of leafless shoot :

A significant cubic effect of periods on dry weight of leafless shoot was recorded in both years . The cubic equations and lines of this character and periods are illustrated in Fig . 6 . No appreciable increments were gained in dry weight of leafless shoot in both seasons up to 14 wks old . However , at sampling date of 16 wks old onwards , significant increments were recorded reaching to a maximum at the age of 24 wks being 52.815 and 53.128 g in the first and the second year ; respectively .

Fig. 6 . Graph of regression of dry weight of leafless shoot on plant age in *Tagetes patula* L. in the first (left) and the second (right) season .

1.3. The Leaf :

The main axis of seedling bears the embryonic leaves ; i.e. , the two cotyledons which are situated on the hypocotyl . Oil glands develop on the abaxial surface of the cotyledons . They are dark green with areas of pale purple in colour , petiolate , simple , oblong in shape , having entire margin , with emarginate apex and rounded at their base . Cotyledons last for about 4-5 wks after sowing .

The first foliage leaf develops 3 wks after sowing at first . It looks like a simple leaf. But, soon segmentation becomes recognizable , denoting the true compound leaves of the plant . Leaves are exstipulate , opposite decussate and imparipinnate . Leaflets may also be segmented during their development as to resemble the main leaf . Leaflets are borne in opposite pairs along the leaf axis, or rachis , and there is a terminal solitary leaflet, which is bigger in its size than other leaflets . The leaf is comprised of about 9-13 sessile, lanceolate to oblong and serrate leaflets , each of 1.5 - 2.5 cm long and 0.4 - 0.5 cm width . Leaflet teeth are tipped with a long weak awn . Each tooth usually has a large gland at its base gives off a rather pleasant penetrating smell . Leaflets are velvety at the adaxial surface and glabrous at

the abaxial one . Venation is reticulate pinnate . The rachis of the leaf and midvein of leaflets are channelled ; i.e., with a longitudinal groove .

The aforementioned leaf characters conformed to those mentioned by Bialey (1969) and Anon . (1982) .

1.3.1. Dry weight of leaves :

The cubic equations and lines illustrating the effect of periods on this trait are shown in Fig.7. No substantial increments were recorded in dry weight of leaves upto plant age of 14 wks . However , the first noticeable increase in leaves dry weight was achieved by 16 - wk - old plants,being 8.509 and 9.127 g in the first and the second season , respectively . Afterwards , the dry weight of leaves increased steadily to reach a maximum of 51.668 g in the first year and 44.545 g in the second one when plants aged 22 wks . On the other hand , sampling dates of 24 and 26 wks were associated with a decrease in leaves dry weight . Such decrease would be expected due to withering and defoliation associated with aging .

Fig. 7: Graph of regression of dry weight of leaves on plant age in *Tagetes patula* L .in the first (left) and the second (right) season .

1.4. The inflorescence :

The terminal bud of main stem developed into an inflorescence toward the end of May . Plants were 9-10 wks old . They kept blooming continuously over a long period which lasted till plants were 23 wks old . Lateral branches played the main role in flowering . The development of both vegetative and reproductive organs continued parallel to each other until the end of the growing season . The flowers are borne in a single capitula about 3.5 cm across , carried on long slender peduncles which are hollow . Peduncle

averages 4.5 - 8.5 cm long . The inflorescence is simple and terminal . It develops many disc flowers in the centre surrounded by only one row of ray flowers . The average number of ray flowers is about 5 to 6 . The capitulum is racemose . Flowers open in acropetal succession , the biggest being around the margin of the capitulum . The two kinds of flowers are arranged on a convex receptacle which is surrounded by an involucre in one series having thick oblong phyllaries with acute teeth and glandular - dotted on the surface . The phyllaries are covered with trichomes similar to those developed on the shoot . The involucre is united nearly throughout a long tube or cup .

The consecutive stages starting from differentiation of flower buds till mature fruits were developed (Fig . 8) took about 17 days .

Fig .8: A photograph of *Tagetes patula* L , showing to the right a portion of flowering branch and to the left successive developmental stages of inflorescence , starting at top right with two stages of flower bud , onset of blooming then full blooming . At lower right mature fruit , disc flower then ray flower .

1.4.1. The ray flower :

One series of 5 to 6 flowers are arranged around the margin of capitulum receptacle . They are ligulate in shape ebracteate , sessile , zygomorphic and pistillate . The calyx is pappus of 1-2 long awned scales and 2-3 shorter blunt ones . It is transparent white in colour . The corolla is ligulate , 2.3 cm in length and 1.3 cm in width . It has golden yellow colour with red marking of striated pattern . Corolla is obvate in shape and terminated by three lobes . The median lobe is emarginate at its apex .

Corolla is velvety with papillae spreaded on its upper surface . The limb is about 1.0 to 1.5 cm long and 0.3 cm across . Androecium absent . Gynoecium is bicarpellary , syncarpous and uniloculate . One ovule developed of basal placentation . The ovary is inferior with long style terminated by stigma which is bifid and yellow in colour .

Floral formula of the ray flowers is as follows :

$$\%, O , CA^{(1-5)} , CO^{(3)} , A^{x} , G^{(2)}$$

1.4.2. The disc flower :

The disc flowers are numerous developed in centre of convex receptacle . It is tubular in shape , ebracteate , sessile , actinomorphic , hermaphrodite and epigynous . The disc flower is about 2.0-2.5 cm long and 2 mm in diameter . Calyx is similar to that mentioned earlier for the ray flower . Corolla is tubular in shape , yellow with red colour at terminal of its internal surface . When tubular flowers are well developed , the corolla consists of 5 united petals terminated with 5 lobes at its apex . It bears trichomes and papillae on its internal surface . Androecium consists of 5 epipetalous stamens , alternating with petals . Anthers are long , syngenesious and filaments free . Gynoecium is similar to that mentioned earlier for the ray flower , sometimes with trifid stigma .

Floral formula of the disc flowers is as follows :

$$O , O , CA^{(1-5)} , CO^{(5)} , A^{5} , G^{(2)}$$

It is important to note that the ovary of both ray and disc flowers is fertile and develops into a fruit . This implies that seeds are produced by both ray and disc flowers .

Description of French marigold inflorescence locally cited is in accordance with that given by Christopher (1958) , Bailey (1969) and Anon . (1982) .

1.4.3. Cumulative number of inflorescences :

Flowering started on 29 May in the first year and 19 May in the second one . To gain information about flowering behaviour, the cumulative number of inflorescences per plant developed at 5 - day intervals were recorded (Fig.9) .

Number of inflorescences per plant increased consistently through September 16 th reaching a maximum of 72.7 in the first year and 48.9 in the second one . Afterwards, fruit development increased continuously and as a result number of inflorescences decreased till the end of flowering(October) .

Fruit formation started on June 18 th in the first year and June 8 th in the second one and continued parallel to formation of inflorescences. However, fruit development was completed at mid November .

Fig .9: Cumulative number of *Tagetes patula* L . inflorescences per plant at 5 - day intervals in two years .

1.4.4. Yield of inflorescences :

Consecutive pickings of inflorescences were carried out fortnightly to record their average number as an indication to inflorescence yield (Table 2). Eight pickings of inflorescences were obtained in the first year, while pickings in the second year were nine. The maximum yield of inflorescences (93 .1) was taken from the seventh picking in both seasons .It is important to note that the substantial increments in average number of inflorescences were restricted to picking 5 to 7.

1.4.5. Fresh weight of inflorescences :

Similar to the trend of results shown by the average number of inflorerscences , average fresh weights recorded for pickings 5 to 7 differed significantly. The seventh picking recorded the highest fresh weight of inflorescences being 34.474g in the first year and 35.987g in the second one.(Table 2)

1.4.6. Air-dry weight of inflorescences :

Inflorescences of successive pickings previously counted and weighed fresh were left for not less than a week under room conditions before being re-weighed to determine their air-dry weight (Table 2) .As would be expected air-dry weight of inflorescences at successive pickings followed the same pattern of behaviour recorded by both average number and fresh weight of pickings. The maximum air-dry weight of inflorescences produced by the seventh picking was 7.241 and 7.647g in the first year and in the second one , respectively .

Table (2): Average number and fresh and air-dry weights, g, of inflorescences of *Tagetes patula* L. throughout successive pickings in two seasons

Date	First season			Second season		
	Av. no.	Fresh wt.,g	Air-dry wt.,g	Av. no.	Fresh wt.,g	Air-dry wt.,g
May 30	3.8	2.236	0.316	---	---	---
June 15	6.7	4.535	0.603	4.8	2.477	0.551
30	13.5	10.773	0.983	5.8	4.461	0.768
July 15	25.5	13.888	2.145	10.8	8.278	1.632
30	35.8	15.968	2.313	13.5	8.509	1.676
Aug. 14	51.1	23.035	4.909	26.0	14.743	3.125
29	93.1	34.474	7.241	49.4	24.417	4.889
Sept. 13	57.8	23.814	4.093	93.1	35.987	7.647
28	---	---	---	58.0	24.586	4.565
Oct. 13	---	---	---	38.3	10.640	3.304
L.S.D.(0.05)	**13.4**	**6.555**	**1.668**	**9.4**	**4.804**	**1.004**

1.5. The fruit :

The fruit is one-seeded inferior achene. The fruit coat is free from that of the seed which is almost filling the fruit .It is simple, oblong in shape and 9 mm in length and 1 mm in width. Fruit is ebony in colour with creamy persistent calyx atop the fruit of 1 to 5 hairs . The fruit base where it attaches with the receptacle is also creamy in colour . The embryo is white in colour, oblong in shape, straight, with short inferior radicle and two expanded cotyledons . Endosperm is absent. The specific weight of 100 fruits averaged 0.197g and their size averaged 0.7 cm^3.

2. Histological studies :

2.1. Structure of the main root :

Transverse section of 2-wk-old root (Fig.10) showed that the main root has an uniseriate epidermis , followed by a cortex of 9-10 layers of parenchyma cells . Triangular intercellular spaces are seen . There is a well defined layer of endodermis , followed by a one - layered pericycle . The root has a diarch radial protostele in which the two primary phloem groups lie on the flanks of the primary xylem strand . Each xylem ridge comprised of about 7 to 8 vessels . The phloem is characterized by its large amount of parenchyma cells in proportion to the sieve tubes with their companion cells. The metaxylem vessels occupy the root center and the pith disappears .

At the age of 4 wks, primary structure is completed .The total number of xylem vessels ranges from 20 to 25 . Some of the parenchyma cells of the protophloem begin at this age to develop thick walls. At older ages, these cells will serve as supporting fibrous strand lining the pericycle . Secondary growth takes place in the common manner .

When plants are 6 wks old , the cortex is ruptured at many regions (Fig. 11) and the endodermis is still intact and keeping pace with the increase in root girth through anticline divisions of its cells . At this age, the phloem forms more fibrous strands , being irregularly distributed among the rest of the primary phloem parenchyma cells which are still keeping pace with the increase in root diameter. The secondary xylem is far prevailing the secondary phloem in amount and the ray cells extending in the phloem show marked dilation. The secondary xylem has an increased amount of vessels present either solitary or frequently in radial clusters of 2 to 5 vessels each ; oblique or tangential clusters may be occasionally seen . It appears also that the ground tissue has a higher amount of parenchyma than fibers in this stage of growth .

At the following ages , the structure of the root is similar to that of the previous age with respect to secondary structure except that the component cells are relatively larger in number due to its larger diameter .

The previous description agrees with that given by Nikiforova (1969)

Fig. 10: Transverse section of the main root of *Tagetes patula* L., 2 wks old , showing the primary structure of the main root . Details :co,cortex;en, endodermis; ep, epidermis; pe, pericycle; Px, protoxylem.(x100)

Fig. 11: Transverse section of the main root of *Tagetes patula* L ., 6 wks old , showing its secondary structure. Details : en, endodermis; fb, fibres;sph, secondary phloem; sx, secondary xylem. (x125).

2.2. Structure of the main stem :

2.2.1. The shoot apex :

The dome-shaped apical meristem consists of two-layered tunica overlying the corpus (Fig.12) . The average height of the shoot apex above the first discernible leaf primordium averages 187.5 µ . While the average of its diameter is 209.8 µ . The average number of cells across this particular region is 19-21 . The distance below the tip where the procambium first differentiates as dissected ring in transection averages 382 µ . The first vascular tissue to differentiate is the xylem at 687.5 µ below the tip of the shoot apex . This is followed by phloem at 818 µ below the tip . Vacuolation is observed at 670 µ below the promeristem .

2.2.2. The median internode of the main stem :

The middle part of the stem loses its characteristic outline since ridges and furrows disappeare (Fig.13) . The epidermis is composed of a single layer of nearly square cells , covered with a thin cuticle . The cortex consists of 5 to 6 layers of cells . The outer 1 to 2 layers are collenchymatous underlying the epidermis . The starch sheath is hardly recognized . The fascicular cambium gives rise to relatively more secondary xylem and relatively less secondary phloem. The interfascicular cambium forms lignified parenchyma to the inside and thin walled parenchyma to the outside .

Fig. 12: Longitudinal median section of the shoot apex of *Tagetes patula* L . showing the two - layered tunica and corpus. Details: c, corpus; t, tunica. (x40)

Fig.13: Transverse section of median internode of the main stem of *Tagetes patula* L . at flowering onset. Details : pi, pith; px, protoxylem; sx, seconday xylem. (x 50)

The lignified parenchyma cells appear as a regular rows occupying the area between the xylem bundles . The fibrous groups become well defined against the secondary phloem, where it appears as dome shaped groups alternating with groups of parenchymatous cells.It is difficult to distinguish between the secondary phloem and the outer primary phloem. The pith appears intact,comprised of 5 to 20 cells in diameter of 865 µ .

The previous study is in harmony with that mentioned by Nikiforova (1969) on *Tagetes patula* L. and *T* . *erect.* L . and McConnell and Struchmeyer (1971) on *Tagetes erecta* L.

2.2.3. Trichomes and stomata :

The epidermis of stem forms trichomes of uniseriate type (Fig. 14 a). This trichome consists of a foot of 2 to 3 cells and a body of 7 to 9 cells in one row. The terminal cell is slightly curved . The papillae developing from single epidermal cell are also observed. They are formed in longitudinal rows (Fig. 14 b) .

The stomata (Fig. 14d and e) are either of anisocytic type each consists of 3 cells (may be unequal in size enclosing the guard cells), or of anomotetracytic type.

Fig 14: Type of trichomes and stomata being developed on stem, leaflet and fruit of *Tagetes patula* L. (a) uniseriate trichome (x200) , (b) papillae (x 400), (c)twinned hairs (x200), (d) anisocytic stoma (e)anomotetracytic stoma (x200) and (f) anomocytic stomata (x400). Details: b, body ;ep, epidermis ; f, foot; gc, guard cell.

2.3. Structure of the leaf :

Transverse section of leaflet given in Fig.15 shows that the two epidermal surfaces are covered with thin layer of cuticle . At the midrib region , the upper epidermis is concave in shape, while the lower one is convex . Stomata are more numerous on the lower epidermis than on the upper one and it is of anomocytic type ; i.e., without any subsidiary cells (Fig.14f) . The mesophyll, apart from the midrib region, consists of 5- 6 layers of cells. The palisade layer consists of 1row of slender cells, which are loosely arranged , hence, narrow intercellular spaces occurred among them. The spongy tissue consists of 4-5 layers of chlorenchymatous cells ,
occupying one - half of the whole thickness of the mesophyll . On the abaxial side at the midrib region , there are 2-3 layers of cells , the one layer abutting the lower epidermis is angular collenchyma . The midrib bundle has relatively narrow phloem on the abaxial side , and wider xylem on the adaxial one . The number of xylem rows in the midrib is about 5-7 each of 3-5 vessels .

2.4. Structure of the ray and the disc flowers :

2.4.1. The ray flower : The ray flowers are arranged on the margin of the receptacle . They are golden yellow in colour with red marking . They are ligulate in shape . The calyx (1- 5 sepals) is present . Each sepal consists of 2 epidermal layers of nearly square - shaped cells and 1-2 layers of rounded parenchymatous cells in between . Each sepal is supplied by a single bundle . The corolla consists of 2 epidermal layers and 7-9 layers of irregular arranged parenchymatous cells in between , There are 6 bundles extending through it . The two united carpels are supplied by 2 bundles running through a mesophyll of 10 - 12 layers of parechymatous cells .

2.4.2. The disc flower : The disc flowers are tubular in shape and yellow in colour with red colour on the internal surface of the corolla . The calyx consists of 1-5 sepals , each is bounded by an uniseriate epidermis of square - shaped cells . The mesophyll consists of 2-3 layers of rounded or angular cells . There is a single bundle running in the center of the mesophyll . The corolla tube consists of 2 epidermal layers of nearly square or barrel - shaped cells with 3-4 layers of parenchymatous cells in between . There are 5 bundles extending through the tubular portion of corolla . The androecium consists of 5 stamens having free filaments , each is more or less rounded in shape , bounded by an uniseriate layer of square or barrel - shaped cells . The ground tissue consists of 5-6 layers of parenchymatous cells . Each stamen is supplied by a single bundle . The gynoecium is composed of 2 united carpels . It is composed of defined epidermis of square cells, rather thick - walled . The ground tissue consists of 13-15 layers differ slightly in shape and size . There are 2 small bundles embedded in the tissue .

It is important to mention that papillae developing from single rounded epidermal cells are found scattered on the internal surface of the corolla of the ray and the disc flowers making it velvety. In addition to uniseriate trichome with a body of 2-3 cells in one row .

2.5. Structure of the fruit :

The fruit (Fig. 16) is oblong in shape . The fruit coat consists of 3 parts . The first is the exocarp comprised of 3 parts . The first is the exocarp comprised of epidermis and subepidermis. Cells are rounded in shape and covered with a thick layer of cuticle . Some of the epidermal cells differentiate into trichome of twinned hair (Fig. 14c). Small glandular hairs of a limited number of cells may also develop. The second is the mesocarp which is laying below the first one, consists of 2 layers of palisade-like cells.

Fig. (15): Transverse section of the leaflet of *Tagetes patula* L. through the midrib. Details : ep, epidermis; pal, palisade tissue; spo, spongy tissue; vb, vascular bundle (x50)

Fig. (16): Transverse section of fruit of *Tagetes patula* L. showing the structure of the fruit and the seed . Details: enc, endocarp; exc, exocarp; mec, mesocarp; sco, seed coat. (x100)

The upper layer of these cells is more lignified than the lower one . The third is the endocarp which is consisting of 1-2 layers of thin - walled cells and prominent intercellular spaces, except 3 - 4 cells across the sides of the fruit . The seed coat consists of more or less spongy parenchymatous tissue . Two traces of vascular bundles can be seen embedded in this tissue . The embryo is straight in shape comprised of 2 cotyledons.

3.The volatile oil :

3.1. Determination of the volatile oil content :

The volatile oil formed in shoot and inflorescences . Its concentration was determined in leafless shoot and leaves at 3 ages (Table 3) . While, in inflorescences , it was determined at 4 consecutive dates resembling different developmental stages (Table 4).

In both seasons, average concentrations of the volatile oil at 10 and 16 wks old in either of leafless shoot or leaves were indifferent. At the age of 22 wks, however, a significant increase in volatile oil concentration was achieved. In leafless shoot average concentration of the volatile oil in the first season increased from 0.10% at 10 wks old to 0.17% at the age of 22 wks . The corresponding values for leaves were higher , being 0.11 and 0.43%.

As to volatile oil concenttration in inflorescences, the highest concentration associated with budding stage (0.44%). Thereafter, average concentration of the volatile oil persistently decreased reaching a minimum value of 0.08% when fruits matured.

Table (3): Percentage of volatile oil in leafless shoot and leaves of *Tagetes patula* L. at consecutive ages in 2 seasons

Plant age, Wks	First season			Second season			X
	Leafless shoot	Leaves	X	Leafless shoot	Leaves	X	
10	0.10	0.11	0.10	0.11	0.13	0.12	0.11
16	0.13	0.10	0.12	0.13	0.10	0.12	0.12
22	0.17	0.43	0.30	0.19	0.50	0.34	0.32
X	0.13	0.12	0.17	0.14	0.24	0.19	

L.S.D. (0.50) for : S×O× A = 0.40%

Table (4): Percentage of volatile oil in *Tagetes patula* L. Inflorescences at different developmental stages in 2 seasons.

Stages	First season	Second season	X
Budding	0.42	0.45	0.44
Flowering	0.29	0.33	0.31
Full blooming	0.23	0.24	0.24
Mature fruit	0.07	0.09	0.08
X	0.25	0.27	

L.S.D. (0.05) for St= 0.05%

3.2. Qualitative analysis of the volatile oil:

The volatile oil of shoot is bright yellow in colour . But in case of inflorescences its colour is orange yellow. The volatile oil is mobile liquid having a pleasant aromatic smell .

Baslas and Singh (1980) found that volatile oil of *Tagetes erecta* L.contained α - pinene, β - pinene, β - phellandrene, δ - limonene, dipentene, 1,8 cineole, menthol, geraniol, linalol, tagetone, nonanal and linalyl acetate.

It was reported by Hethelyi *et al*. (1987) that volatile oil of French marigold contained Z - and E - ocimenones in equal proportion, myrcene, limonene, β- caryophyllene and piperitone .

Components stated to be produced by this plant and which were possibly secured as authentic samples were spotted on the chromatograms according to their retention time (Fig.17).

GLC analysis (Table 5) showed that the volatile oil of French marigold was a complex mixture containing about 33 or 31 components in case of shoot or inflorescences, respectively.

Table (5):Retention times , relative retention times to tagetone and percentages of volatile oil components in *Tagetes patula* L. shoot , 22 wks old , and inflorescences at flowering onset .

Shoot						Inflorescences			
Comp-onents	r.t., min	r.r.t./ tagetone		%		Comp-onents	r.t., min	r.r.t./ tagetone	%
		Leaves	Leafless shoot	Leaves	Leafless shoot				
1	2.15	8.08	-	0.327	Traces	1	2.78	0.16	0.122
2	2.76	0.11	0.11	0.252	0.231	2	4.26	0.24	0.266
3	4.24	0.16	0.16	0.827	0.453	3	5.06	0.28	0.452
4	5.06	0.20	0.19	0.504	0.651	4	5.91	0.33	3.440
5	5.87	0.26	0.22	10.341	5.845	5	6.67	0.37	13.434
6	6.64	0.23	0.25	8.204	10.334	6	7.13	0.40	5.352
7	7.09	0.28	0.27	3.163	2.166	7	8.03	0.45	15.077
8	7.99	0.31	0.30	38.539	20.387	8	12.64	0.71	0.428
9	11.27	0.44	0.42	0.446	0.042	9	13.52	0.76	1.832
10	11.70	0.45	0.44	0.257	0.517	10	14.15	0.79	3.956
11	12.55	0.49	0.47	1.085	0.731	11	15.05	0.84	0.158
12	13.46	0.52	0.50	2.678	6.013	12	15.89	0.89	0.315
13	14.10	0.55	0.53	3.501	5.881	13	17.19	0.96	0.144
14	15.81	0.61	0.60	0.342	0.432	14	17.85	1.00	33.611
15	17.12	0.66	0.66	0.191	10.055	15	19.71	1.10	0.873
16	17.67	0.69	0.71	3.525	0.178	16	20.11	1.13	1.999
17	19.06	0.74	0.73	0.336	1.014	17	20.59	1.15	5.191
18	19.55	0.76	0.75	1.315	1.571	18	21.19	1.19	2.012
19	19.81	0.77	0.77	2.204	2.212	19	21.75	1.22	0.852
20	20.35	0.79	0.78	1.138	2.612	20	22.61	1.27	0.195
21	20.94	0.81	0.81	0.747	2.528	21	23.70	1.33	0.422
22	21.56	0.84	0.84	0.704	1.422	22	24.08	1.35	0.134
23	22.49	0.87	0.88	0.337	0.906	23	25.09	1.41	0.970
24	23.57	0.92	0.90	1.601	2.681	24	25.87	1.45	3.338
25	23.98	0.93	0.92	0.097	0.505	25	27.49	1.54	1.510
26	24.97	0.97	0.97	1.032	2.743	26	28.67	1.61	0.216
27	25.75	1.00	1.00	13.714	12.763	27	30.37	1.72	1.110
28	27.43	1.07	0.07	1.150	2.707	28	31.92	1.79	0.147
29	28.61	1.11	1.09	0.433	0.639	29	33.31	1.87	0.419
30	30.37	1.19	1.19	0.184	0.595	30	35.20	1.97	1.349
31	31.89	1.24	1.23	0.082	0. 698	31	36.35	2.04	0.713
32	34.77	1.35	1.34	0.425	0.197	32	-	-	-
33	36.32	1.41	1.41	0.260	0.291	33	-	-	-

Components of the volatile oil of various sampling dates varied in ratio of their respective concentrations. Tagetone comprised the major constituent in volatile oil obtained from inflorescences (33.611%) . But for shoot, γ -

terpinene(component 8) replaced it, being 38.539 and 20.387% for leaves and leafless shoot; respectively, and tagetone came second (component 27) the corresponding values were 13.714 and 12.763 % . Other constituents , however , varied to different extents .

Fig. 17: Gas chromatogram of volatile oil of *Tagetes patula* L. inflorescences at flowering onset stage . (Instrument : G.C. Hewlett Packard UP 5830 A)

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دراسات مورفولوجية وتشريحية وكيميائية على القطيفة *Tagetes patula* L.

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إن المعلومات النباتية المتاحة عن أنواع الفصيلة المركبة قليلة ، لذلك تهدف هذه الدراسة الى تقديم معلومات تفصيلية عن التركيب الخارجى ، والداخلى ، وكذلك تركيب الزيت العطرى لنبات القطيفة خلال المراحل المتتالية لنمو النبات فى موسمين متتاليين .

وفيما يلى ملخص لنتائج هذه الدراسة :

تنتشر بذور القطيفة على هيئة ثمار فقيرة ، مستطيلة الشكل ، طولها 9 مم ، وعرضها 1 مم ، مستديمة الكأس ، الإنبات هوائى ، ونسبة الإنبات 36,75 %. تنتهى مرحلة البادرة بعد 3 -4 أسابيع من الزراعة.

تتكشف القمة النامية للساق الرئيسية الى نورة ، وتمثل الفروع الجانبية (5-4) غالبية النمو الخضرى ، الساق ، والفروع الجانبية قائمة ، ملساء ، تحمل قليلا أو كثيراً من الشعيرات ، ومصمتة ، و مضلعة ، أرجوانية اللون مصحوبة بنسبة بسيطة من الاخضرار ، يصل طول الساق الرئيسية إلى نحو 15 سم ، و تكوّن الفروع الجانبية الارتفاع الحقيقى للنبات ، حوالى 45 سم . متوسط عدد السلاميات للساق الرئيسية 7، و متوسط سمك السلامية القاعدية حوالى 12 مم لا يزيد الوزن الجاف للساق والفروع بدون الأوراق زيادة ملحوظة حتى عمر 14 أسبوعاً ، و تحدث زيادة معنوية بالوزن الجاف فى الاعمار المتتالية حتى نهاية موسم النمو.

تتكون الورقة الخضريه الأولى بعد 3 أسابيع من الزراعة ، والأوراق مركبة متقابلة متصالبة ، ريشية فردية، ويحقق الوزن الجاف للأوراق زيادة معنوية عند عمر 16 الى 22 أسبوعاً .

يبدأ التزهير عند عمر 10 أسابيع ، و يسير كل من النمو الخضرى ، والتكاثرى جنبا الى جنب حتى نهاية موسم النمو ، وتلعب الفروع الجانبية دوراً رئيسياً فى التزهير ، النورة هامة ، تتكون لدى أطراف الساق ، والفروع الجانبية ، وتتكون النورة من الازهار الشعاعية ، والقرصية التى تنمو على التخت المحاطة بالقلافة ، وتترتب القنابات فى صف واحد ، لون الأزهار أصفر ذهبى مع وجود هالة حمراء ، الأزهار القرصية لونها أصفر مع وجود لون أحمر على السطح الداخلى ، تنتج البذور بكل من الازهار الشعاعية، والقرصية . يصل عدد القطفات المتتالية للنورات كل أسبوعين الى 8-9 وكان أعلى محصول للنورات فى القطفة السابعة ، و الثمار فقيرة ، مستطيلة الشكل ، طولها 9 مم ، وعرضها 1 مم ، ولونها أبنوسى ، ويوجد كأس مستديم فى قمة الثمرة من 1-5 شعيرات لونها قشدى ، كما تكون قاعدة الثمرة أيضا قشدية اللون .

يتميز التركيب التشريحى للجذر بوجود البشرة تليها القشرة من 3-4 طبقات من الخلايا تنتهى بالأندودرمس ، ثم يلية طبقة البريسيكل التى تحيط بالاسطوانة الوعائية ثنائية الاذرع . يشغل الخشب المركز ، وبرانشيمة اللحاء سميكة الجدر نوعا ما . تتحول هذه الخلايا الى ألياف للتدعيم ، يبدأ النمو الثانوى بعد 4 أسابيع ، ويستمر بالطريقة المعتادة للنمو.

تتكون القمة الخضرية من تيونيكا ذات طبقتين تحيط بالكوربس ، ويتكشف الخشب فى النسيج الوعائى يليه اللحاء ، تكون الساق الرئيسية أسفل القمة النامية مباشرة عادة خماسية، وتتكون السلامية الوسطية للساق الرئيسية فى بداية التزهير من طبقة البشرة يليها 6-9 طبقات ، الثلاث طبقات الاولى منها كولنشيمية ، يعطى الكامبيوم الوعائى خشب ثانوى أكثر من اللحاء الثانوى ، ويعطى الكامبيوم البين حزمى خلايا برانشيمية ملجننة للداخل ، وخلايا برانشيمية رقيقة الجدر للخارج

يتكون نصل الوريقة من بشرتين ، العليا مقعرة ، والسفلى محدبة ، الثغور من النوع Anomocytic تتكون طبقة النسيج العمادى من خلايا غير منتظمة فى ترتيبها . يتكون النسيج الإسفنجى من 4-5 طبقات من خلايا كلورنشيمية تشغل نصف سمك النسيج الوسطى . تكون حزمة العرق الوسطى ذات مساحة ضيقة من اللحاء وواسعة من الخشب. يتراوح عدد صفوف الخشب فى العرق الوسطى من 5-7 صفوف ، وكل صف يتكون من 3-5 أوعية .

تم دراسة التركيب التشريح للأزهار الشعاعية ، والأزهار القرصية ، وكذلك الثمرة . يتكون الغلاف الخارجى للثمرة من بشرة ، وتحت بشرة ، بعض خلايا البشرة تتكشف الى شعيرات زوجية Twinned hairs بالإضافة الى شعيرات غدية . يتكون النسيج الوسطى من طبقتين عمادية الشكل . الطبقة الاولى ملجننة اكثر من الطبقة التى تليها . يتكون الغلاف الداخلى من 1-2 طبقة من خلايا رقيقة الجدر مع سيادة المسافات البينية . يتكون غلاف البذرة من برانشيمة أسفنجية .

تعطى النورات ، والمجموع الخضرى زيتاً عطرياً يصل الى $1/2$% ، ويحتوى الزيت العطرى للنورات ، على 31 مركباً تصل الى 33 مركباً فى حالة المجموع الخضرى ، المركب الرئيسى للزيت العطرى بالنورات التاجيتون ، وللمجموع الخضرى جاما - تربنين.