Effect of light level on the growth and essential oil production of two herbs :: sage (Salvia officinalis) and thyme (Thymus vulgaris) /

EFFECT OF LIGHT LEVEL ON THE GROWTH AND ESSENTIAL OIL PRODUCTION OF TWO HERBS: SAGE (*Salvia officinalis*) AND THYME (*Thymus vulgaris*)

A Thesis Presented

by

YAN-LI LI

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ABSTRACT

EFFECT OF LIGHT LEVEL ON THE GROWTH AND ESSENTIAL OIL PRODUCTION OF TWO HERBS: SAGE (*Salvia officinalis*) AND THYME (*Thymus vulgaris*)

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Sage (*Salvia officinalis)* and thyme (*Thymus vulgaris*) are popular herbs in the United States which are widely used in perfumery , culinary and as medicinal plants. The important product of these herbs, the essential oil, is produced during secondary metabolism. Accumulation of this essential oil depends directly or indirectly upon light.

Studies on growth and essential oil production and composition in sage and thyme under light levels at 15%, 27%, 45% and 100% of full sunlight were made to determine the effect on growth and essential oil production. Different growth terms for plants were used to establish plants of approximately equal size, then evaluated low of light on essential oil synthesis. Leaf length, width, and density of peltate hairs were significantly decreased under low light levels with both herbs. Height and internode length of plants grown at reduced light levels were greater than for plant grown at 100% of full sunlight.

The total oil in the sage was highest in the plants grown at 45% of full sunlight. It decreased at 27% or 100% of full sunlight, even though these treatments had similar dry matter accumulation. The major constituents in the essential oil of sage did not significantly differ among the treated light levels, expect for (+)-thujanone and epimanool. Sage grown at 45% of full sunlight produced the highest content of (+)-thujanone, and had a decreased accumulation of camphor. High light levels depressed epimanool synthesis.

The highest content of essential oil in thyme occurred at 100% of full sunlight and decreased with decreasing light levels, regardless of biomass accumulation. Thymol, the major and most useful compound in thyme essential oil, and myrcene levels significantly increased as light levels increased and reached the highest accumulation in the essential oil under 100% of full sunlight. In addition, high level light tended to depress ß-cymene accumulation. The content of isothymol and γ-terpene in the essential oil was not directly related to light levels. The effect of light level on production of essential oil undoubtedly differs for various herbs. Herb essential oil production does not depend only on production of biomass.

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CHAPTER I

INTRODUCTION

Sage (*Salvia officinalis)* and thyme (*Thymus vulgaris*) are currently among the most popular herbs being used in the United States. These herbs not only serve culinary purposes, but also are aromatic materials in perfumery and valuable medicinal plants. To date the consumption of sage and thyme has increased in the United States to the point where some must be imported from other countries. Both the producer and consumer will benefit from increased production of sage and thyme crops and from improved essential oil content of these herbs. To successfully increase domestic production of these herbs detailed studies on growth and development, and influence of environmental factors on both growth and production of essential oil is required.

Sage

Sage is held in high esteem in the food industry due to its warm spicy fragrance and a slightly bitter but pungent flavor and odor. The leaves, either fresh or dried, are used alone or in herb mixtures as a flavoring in a variety of food. In addition, as a medicinal herb, sage is at the top of the list for household remedies including relief of itching, lowering of fevers, and relief of nervous headaches.

Sage is native to the Mediterranean and naturalized in other parts of southern and central Europe. United States requirements are met by imported Dalmatian sage from Yugoslavia or by commercially cultivated herb from California, Oregon and Washington (Embing & Haziyer 1977; Tucker et al., 1980). The value of the sage is based on the content of essential oil. A minimum of 1 ml of essential oil per 100 g of dried tissue is required by federal specifications (Farrell,1985a).

The essential oil is one of the most valuable components of the herb, and has been extensively investigated in recent years (Charles & Simon, 1990; Croteau et al., 1987; Falk *et al.*,

1990; Kustrak *et al.*, 1984; Rhyu, 1979; Tucker *et al.*, 1980). Major constituents of the essential oil of sage are found in Table 1.

Table 1.1. The major consitituents of essential oil in sage.

Constituent*	% of Sage Oil	Constituent*	% of Sage Oil
Borneol	2.4	Humulene	16.4
Isoborneol	----	Limonene	2.3
Bornyl acetate	3.2	Linalool	1.2
Comphene	3.8	Linalyl acetate	----
Camphor	10.3	Myrcene	1.1
ß-Caryophyllene	9.3	α-Phellandrene	0.1
Cineol(1,8)	5.4	α-Pinene	3.6
p-Cymene	0.1	ß-Pinene	2.1
Estragole	0.4	α-Thujene	0.5
Fenchone	0.1	α-Thujone	20.5
α-terpineol	----	ß-Thujone	3.2

* The Data are from Kustrak *et al.*, 1984 and Rhyu, 1979.

Both the volume and components of sage essential oil differs with geographic location (Embing & Haziyer, 1977). The average oil yield of Dalmatian sage for a growing season is about 1.4%, while Italian and American sage from Washington, Oregon or California yields between 0.6 and 1.0%. Table 2 lists differences in the composition of sage essential oil from three locations.

Table 1.2. The composition of sage oil in relation to growth regions*.

Dalmatian Oil	Italian Oil	Washington Oil
α-Pinene 3.3%	Monoterpene 15%	----
ß-Pinene 5.6%	Sesquiterpene 20%	----
Cineol 14.8%	Cineol 15%	----
Thujone 50.0%	Thujone +Camphor 31%	Thujone 35.4-46.7%
Camphor 8.2%	----	----
Borneol 6.6%	Free alcohol 11.2%	Borneol 13%
Borneol acetate 1.7%	----	Esters 3-6%

The Data are from Embing & Haziyer, 1977.

Karawya *et al.* reported a total terpene hydrocarbon content of 78.2% for sage from Egypt, in which the major constituents were ß-phellandrene (18.5%) and camphene (11.4%). No thujone was detected. Camphor content was only 0.7%, while 1,8-cineol amounted to 17.1%. Dalmatian sage oil, called "high test oil", contains the following compounds: high thujone content, a total ketone content of 42-61%, ester (borneol acetate) 1.5-4%, alcohol (borneol) 7-16%, and sesquiterpene 10% or less. Washington State oil and Italian oil are similar to "low test oil" of Dalmatian sage, their major constituents include esters (2.2-4.9%), alcohol (11-15%), and ketone (22-40%).

The price of sage on the market depends on the thujone content, more than 1.5% essential oil and a high percentage of thujones (50%) and a low percentage of camphor (20%), is desirable (Putievsky et al. 1986). A high content of 1.8-cineol is also commercially important.

Thyme

Thyme is extensively used as a pot herb in cooking, perfumery, and in liquor distillery. Thyme oil is used in the perfumery industry and in soap and detergent. Thymol is a major commercially important aromatic chemical in the essential oil of thyme. Thymol has a powerful medicinal odor, and has antiseptic, antibacterial and disinfectant properties valued for dental preparations, oral hygiene products, in vermifuges, and in antigastrointestinal products (Farrel, 1985).

Thyme is native to southern Europe and the Mediterranean countries. Spain is the main thyme oil producing country, although it is also widely cultivated in the United States and other parts of the world. The US Federal Specification for the value of thyme is that it contains no less than 0.9 ml of essential oil per 100 g of dried plants.

Eighty-two compounds have been identified in the essential oil of wild thyme from France (Parkanyi, 1993). Thyme oil was found to contain: 10 monoterpenes, 17 sesquiterpenes, 9 oxides, 13 carbonyl compounds, 11 esters, 17 alcohols, 5 miscellaneous compounds (including indole). The chief constituent of thyme oil is thymol. Other components are: ß-pinene, *p*-cymene, caryophyllene, camphene, α-thujene, myrcene, Δ-3-carene, ß-phellandrene, α-terpinene, α-terpinene, γ-terpinene, *cis*-ß-ocimene, terpinolene, α-humulene;linalyl acetate, α-terpinyl-acetate, geranyl acetate, *trans*-sabinene hydrate, limonene+cineole, *trans*-linlool oxide, *cis*-linalool oxide; carvacrol, *l*-linalol, *l*-borneol, geraniol, amyl alcohol, terpineol, methyl carvacrol (Farrell, 1985; Papgeorgiou & Agryiadou, 1981; Poulose & Croutea, 1978; Yarniv & Palevitch, 1982).

As with sage, the reported constituents of thyme oil are different in plants from various parts of Europe (Farrell, 1985b; Papgeorgiou & Agryriadou, 1981; Poulose & Crouteau, 1978; Yarniv & Palevitch, 1982).

CHAPTER II

LITERATURE REVIEW

Plant growth and development are determined basically by genetic factors. These factors affect the essential oil production of herb plants as well. Many species of herbs, spices or medicinal plants are known to have some special anatomical structures on leaves, such as the glandular hairs, which are responsible for the secretion of the essential oils (Dudai, 1988; Fahn, 1979; Gershenzon, 1989; Venkatachalm *et al.*, 1984; Werker *et al.*, 1985a & b; Yamaura, 1989; Zarate, 1994). The content and components of essential oil can be different in different parts of the plant and at different growth stages (Arras & Grella, 1992; Huopalahti & Linko, 1983; Putievsky *et al.*,1983, 1986 a & b, & 1988; Singh *et al.*, 1989).

Influences of Light

Essential oil is one of the components of secondary metabolism in herbs. Carbohydrates produced during photosynthesis are used to synthesize secondary compounds, thus, accumulation of essential oil in herbs directly or indirectly depends upon production of carbohydrates during photosynthesis. For this reason, light quality and quantity play a greater role in essential oil production than other environmental factors. Light effects influencing growth and essential oil production include light level, wavelength, photoperiod, and interactions of these factors.

Light Level

The compounds produced during photosynthesis may form the backbone for the synthesis of secondary compounds. A relatively close connection between light and special product formation can be postulated. Morphological and physiological responses to different levels of irradiance may be significantly different.

Primary morphological responses of plants to low light level are increased internoded length, thin and chlorotic leaves, decreased leaf numbers and decreased leaf area due to lack of assimilates (Evans, 1973; Firmage, 1981; Hälvä *et al.*, 1992a; Morgan & Smith, 1976 & 1981; Morgan, 1981).

The essential oil content of herbs grown during summer months is higher than in plants grown during winter (Singh, J.N. & D.P. Singh, 1969). In *Origanum syriacum,* the essential oil content changes widely with season (Dudai *et al.*, 1992). A similar response has been reported in *Salvia officinalis, Origanum vulgaris* and *Pelargonium graveolens* (Putievsky *et al.*, 1986 & 1988; Ravid & Putievaky, 1984). Increased oil production under high light level has also been observed in *Mentha piperita, Matricaria recutita* and *Thymus vulgaris* (Clark & Menary, 1980b; Langston & Leopold, 1954; Saleh, 1973; Yamaura *et al.*, 1989). Reducing solar radiation by shading the plant causes a significant decrease in the essential oil content of herbs. Under controlled conditions, the oil content of *Matricaria chamomilla* is 0.88% at an irrdiance of $9.5x10^4$ erg m^2/sec; a reduction in the light by two-thirds resulted in oil accumulation of only 0.4% (Saleh, 1972). The oil yield of Philippine mint (*Mentha cordfolia*) was 28% lower in plants grown under 2% of full sunlight than plants grown at 25% of full sunlight (Cantoria *et al.*, 1974).

Japanese mint (*Mentha arvensis)* grown under artificial shade was observed to have greater leaf area than plants grown without shade. No significant differences in oil yield were reported among the different light intensities at the final harvest (Craker & Seiber, 1982; Duriyaprapan & Britten, 1982; Hotin, 1968). De Santo (1973) also grew peppermint (*M.piperita)* under full sunlight and three levels of shading and reported compensatory effects so that growth rates were similar at 100% of sunlight and 44% of full sunlight.

Plant essential oil production is modified by changes in the level of basic metabolites. Hälvä *et al.* (1992a) determined the total oil concentration of dill (*Anethum grvaeolens*) grown under different light levels. The increase in both plant weight (fresh and dried) and oil production with increasing light

level showed that oil production is directly connected with biomass production, and thus to photosynthesis.

Not only would light level affect the amount of essential oil of herbs, but it would also affect the composition of the oils. When researching the metabolism of monoterpenes of peppermint (*Mentha pipperita*), Burbott & Loomis (1967) and Clark & Menary (1980a) reported that full sunlight level combined with long day length (18 hrs) and cool night temperature (8^0C) enhanced the formation of menthone but depressed the accumulation of menthofuran and pulegone in leaves. On the other hand, observations by Dudai *et al.* (1992) indicate that only in the short day length treatment, did light intensity affect the composition of essential oil, so that the relative content of the phenolic monoterpenes decreased while that of p-cymene increased.

Light Spectrum

The spectral distribution of light not only can modify plant growth and development, but can also affect plant secondary metabolism in several ways. In general, there are three major types of action spectra for plant responses: (a) maximum response occurring in both red and blue light, including photosynthesis, tropism and plant movement; (b) the maximum response occurring in red light, as with chlorophyll formation, photoperiodism, seedling morphogenesis and dormancy responses; (c) maximum response in blue light, as with phototropism and polarity effects (Bickford, 1972).

Plants detect light quality with chemical photoreceptors. In addition to chlorophyll, which is responsible for photosynthesis, phytochrome and blue light absorbing photoreceptors are involved in the photomorphogenic control of plant growth and development (Holens & Smith, 1977a & b).

Phytochrome detects the relative proportions of red and far-red light in the natural environment. Detection of this phyochrome is effective after a short exposure to red or far-red light. Certain responses, however, require longer or repeated exposure. The activity of phytochrome can be affected by gene function, enzyme activity, hormone level and membrane functions (Funk & Croteaw, 1993).

Phytochrome directly or indirectly regulates photomorphogenic responses such as dormancy, germination, flowering, pigment development and leaf expansion (Smith, 1981 & 1982). Treatment with far-red light, increases dry matter and reduces leaf area and branching (Child & Smith, 1987; Rajan, 1970). Plant dry matter and leaf area, however, tend to increase even under low levels of red light and short exposure conditions (Hälvä, *et al.*, 1992).

The blue receptor is responsible for detecting the quantity of light, and is partly controlled by phytochrome. Plant responses to blue light include inhibition of vegetative growth (shorter internodes, thicker leaves and smaller leaf area), chloroplast development, and synthesis of pigment, protein, and enzymes (Hälvä, *et al.*, 1992b; Casal & Smith, 1989; Cosgrove, 1982; Thomas, 1981; Warrington & Mitchell, 1976a & b).

Light spectra affect essential oil production in plant in many ways. In the research of light quality, growth, and essential oil in dill (*Anethum graveolens*), Hälvä *et al.* (1992b) demonstrated that red light treatment for 4 hrs increased oil production companed to natural and blue light treatments. Additionally, the major constituents, (*α*-phellandrene, *β*-phellandrene, *p*-cymene), except myristcin, increased in plants receving 4 hrs red light as compared with plant exposed to natural light, blue light, or 2 hrs of red light. Saleh (1972) reported that red light treatment resulted in the highest total oil and chamazulene content of chamomile, expressed on a plant basis, due to the increased number of flowers and dry weight. Observations of Labiate plants by Venkatchalam *et al.* (1984) indicated that the number of trichomes was correlated with the amount of monoterpenes. Monoterpene production was stimulated by red light in thyme *(Thymus vulgaris)* seedlings, in part, due to an increase in the number of newly developed trichomes following irradiation. Trichome formation is controlled by phytochrome (Tanaka, 1989). In maritime pine (*Pinus pinaster*), red light stimulated the synthesis of mono- and sesquiterpene hydrocarbon (Gleizes *et al.*, 1980). Irradiation of etiolated sunflower seedlings with pulses of red light

led to an increased accumulation of sesquiterpene lactones, whereas its effect was partly inhibited by a subsequent treatment with far-red light (Spring *et al.*, 1986).

Photoperiodism

Photoperiod plays a fundamental role in regulation of plant growth and development processes. A morphological response to photoperiod is clear in peppermint (*Mentha piperita*) (Langston & Leopold, 1954; Stewart, 1962). Short days result in decumbent plants, small leaves and many stolons, while, long days results in erect plant, large leaves and flowers.

Conversion of a plant from the vegetative phase to the reproductive phase depends on photoperiod. In an earlier study of *Majorana syriaca*, it was reported that the critical day length for flower initiation was 12 hrs, but full flowering required a longer photoperiod (Dudai *et al*, 1989). Photoinduction of earlier flowering under long days may produce lower vegetative yields (Putievsky, 1983a; Franz, 1986).

The effect of photoperiod on growth and essential oil production is different among different plants. Long day conditions enhanced growth of peppermint (*Mentha piperita*) with a corresponding increase in the total amount of oil (Burbott & Loomis, 1967). Basil (*Ocimum basilicum*), which is long day plant, produces the highest yields with a 24-hr light period. Higher production is achieved with a 16-hr light period combined with high temperature, but high temperature, combined with short days, decreased yield (Skrubis & Markakis, 1976; Putievsky, 1983b). A similar effect has been observed in many plants, such as oregano (*Origanum vulgaris)*, dill (*Anethum graveolens*), chamomile (*Matricaria chamomilla)*, and thyme (*Thymus vulgaris*) (Putievsky, 1983a; Hälvä, *et al*., 1992a & b; Salen, 1972 & 1973).

Photoperiod is also reported to affect essential oil composition. Peppermint plants subjected to long photoperiods contain larger amounts of menthane and menthol, major compounds in peppermint oil, and less menthofuran, an undesirable compound. In contrast, plants subjected to short photoperiod

contain less methene and menthol, and more menthofuran (Burbott & Loomis 1967). In addition, long days stimulate the accumulation of cineole, *ß*-pinene, sabinene, and *trans*-sabinene hydate, and depress the production of limonene, pulegone and menthyl acetate in Tasmanian peppermint (Clark & Menary, 1979a&b, & 1980a).

Interactions among Light Level, Spectrum and Photoperoid

Significant interactions occur between light intensity and photoperiod. The effect of light intensity is greater under long days than under short days. A low light intensity combined with 18 hrs of light gave peppermint (*Mentha piperita)* very poor growth and lower menthone, which is the principle compound in the essential oil (Burbott & Loomis, 1967) (production of peppermint oil requires a daylength of 15 to 16 hrs).

A light treatment of 16 hrs stimulates the accumulation of scopolamine in *Datura tabula*, but formation of scopolamine may be inhibited by low light intensity. A short day length is analogous to a low light intensity and universally inhibits the accumulation of poppy alkaloids (Bernàth 1986).

Glandular Hairs: the Biosynthesis Site of Essential Oil

The production of essential oil and resins in plants is generally associated with the presence of specialized secretary structures such as glandular trichomes and oil or resin ducts (Fahn, 1979). Such structures contain the monoterpenes, sesquiterpenes and diterpenes, so there is little doubt that they are the primary sites of terpene accumulation (Gershenzon, *et al*, 1989; Werker *et al*, 1985a, b & c; Yamaura *et al*., 1989).

The primary secretory organ is the glandular trichome, the detailed structure of which varies widely with species (Werker *et al*., 1985b). Peppermint, for example, possess two types of secretory trichomes, both types have a unicellular base and stalk bearing a head consisting of either one or eight secretory cells (Battaile & Loomis, 1961). At least four discrete gland types have been ascribed to sage

(long-stalked glands and sessile glands bearing a heand with one-, two-to four, and eight cells) (Venkatachalam, 1984).

There is direct and indirect evidence for the biosynthetic capabilities of glandular trichomes: (a) these structures may incorporate labeled precursors such as sucrose, acetate and mevalonate into terpene (Croteau, 1984); (b) monoterpene synthesis has been found in trichomes using undifferentiated cells in culture, coupled with evidence of monoterpene transport in intact plants (Battaile & Loomis, 1968; Charlwood, 1983); (c) trichomes are the location of the enzymes catalyzing the reaction of accumulating monoterpene, such as (-)-carvone in spearmint (Gershenzon, 1989).

Ultrastructural studies have concluded that terpene synthesis in the glandular trichome occurs principally in the secretory cells rather than the stalk of the basal cell (Zarate & Yeoman, 1994). At the subcellular level, carveol dehydrogenase activity in spearmint secretory cells was associated with the presence of a smooth endoplasmic reticulum. Another type of organelle often linked with monoterpene synthesis is the leocoplast, a plastid of complex shape without thylakoids (Gershenzon, 1989; Cheniclet *et al.*, 1985).

Accumulation of essential oils in glandular trichomes partly depends on the organ of the plant on which they occur and the stage of development. The content of essential oil and the number of peltate hairs were higher in the flowers than the leaves of *Saliva sclarea* and *S. dominica* (Bosabaliis & Tsekos, 1982; Werker *et al.*, 1985c).

Glandular trichome formation may be affected by light. The formation of peltate glandular trichomes occurred prior to monoterpene accumulation in both green and etiolated thyme seedlings (Yamaura *et al.*, 1989). The yield of thymol, the main constituent of essential oil in thyme, increased with increasing length of irradiation.

Influences of Plant Part and Development Stage

The content and composition of essential oil of herbs are associated with various plant parts and development stages. The essential oil content and leaf percentage of cultivated *Salvia fruticosa* were the highest in the summer, while the total fresh weight was the highest in the spring (Putievsky, *et al.*, 1986b). During full bloom, the stem contained a low level of essential oil, compared with its content in the leaves and inflorescences of *S. fruticosa*. The composition of the essential oil was similar in the stems and in the inflorescences of, but differed from, that found in the leaves. In sage, however, flowering was found to have little influence on the content and composition of essential oil. (Putievsky, *et al.*, 1986a). Young leaves and flower buds of *Pelargonium graveolens* contained more oil than old ones (Puttievsky *et al.*, 1983). The total amount of essential oil in dill (*Anethum graveolens*) reached the lowest value during vegetative growth, due to the fact that dill grows much more rapidly than the biosynthesis of secondary production, such as essential oil (Huopalahti & Linko, 1983). However, the total content of essential oil of dill increased continuously during the whole growth period (El-Gengaihi & Homok, 1978).

There are variable components in essential oil in the different parts of plants or at different growth stages. Investigations on the changes in the composition of the essential oil of peppermint indicated that the greatest changes occurred in the preflowering bud stage. The pre-bud oil had the highest content of menthol and menthone together (Baslas, 1970). Rabak (1916) reported the formation of ester and menthol take place most readily in the leaf tops of the plant, and the metabolic processes show increasing activity as the plant matures (Verzar-Petri *et al.*, 1978). In wild thyme (*Thymus capitatus*), caracrol, *p*-cymene and -terpinene, major compounds of essential oil, showed changes related to the different growth stage. Carvacrol level was high before and after flowering, while the converse occurred for *p*-cymene and terpinene. γ-Terpinene was at a maximum when the plant was young, but decreased

with an increase in carvacrol. These changes in the three constituents would seem to confirm a close biogenetic relationship (Arras & Grella, 1992).

Position and age of leaves may affect the essential oil production. For example, in lemongrass (*Cymbopogon flexuosus*) only the initial growth period of leaves was associated with active oil synthesis. The highest oil yield was obtained in the second leaf. Furthermore, the level of the oil decreased in leaves nearing maturity, possibly because of catabolism (Singh *et al.*, 1989, 1990 & 1991).

CHAPTER III

MATERIALS AND METHODS

Plant Materials

Sage and thyme (Weel-Sweet Herb Farm, 317 Mt. Bethel Rd., Murray, NJ 07865) were sown in pots in the autumn of 1993. Seedlings with 2-4 leaves, were transplanted into 6 inch pots filled with a commercial potting mixture (Pro Mix Bx, Premier Brand Inc. Yonkers, N.Y. 10740) and grown for 4 or 5 months. Experimental plants were obtained by vegetative propagation the follow spring. Growth conditions in the greenhouse consisted of ambient sunlight with day time temperature of 22 ± 2^{0}C and night temperature of 18 ± 2^{0}C. The herbs were watered as needed and fertilized once weekly with a complete fertilizer (0.1% $N:P_2O_5:K_2O = 20:20:20$, with microelements: H_3BO_3 9.15 ppm; $MnCl_2$, 5.80 ppm; $ZnSO_4$, 0.70 ppm; $CuSO_4$, 0.25 ppm; H_2MoO_4 0.06 ppm). The plants were allowed to grow for 40 (thyme) or 50 days (sage) before treatments were initiated.

Different Light Level Treatment

Different light levels were obtained by using shade cloths (PAK Unlimited Inc, A.H. Hummert, 2746 Choteau Ave, St. Louis, Missouri). The shade cloths (polypropylene fabric) transmited either 45%, 27%, or 15% of full sunlight. The control plants received 100 % of full sunlight in the greenhouse. The light level for each treatment were determined with a quantum sensor (type: L1-170, Lambda Inst. Co. Inc.) at 8:00 am, 12:00 and 5:00 pm.

Growth Characteristics

The growth characteristics determined included: fresh and dry weight, plant height, branch number, length, width, and number of leaves, and the number of glandular cells per unit of area (Fig. 3.1).

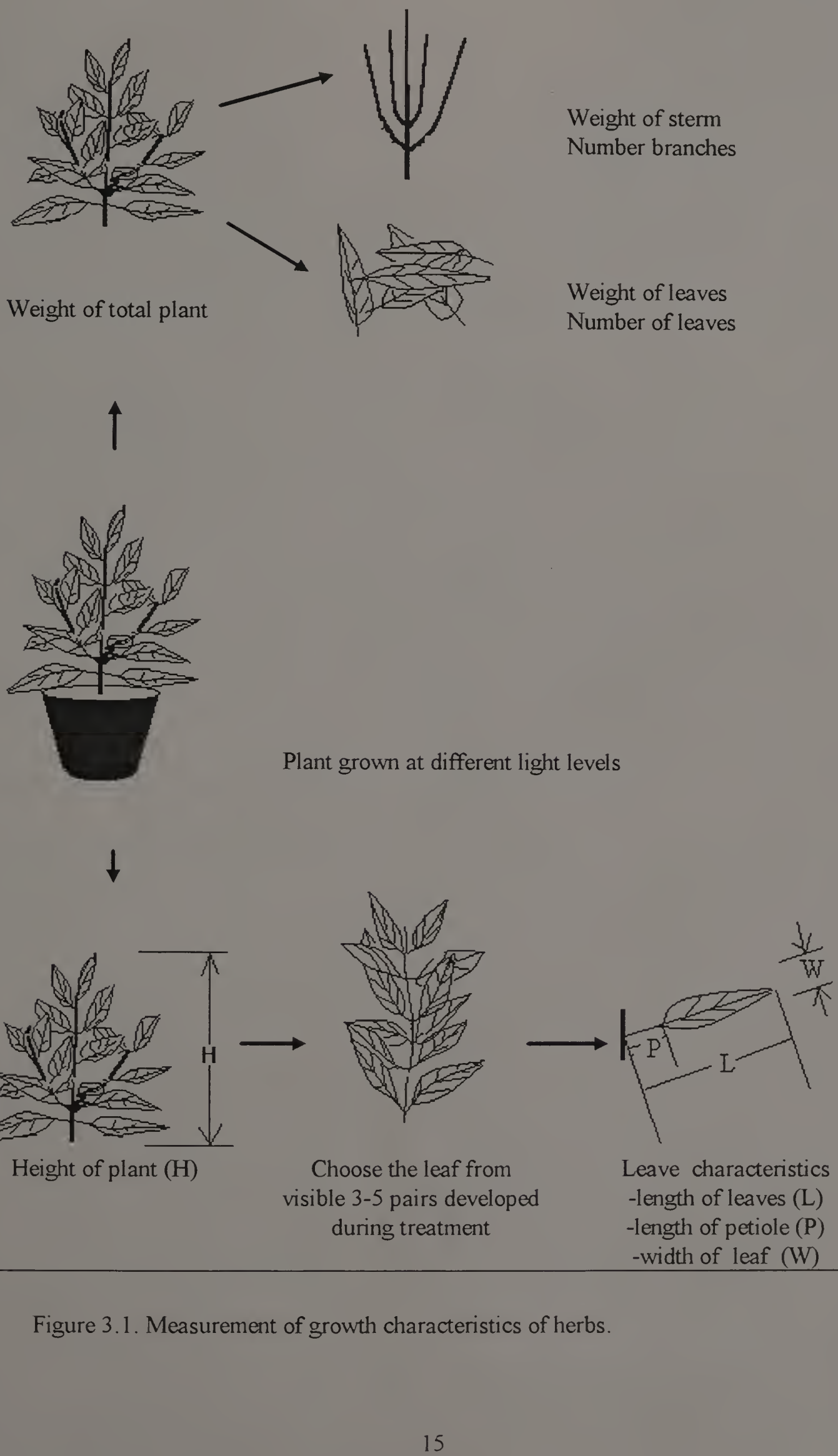

Figure 3.1. Measurement of growth characteristics of herbs.

Before harvest plant height was measured from soil level to uppermost leaf stretched straight. The total number of branches and leaves of each plant was counted. The length and width of each leaf was determined for the uppermost 3-5 pair of leaves because these leaves developed at the different treatment light levels. The number of peltate hairs per unit of area (number/mm^2) were measured on the abaxial side of the uppermost 3-5 pair of leaves. At harvest the aboveground parts were taken and weighed immediately and after drying at 65°C for 24 hrs. Fresh and dry weights of total plant and leaves only were determined separately.

Apporximatly 30 g leaf tissue was kept in sealed plastic bags at -20°C for analysis of essential oils.

Extraction of Essential Oil

Essential oil was extracted by steam co-distillation with pentane in a modified Likens-Nickerson apparatus. About 20 g samples were transferred to a 500 mL distillation flask containing 200 mL of distilled water, spiked with 10 µL of a surrogate standard containing 3.33 µg/µL of naphthalene-d8 and 2.96 µg/µL of phenol-d6. Distillation was allowed to continue for 2 hrs. The pentane was concentrated under a stream of dry nitrogen to 4 mL and stored at -20°C prior to GC/MS analysis. Identical extraction conditions were applied to a blank consisting of 200 mL distilled water.

All solvents and standards were analysis grade obtained from Fisher Scientific and were used without further purification.

GC and GC/MS Analysis

A Hewlett-Packard Model 5890 series II GC was used. The chromatograph was equipped with a 30 m X 0.32 mm (i.d.) DB-WAX (J & W Scientific, Folsom, CA) fused silica capillary column coated with 0.25 mm film and a flame ionization detector. The helium carrier gas pressure on the column was

Injections (2 μL each sample) were at 250°C in the splitless mode using an automatic injection system. For GC analysis, 1 mL of a 10-fold dilution of each sample was transferred to a 1 mL vial, to which 14 μL of 1.42 mg/mL C_{30} was added as an internal standard.

The quantity of each compound in the essential oil was determined by comparison of integrated peak area to the peak area of internal standard C_{30}. In the automatic integration, the peak areas that were larger than 1000 and the percentage of total area were larger 0.1% in chromatogram were measured, and subject to sum up as total essential oil.

The column and temperature program described above was used for GC/MS analysis. The capillary column was directly coupled to the ion source. Mass spectra were obtained in the electron impact (70 eV) ionization mode with a Hewlett-Packard 5989A GC/MS system.

The compounds of essential oil were identified by finding reference spectra that most closely matched a submitted spectrum in the NIST Mass Spectrum Search Program (U.S. Department of Commerce Technology Administration, 1995).

Statistics Analysis

A randomized complete block design with three replicates was employed for this experiment (Damon & Harvey, 1987; Steel & Torrie, 1980). The data collected was subjected to analysis of variance with comparison of means using Statistix.

CHAPTER IV

RESULTS

Yields and Morphological Characteristics

The vegetative yields and development characteristics of both sage and thyme were effected by sunlight level. Ten days after treatment initiation, stems of herbs under reduced light were thinner and leaves were paler than plants grown at full sunlight. In order to obtain equal biomass from all treatments for comparison of essential oil production, extended growth periods were required for plants grown at reduced light to accumulate biomass equivalent to that of plants grown under full sunlight (Table 3).

Table 4.1. Growth time of sage and thyme at different light levels.

	Growth Term (days)	
Treatment	Sage	Thyme
15% of full light	90	85
27% of full light	80	75
45% of full light	72	65
100% of full light	65	55

Sage produced similar fresh and dried yields for all treatments except those grown at 15% of full sunlight. The leaf fresh weight of sage was similar, but dried leaf weight of plant grown at 100% of full sunlight was the largest (Fig. 4.1). Height and internode length increased as light level decreased from 100% to 27% of full sunlight. At 15% of full sunlight, height and internode length were significantly less (Fig. 4.2). The numbers of leaves, branches and nodes of the main stem were different in the plants grown at different light levels, however, there was no interaction between the change of light levels and these parameters (Fig. 4.3). Length and width of leaves were largest in plants grown in 100%

full sunlight, and decreased as the light levels decreased (Fig. 4.4). The density of peltate hairs on the abaxial side of leaves (the number of peltate hairs per mm ^{2} leaf area) decreased as light level decreased (Fig. 4.5).

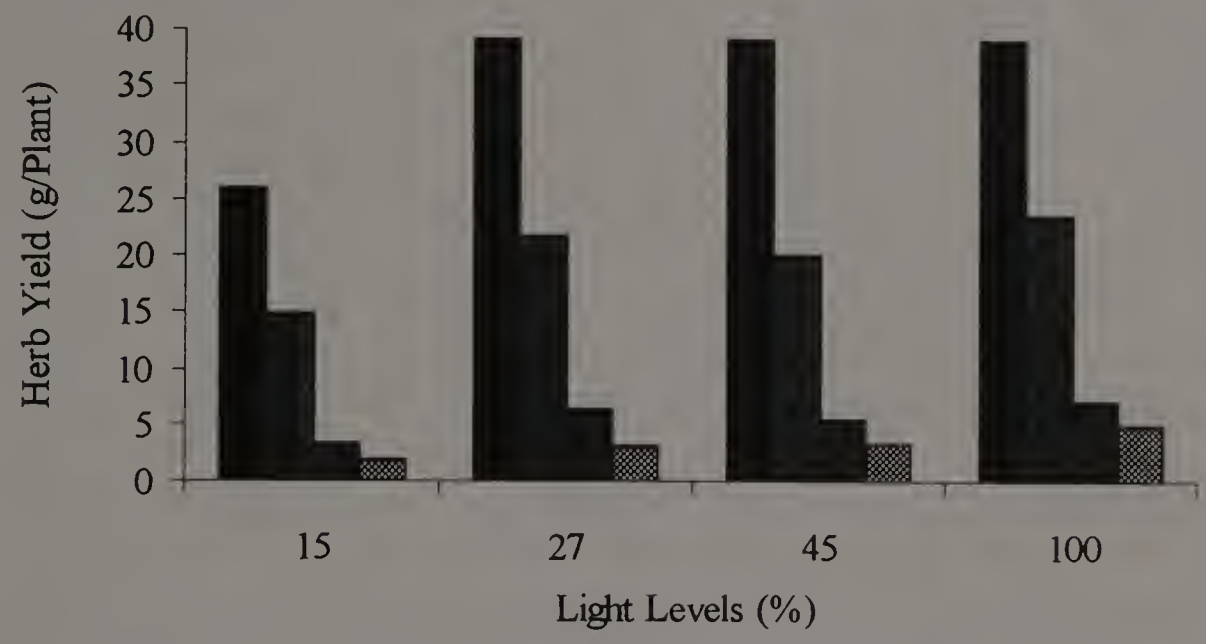

Figure 4.1. Yields of sage grown at different light levels and different growth term.

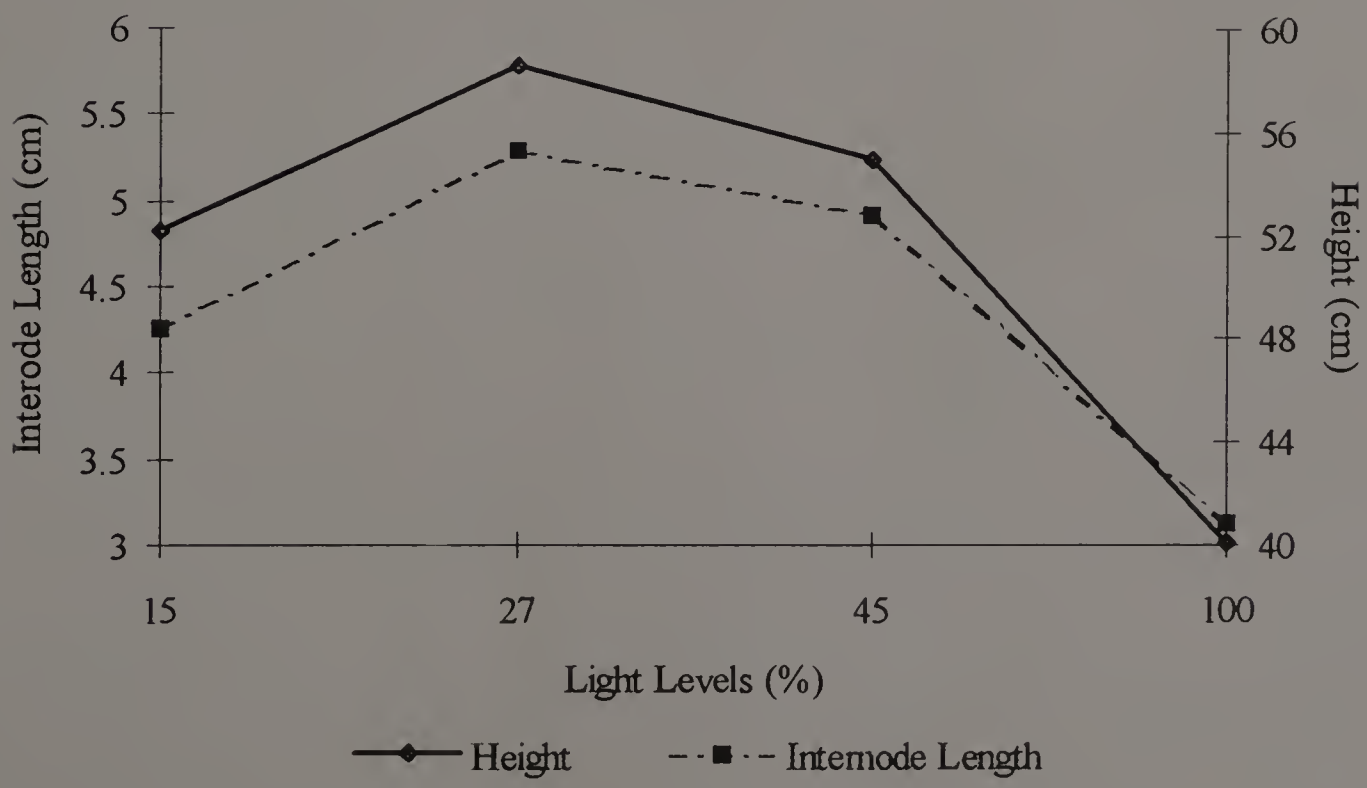

Figure 4.2. The height and internode length of main stem of sage grown at different light levels.

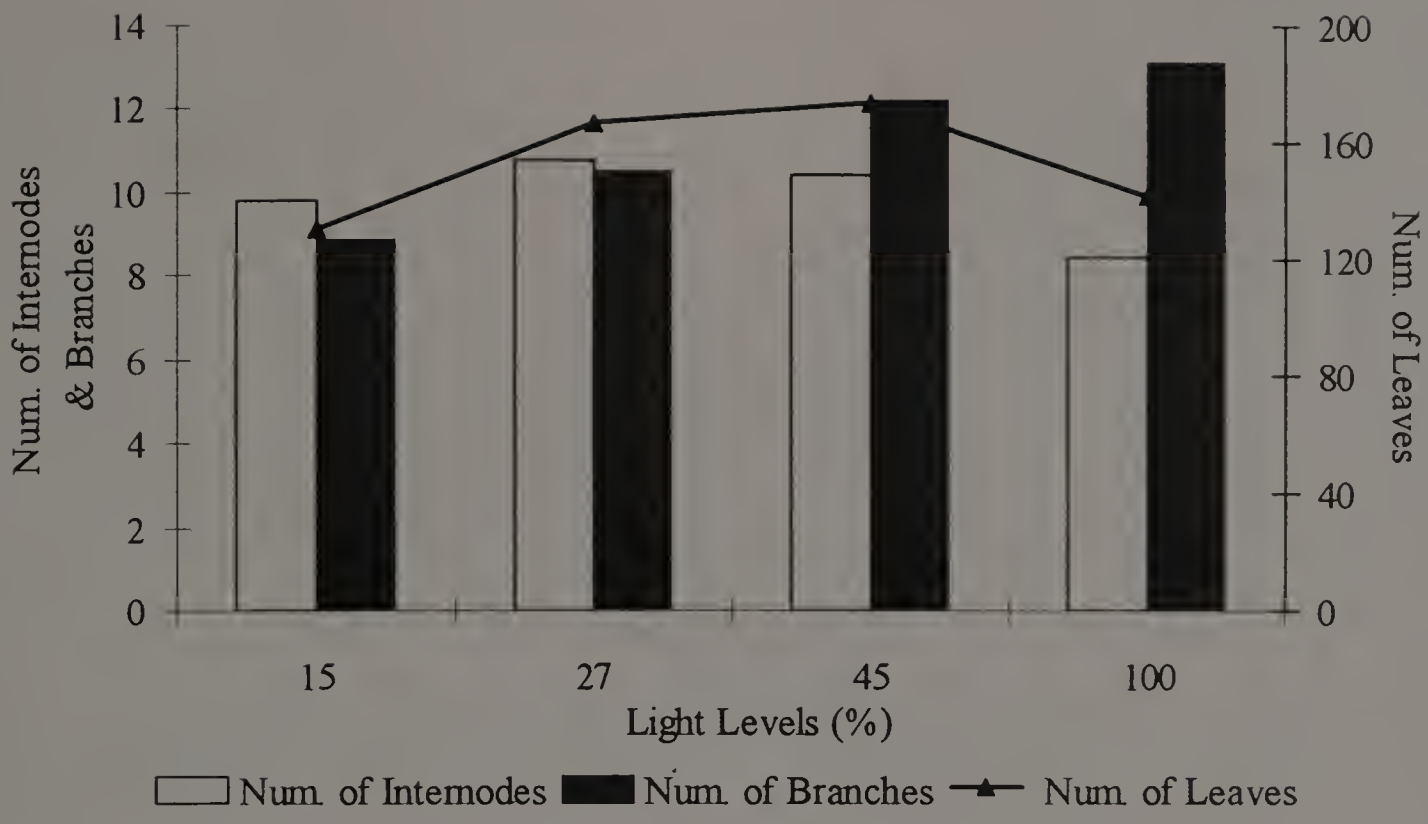

Figure 4.3. Number of internodes, branches of main stem and leaves of sage grown at different light levels.

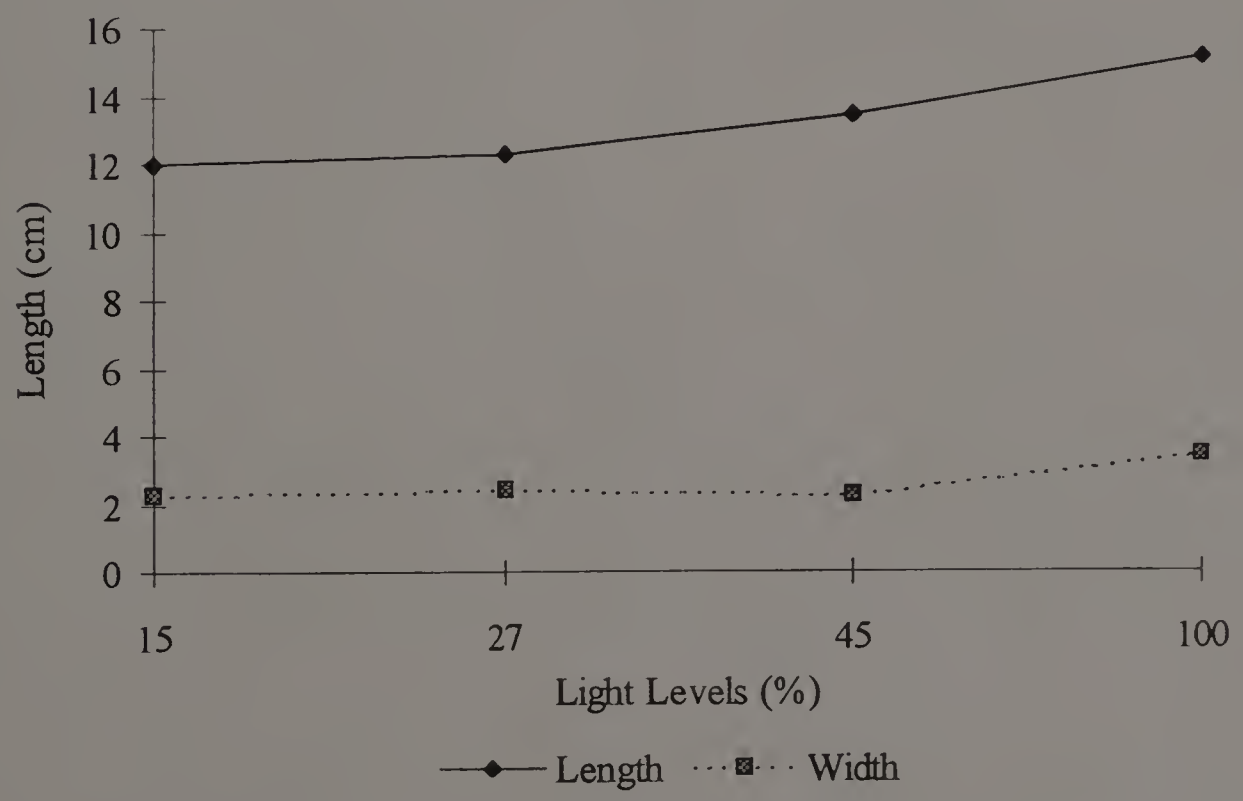

Figure 4.4. Leaf length and width of sage grown at different light levels.

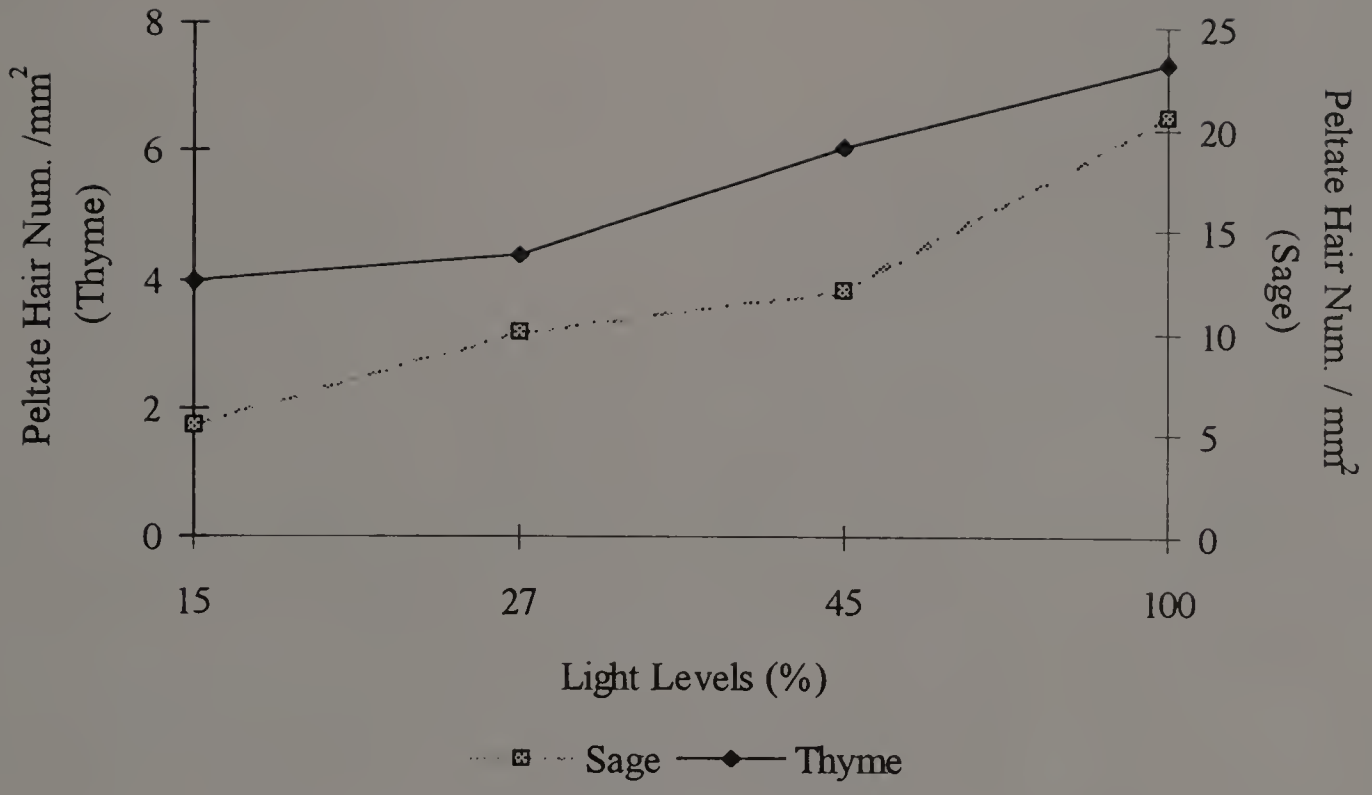

Figure 4.5. The density of peltate hairs of leaves of sage and thyme grown at different light levels.

Thyme produced similar fresh yields, but dry weight was significantly lower in the plants grown at the two lower light levels than in the plants grown at two of higher light levels (Fig. 4.6). Due to the small size of the plants, only plant height, internode length and leaf length and width were measured. The increase in node length of thyme was similar to sage, but plant height continued to increase with decreasing light levels (Fig. 4.7). The length and width of leaves (Fig. 4.8), and the density of peltate hairs (Fig. 4.5) also decreased with reduced light levels, as was observed with sage.

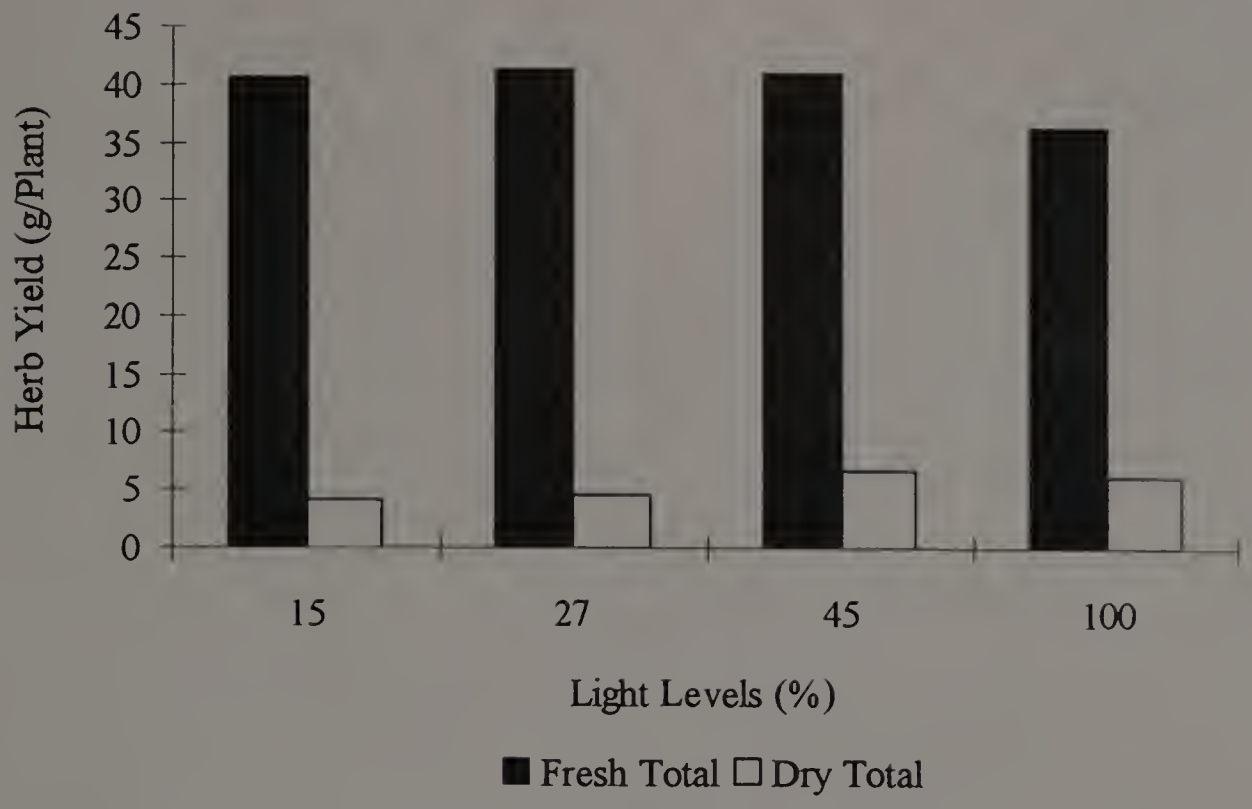

Figure 4.6. Yields of thyme grown at different light levels.

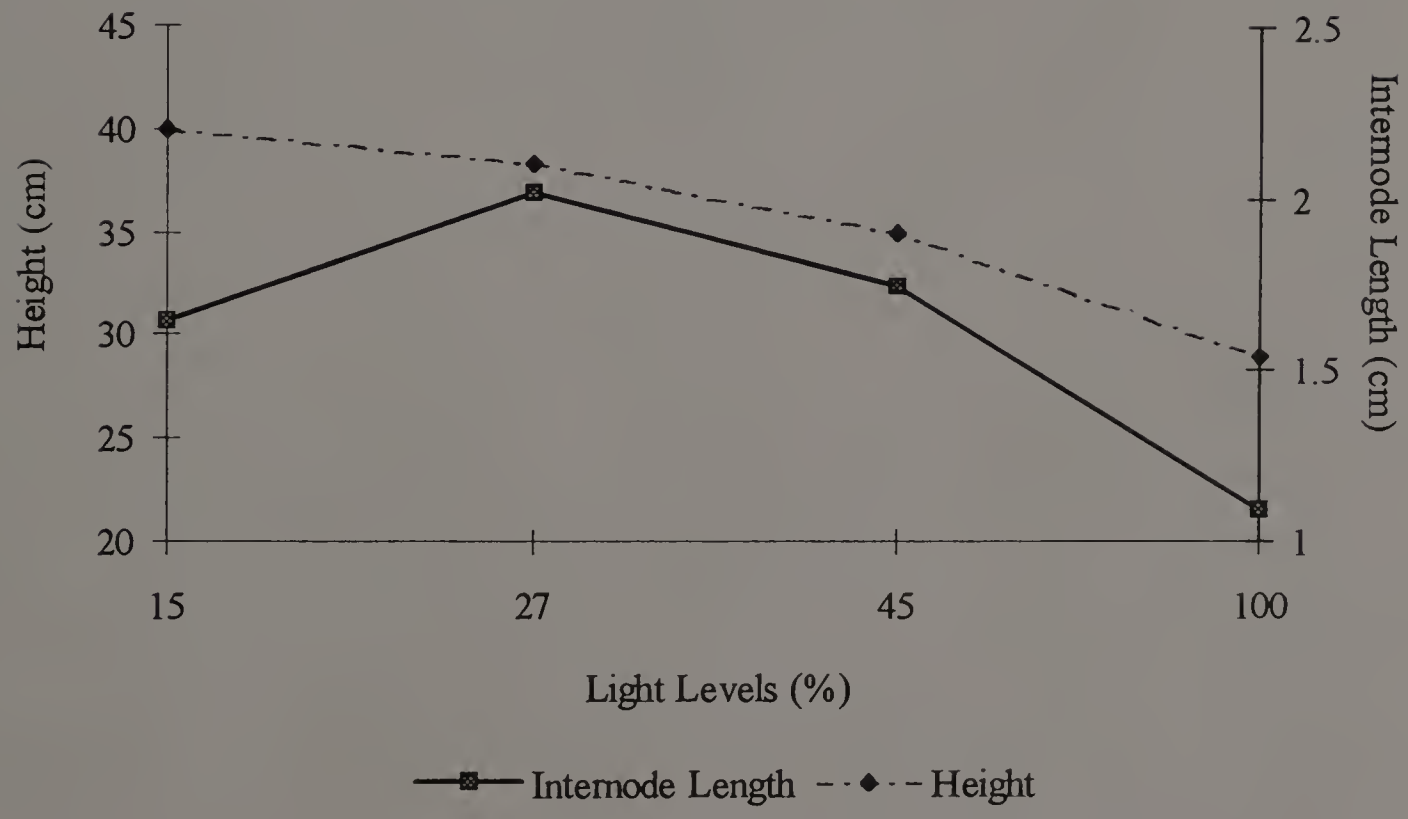

Figure 4.7. The height and internode length of main stem of thyme grown at different light levels.

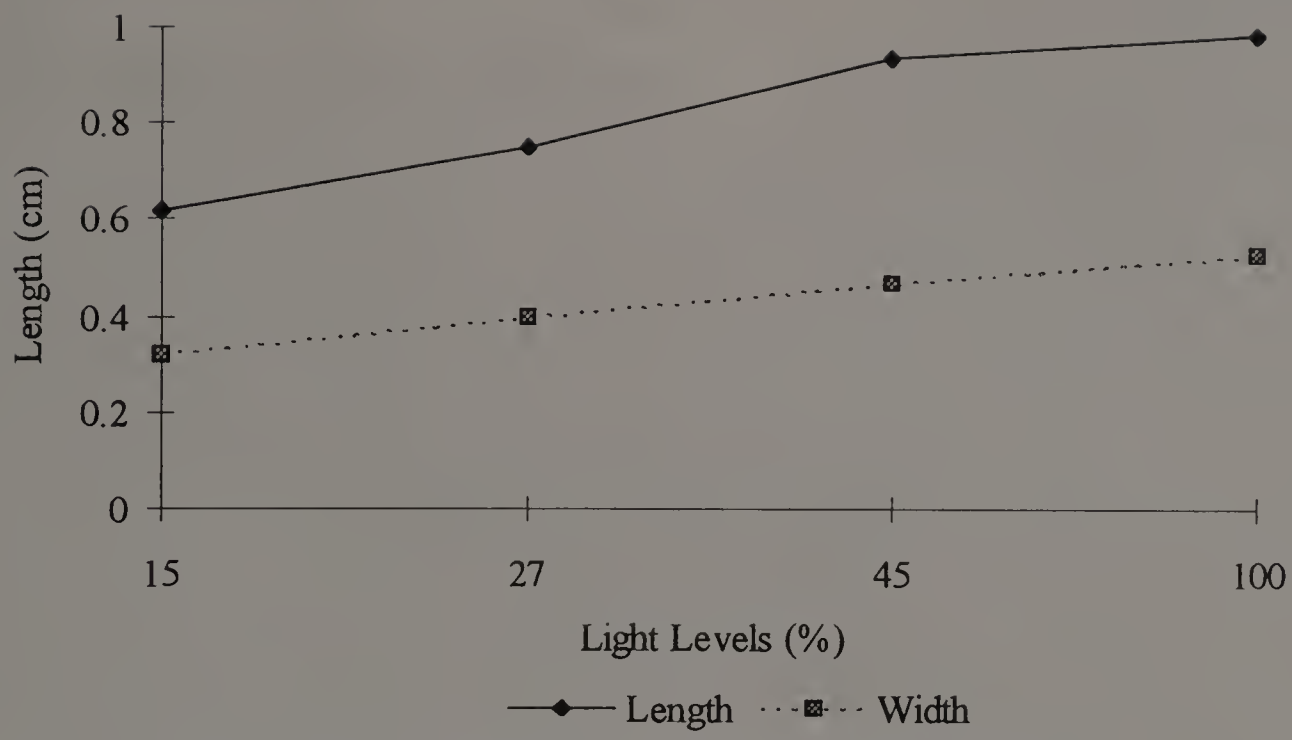

Figure 4.8. Leaf length and width of thyme grown at different light levels.

Analysis of Essential Oil

Eight-nine compounds were identified in sage grown at 100% of full sunlight (Fig. 4.9). These consisted of 7 groups of compounds: 45 monoterpenes, 11 sesquiterpenes, 10 hydrocarbon compounds, 10 carbonylic acids, 5 alcohols, 3 aldehydes, 4 ketones, 1 aminobenzoate and 2 compounds which could not be identified (Appendix Table 1).

Based on the fragments at 95 (100), 81 (77), 41 (72), 108 (47) and 152 (37), the compound which retention time is at 12.62 minutes was identified as camphor (Fig. 4.10). Camphor, the major constituent in essential oil of sage, is a monoterpene, molecular weight 152, and about 26 % of total oil in plants grown at 100% of full sunlight.

An ANOVA and mean comparison test indicated the total oil content of sage grown at 45% of full light reached the greatest value (0.38%, 3768μg/g fresh leaves), even higher than that of sage grown under 100% of full light (0.34%, 3435μg/g fresh leaves), and the total oil concentration decreased with decreased light level from 27% to 15% of full light (Fig. 4-11).

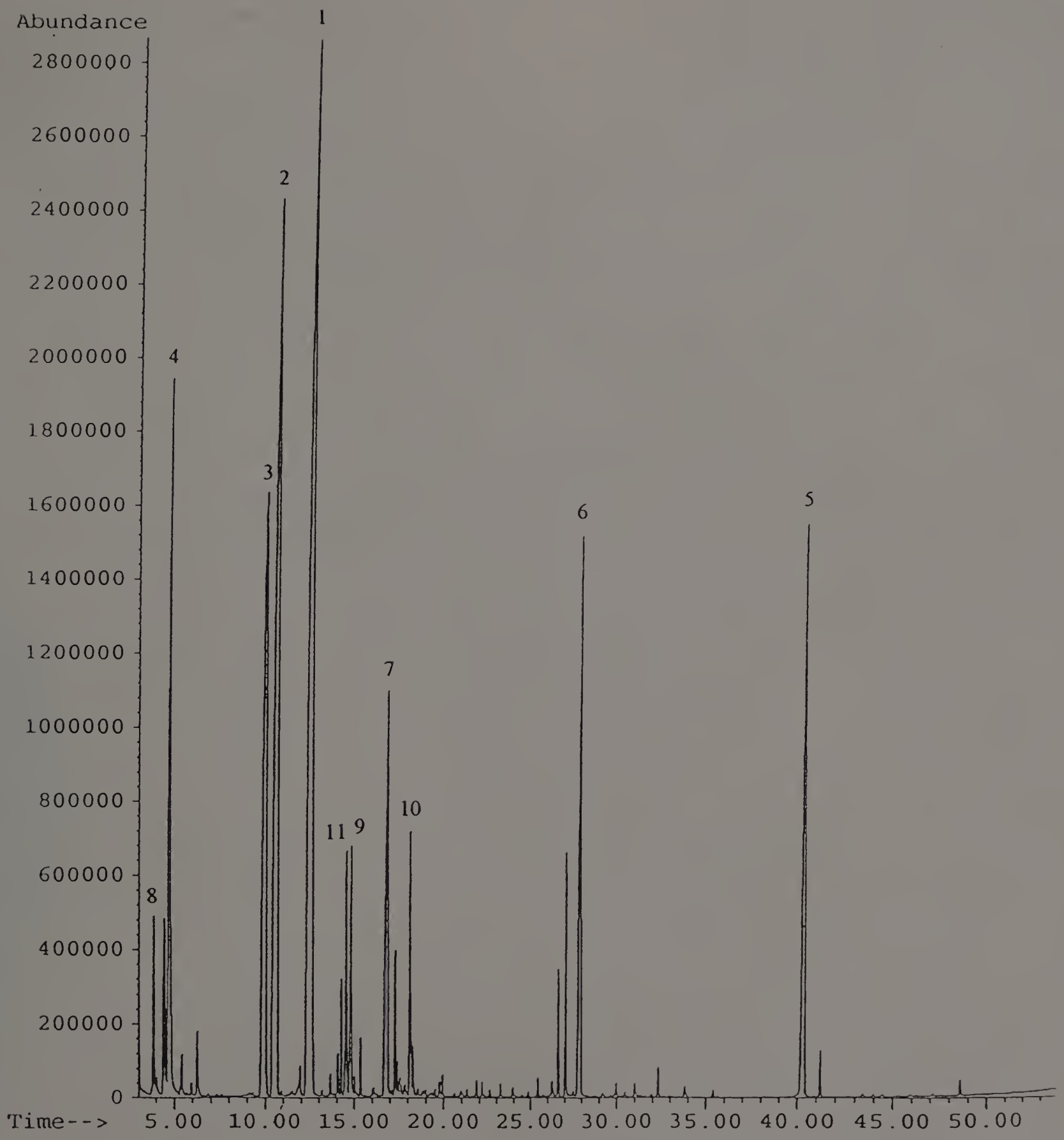

Figure 4.9. Total ion current chromatogram of sage grown at 100% of full sunlight.

1. Camphor, 2. (+)-Thujanone, 3. (-)-Thujanone, 4. 1,8 Cineole 5. Epimanool,

6. Dihydro-cis-, α-copanene-8-ol, 7. α-Caryohyllene, 8. Limonene, 9. ß-Caryophyllene,

10. Borneol, 11. Borneol, acetate (1S,2R,4S)-(-)-

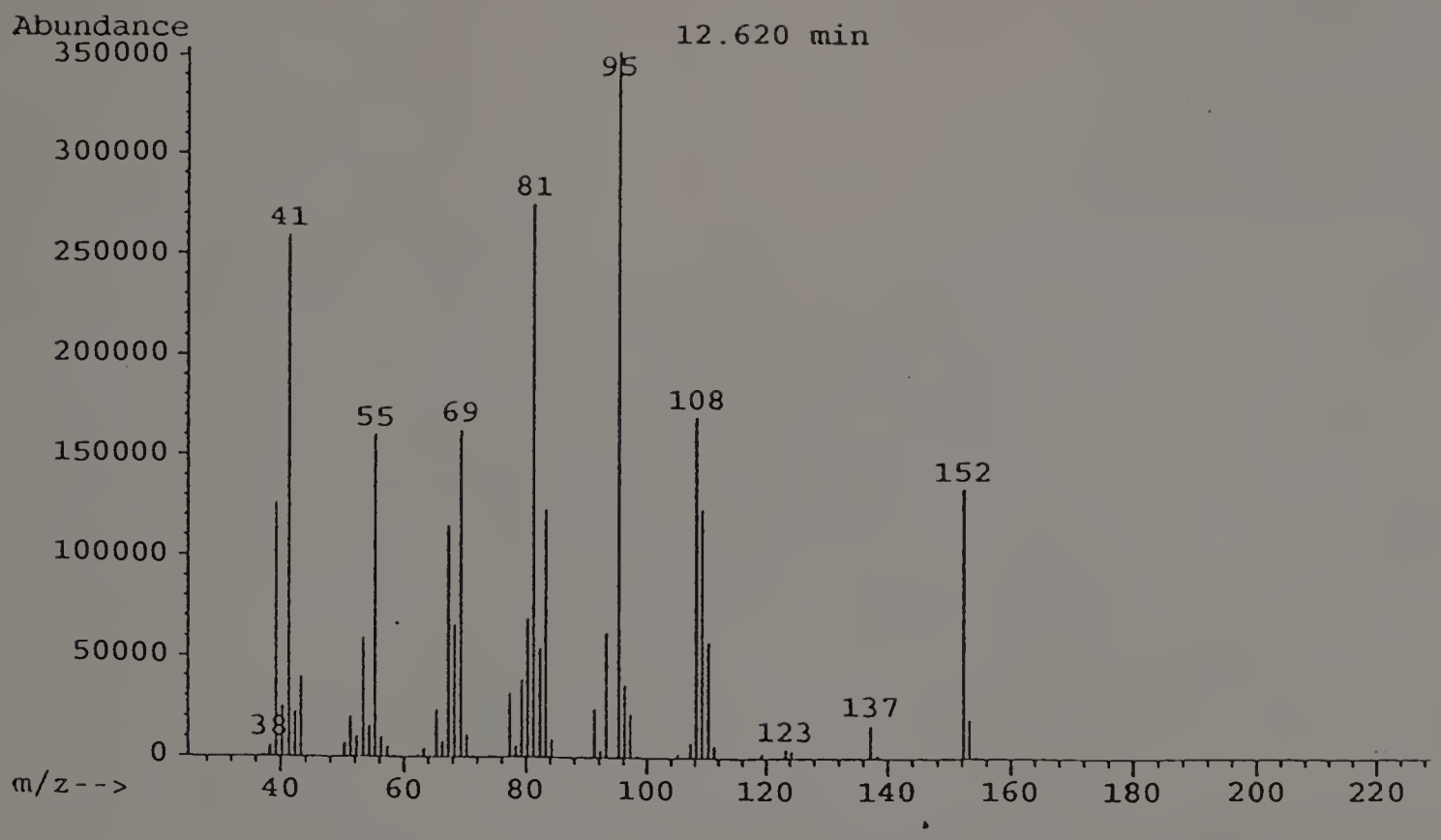

Figure 4.10. Mass spectrum of camphor in essential oil of sage at 100% of full sunlight.

A comparison of the effects of light level during growth on the 20 major compounds (content over 0.1% of total oil) of the essential oil of sage is presented in table 4. (+)-thujanone was significantly higher in sage grown at 45 % of full sunlight, but epimanool decreased with increase in light levels (Fig. 4.12, Appedix Table 23 &24). Camphor, as the largest compound, on a volume basis, was less at 45% of full sunlight than at 100% or 27% of full sunlight.

Fifty four compounds were identified in the essential oil of thyme. The compounds consisted of: 26 monoterpenes, 12 sesquiterpenes, 2 hydrocarbonyl compounds, 5 alcohols, 5 carbonylic acid, and 3 oxides, one of which was not identified (Fig. 4-12, Appendix Table 2). Some compounds were similar to sage, but some were different.

The greatest compound at retention time of 30.65 minutes was idtdified by GC/MS (Fig. 4.13). Its fragments 135 (100), 150 (43), 91 (28), and 115 (20) were very familiar to that of the other compound at 31.08 minutes 135 (100), 150 (39), 91 (20), and 115 (10) (Fig.4. 14). They are complex

Table 4.2. The content of major compounds of sage grown at different light levels.

Compounds of Essential Oil *	100% of full sunlight	45% of full sunlight	27% of full sunlight	15% of full sunlight
	(% of Total Oil)			
Camphor	26.32	23.61	24.88	19.76
(+)-Thujanone**	7.68	10.88	5.37	4.19
(-)-Thujanone	18.12	15.52	15.64	13.07
1,8 Cineole	7.18	7.65	7.07	7.11
Epimanool**	4.59	7.56	8.45	11.07
Dihydro-*cis*-, a-copanene-8-ol	4.49	3.55	3.24	3.11
α-Caryohyllene	4.69	4.55	4.67	4.84
Limonene	1.60	1.68	1.88	1.56
ß-Caryophyllene	1.76	1.30	1.14	0.99
Borneol	1.11	1.16	0.81	0.78
Borneol, acetate (1S,2R,4S)-(-)-	1.58	1.85	2.51	3.28
5-Ketoboryl acetate	0.84	1.12	1.22	1.01
ß-Myrcene	0.63	0.83	0.78	0.61
2-Hexenal	0.82	0.50	0.60	0.59
Isothujol	0.29	0.33	0.09	0.25
2-Methyl-2-((e)-4-methyl-1,3-pentadienyl)1,3-	0.44	0.45	0.49	0.24
Terpinolen, (p-metha-1,4(8)-diene)	0.48	0.55	0.56	0.41
ß-Linalool	0.37	0.31	0.30	0.20
Cyclohexene, 4-methylene-1-(1-methylethyl)	0.46	0.36	0.34	0.33
α-Terpine	0.29	0.30	0.29	0.28
	83.75	84.04	80.33	73.69

*: Compounds were identified by GC/MS.

**: There is significant difference at $p \leq 0.05$.

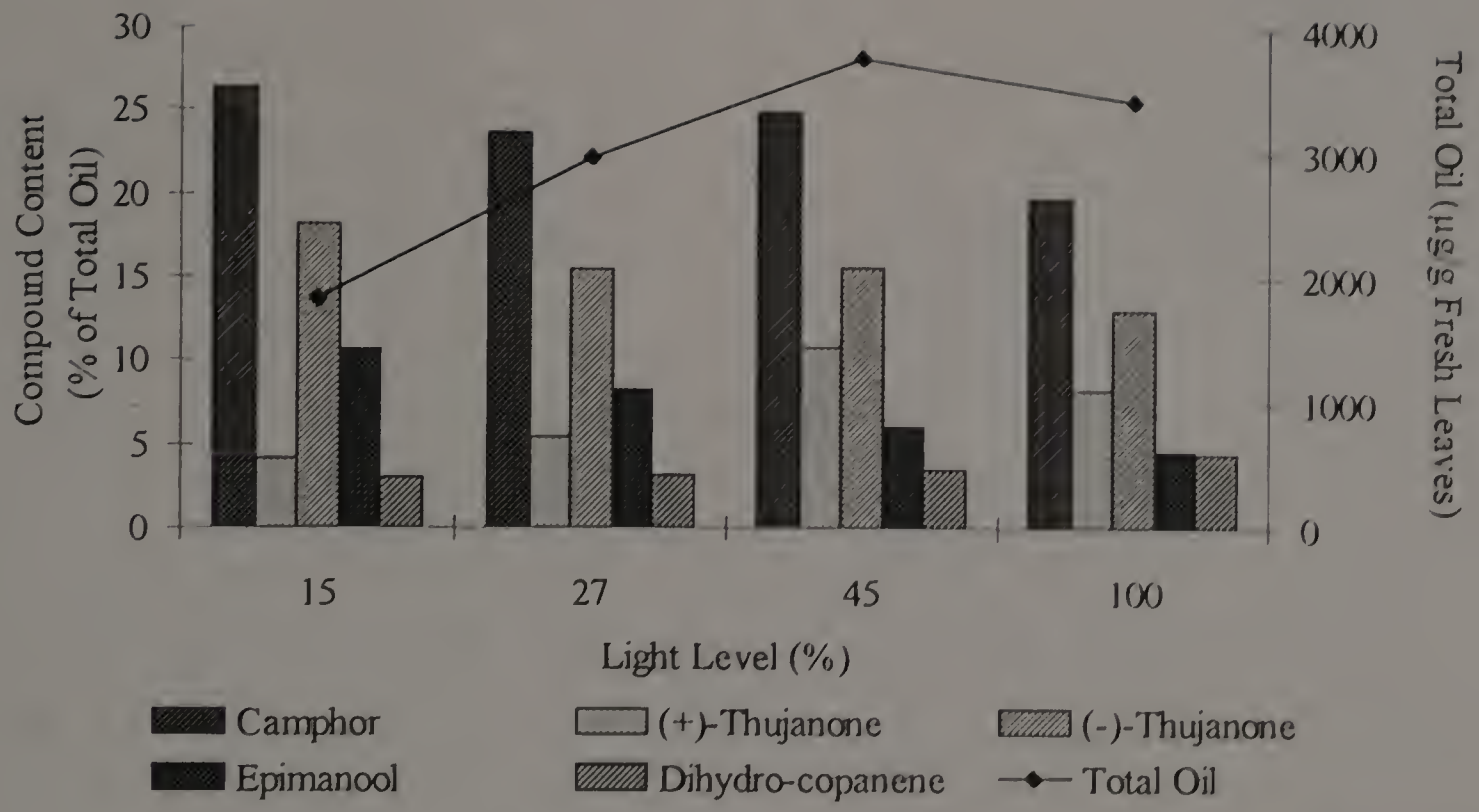

Figure 4.11. Total oil and content of major compounds of essential oil of sage grown at different light levels.

isomers, thymol and isothymol. Thymol was confirmed by determining the retention time of standard of thymol in GC analysis. Thymol at retention time of 30.65 minutes has molecular weight 150, and makes up 54% of essential oil in thyme.

The total oil concentration was highest in the plants grown at 100% of full light (0.49%, 4884 µg/g fresh leaves) and decreased significantly with decreasing light levels. The difference between the highest value (100% of full sunlight) and lower value (27% of full sun light) is about 5 times (Fig. 4-15)

Nineteen major compounds were detected and compared in the various light treatments (Table 5). The major compounds tended to follow similar trends as those of the total oil of thyme decreasing with reduced light levels. Thymol, the primary compound was about 10% higher at 100% of full light than at 27% of full light (Fig. 4-15).

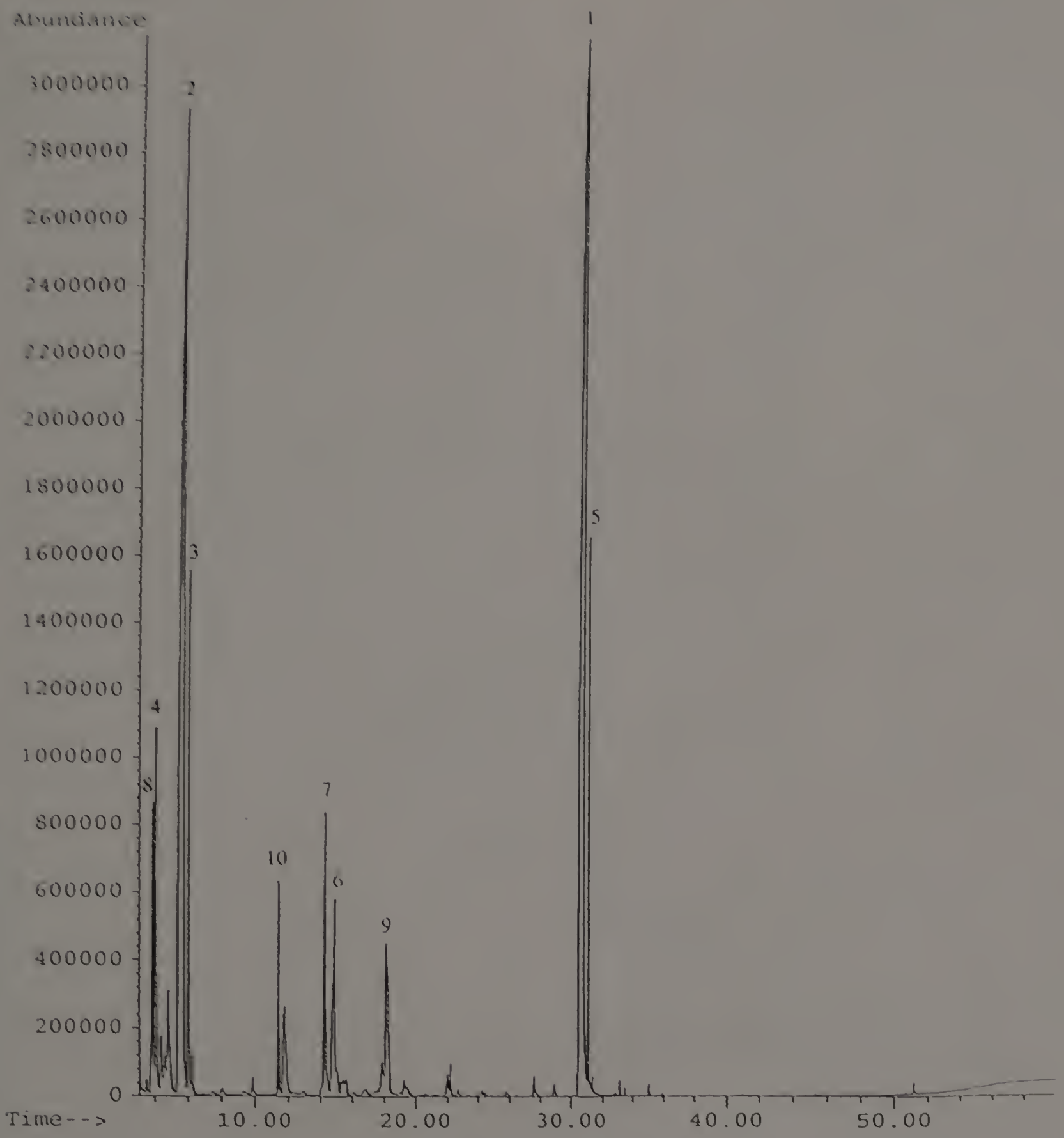

Figure 4.12. Total ion current chromatogram of thyme at grown at 100% of full sunlight .

1. Thymol, 2. g-Terpinene, 3. p-Cymene, 4. α-Terpinene, 5. Isothymol, 6. Caryophyllene, 7. ß-Linalool, 8. ß-Myrcene, 9. (-)-Borneol, 10. 2-Cyclohexen-1-ol, 1-methyl-4-(1-methethyl), trans.

Table 4.3. The content of major compounds of thyme grown at different light levels.

Compounds of Essential Oil *	100% of full sunlight	45% of full sunlight	27% of full sunlight	15% of full sunlight
	(% of Total Oil)			
Thymol**	52.75	45.95	43.45	42.73
γ-Terpinene**	21.09	21.96	18.87	14.42
p-Cymene**	3.39	5.50	8.53	12.98
α-Perpinene	2.55	2.57	2.54	2.41
Isothymol**	2.48	1.89	2.23	2.25
Caryophyllene	1.98	2.53	2.18	2.78
ß-Linalool	1.72	1.42	1.38	1.33
ß-Myrcene**	1.37	1.30	1.08	0.85
(-)-Borneol	1.09	1.26	1.42	1.33
2-Cyclohexen-1-ol, 1-methyl-4-(1-methethyl), trans,	1.01	0.89	0.93	0.94
1,8 Cineole	0.73	0.43	0.45	0.51
7-Octanen-4-ol	0.84	0.90	0.84	1.00
Bornеol	0.40	0.46	0.53	0.48
γ-Cadinene	0.33	0.32	0.38	0.40
D-Limonene	0.33	0.35	0.38	0.37
ß-Cis-ocimene	0.33	0.41	0.32	0.31
α-Fenchyl acetate	0.30	0.51	0.38	0.56
Phenol, 2-methy-5-(1-methylethyl)-, acetate	0.20	0.40	2.02	1.21
z-Carene	0.22	0.29	0.43	0.59
	93.10	89.35	88.35	87.43

*: Compounds were identified by GC/MS.

**: There is significant difference at $p \leq 0.05$.

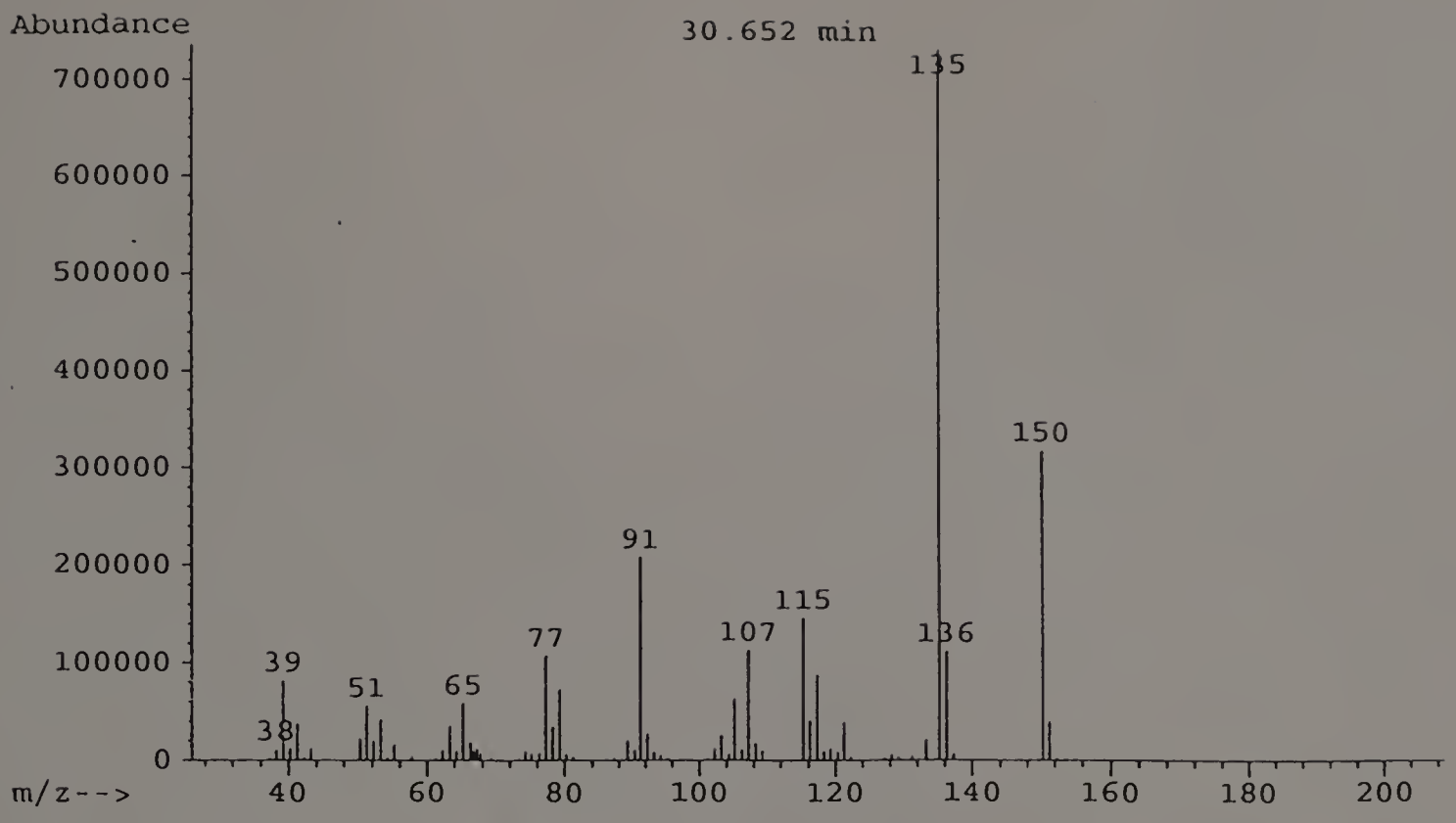

Figure 4.13. Mass spectrum of thymol in essential oil of thyme at 100% of full sunlight.

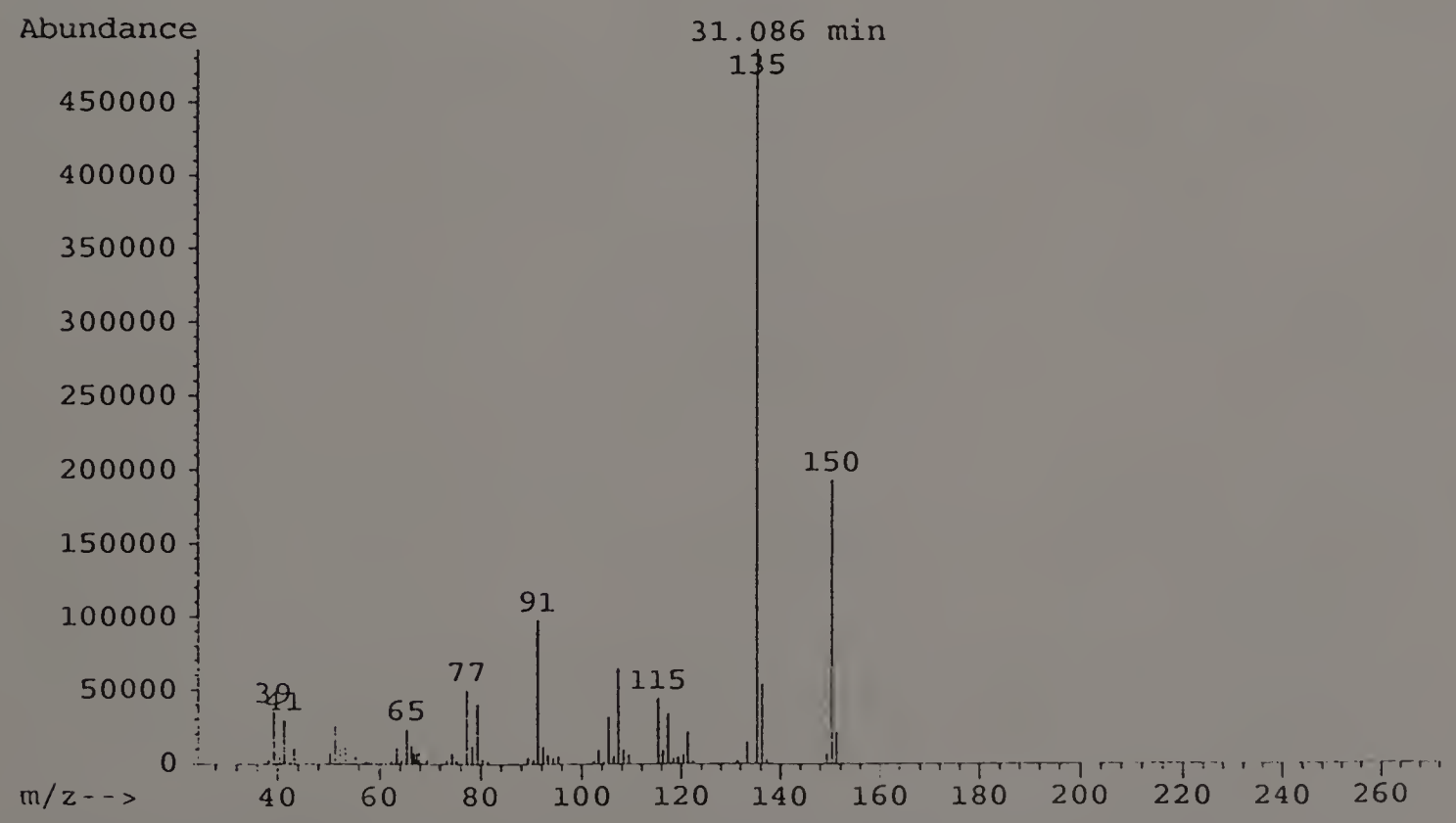

Figure 4.14. Mass spectrum of isothymol in essential oil of thyme at 100% of full sunlight.

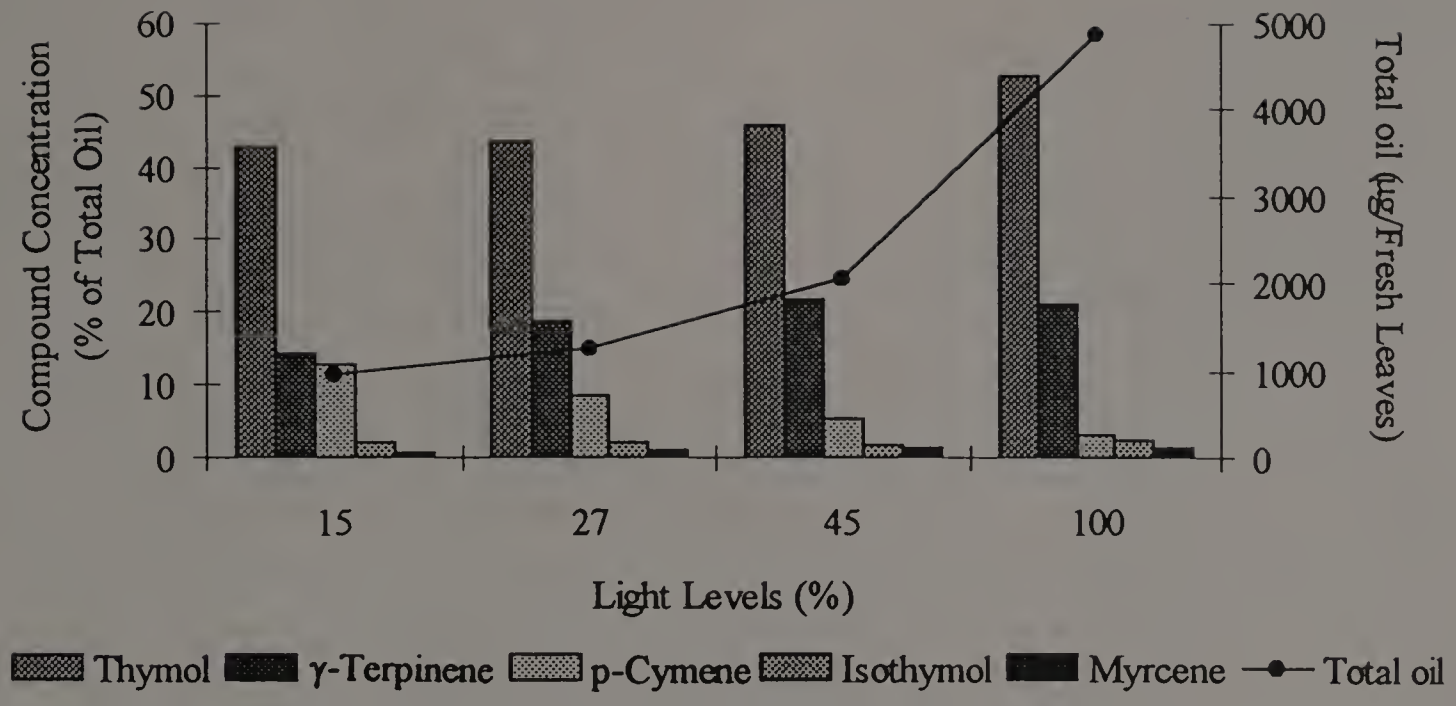

Figure 4.15. Total oil and content of major compounds of essential oil of thyme grown at different light levels.

CHAPTER V

DISCUSSION

The Effects of Light Levels on Herb Growth and Development

The effects of light levels on herb growth and development are very significant, and were observed in a short growth term. Treatment of sage and thyme with different light levels for about ten days, resulted in morphological responses to low light levels. The plant internodes lengthened and leaves were pale green.

Leave length, width and density of peltate hairs significantly decreased with decreasing light level. These results are consistent with reports of Hälvä in 1992. The height and internode length of plants grown at reduced light levels were larger than that at 100% of full sunlight. Sage grown at 27%, 45% and 100% of full sunlight produced similar yields, while plants grown at 15% of full sunlight yielded significantly less biomass.

Fresh weight per plant of thyme grown at low light levels were similar to those of plants grown at 100% of full sunlight, but their dry weights were different. The dry weights of plants grown at the two lower light levels were less than at the higher light levels.

Extended growth period of both herbs at different treatment light levels were based on the preliminary experiment. Sage and thyme grown for 50 and 45 days were treated with different level light, respectively, and harvested after15 and 10 days. When both of herbs grown at 45% of full sunlight increased 7 or 10 days of growth period, and that grown at 27% of full sunlight increased 15 or 20 days, plant reached the simillar fresh (sage and thyme) or dry weight (sage) with that grown at 100% of full sunlight. But if plants at 15% of full sunlight had be continousely increased growth period, the plants would be died. These plants also exhibited unhealthy symptoms, such as tender plant and yellow leaves, indicating that at 15% of full sunlight sage had a photosynthetic rate so low that it could barely grow.

The Effects of Light Levels on the Essential Oil Production

Sage and thyme had different responses to light level treatments in this experiment. The total oil concentration of sage tended to be highest in the plants grown at 45% of full sunlight and decreased at 100% or at 27% of full sunlight, even though all plants had similar biomass accumulation. These results have not been comfirmed by the current literature, and are very interesting because the response of essential oil components was clearly different from the responses of most of the morphological characteristics. Leaf area and density of peltate hairs continued to decrease as light level decreased. Low light levels affected not only accumulation of essential oil of sage but also plant biomass accumulation by initiating other physiologic and biochemical changes. The total oil concentration of sage grown at 15% of full light was the lowest, because of unhealthy plants.

The content of major compounds of essential oil of sage was not significantly different in the plants grown at different light levels expect for (+)-thujanone and epimanool. (+)-Thujanone was the highest in the plants grown at 45% of full sunlight in this experiment. Camphor, a toxic monoterpene in plant (Brown *et al*., 1978), appeared to have decreased at 45% of full sunlight, although the differences among the four light levels were not statistically significant (ANOVA, $P > 95\%$).

Essential oil production in thyme responded differently to light level. The total oil concentration and some of the major compounds of essential oil were highest in plants grown at 100% of full light, and then decreased with decreasing light levels, regardless of dried weight. These results were consistent with that of Yamaura *et al*. (1989).

Not only did light level affect total essential oil accumulation in thyme, but light level also affected the components of thyme oil. Thymol and myrcene, as the major compounds in thyme oil, significantly increased as light levels increased, and reached highest value at 100% of full sunlight. The high light level, however, depressed p-cymene accumulation. However, accumulation of isothymol and γ-terpene were not directly affected by light levels. Thymol, isothymol, myrcene, ß-cymene and γ-

trepene are all important monoterpenes in plants, and thyme could be considered an appropriate material for studying the effect of light on monoterpene accumulation in plants.

The Relations among Herb Yields, Growth Stage and Essential Oil Accumulation

In some studies, such as the dill studies of Hälvä *et al.* (1992b), herb yield and essential oil production were determined as plants were harvested prior to flower bud formation. These researchers reported that essential oil production was directly related to biomass production and thus to photosynthesis because both herb yields and essential oil production increased with increases in light level. In this experiment, however, we did not observed this direct relationship between herb yields, essential oil production and light levels. Both herbs did not have the same essential oil accumulation as plant yielded similar biomass at different light treatment. It would appear that the effect of light level on the production of essential oil does not depend simply on accumulation of biomass.

In this experiment, the essential oil of sage and thyme were analyzed in a short vegetative growth term in which the two herbs were treated with different light levels. Accumulation of essential oil in the two herbs directly depended on light levels, regardless of whether the yields were similar or not. This result is different from the studies on dill by Hälvä *et al.* (1992b). It is possible the growth stage as well as light level affect the essential oil production of these herbs.

The Relations among Leaf Area , Peltate Hairs and Essential Oil Accumulation

Leaf area and density of peltate hairs are major parameters effecting essential oil production in herbs. As in the case of dill (Hälviä *et al.*, 1992b), total essential oil of thyme increased with leaf area, however, the accumulation of sage oil did not respond in the same way, the highest total oil of sage occurred at 45% of full sunlight, but leaf area cintinued to increase with the largest leaves at 100% of full sunlight.

Peltate hairs are important site of synthesizing and accumulating essential oil in herbs, especially for accumulation of monoterpene, sesquiterpene or diterpene (Gershenzon, *et al.* 1989; Werker *et al.*, 1985a, b & c; Yamaura *et al.*, 1989). Bosablidis & Tsekos (1982) observed that the content of essential oil and the number of peltate hairs were higher in flowers than leaves. In this experiment, the density of peltate hairs of both sage and thyme significantly increased as light level increased, the highest value occurred at 100% of full sunlight. Thyme produced its essential oil in the same way; a response reported earlier by Yamaura *et al.* (1989). In sage, however, essential oil accumulation and peltate hair density did not reach the highest point at the same light level. The highest of total essential oil accumulated in plants grown at 45%, rather than 100% of full sunlight. This probable indicates that essential oil production in sage does also not simply depend on the number of peltate hairs of leaves. Peltate hairs concentrate the enzyme catalyzing the reaction of accumulating monoterpene (Gershenzon, *et al.* 1989). It is possible that the effect of light level on the accumulation of essential oil depends on the enzyme activity.

The Relations between Various Herb and Essential Oil Accumulation

From the observation and analysis above, the effects of light level on essential oil production are different between sage and thyme regardless of their biomass accumulation. The appropriate light level of thyme is at 100% of full sunlight, and for sage 45% of full sunlight. The concentration of essential oil at hearvest in dill grown at different light levels increases with light levels (Hälvä *et al.*, 1992b), but in Japanese mint is not found light level affected essential oil yields also at final harvest (Duriyaprapan & Britten, 1982). These differences in response to light level for essential oil production seam to depend on herb species, and are basically determined by herb genetic factors.

APPENDIX A

THE COMPOUNDS OF ESSENTIAL OIL IDENTIFIED BY GC/MS

Table A.1. The compounds of essential oil identified by GC/MS in sage grown at 100% of full sunlight.

Pk#	Retention Time (Min)	Compounds of Essential Oil	Molecular Formula	% of Total Oil
1	12.626	Camphor	$C_{10}H_{16}O$	28.015
2	10.655	(+)-Thujanone	$C_{10}H_{16}O$	19.997
3	10.006	(-)-Thujanone	$C_{10}H_{16}O$	11.892
4	4.749	1,8 Cineole	$C_{10}H_{18}O$	8.148
5	40.381	Epimanool	$C_{20}H_{34}O$	6.056
6	27.933	Dihydro-cis-, α-copanene-8-ol	$C_{15}H_{26}O$	4.875
7	16.874	α-Caryohyllene	$C_{15}H_{24}$	4.592
8	4.424	Limonene	$C_{10}H_{16}$	1.797
9	14.818	ß-Caryophyllene	$C_{10}H_{24}$	1.766
10	18.134	Borneol	$C_{10}H_{18}O$	1.635
11	14.530	Borneol, acetate (1S,2R,4S)-(-)-	$C_{12}H_{20}O$	1.349
12	27.102	5-Ketoboryl acetate	$C_{12}H_{18}O$	1.155
13	3.873	ß-Myrcene	$C_{10}H_{16}$	0.908
14	4.812	2-Hexenal	$C_6H_{10}O$	0.874
15	17.296	Isothujol	$C_{10}H_{18}O$	0.633
16	26.636	2-Methyl-2-((e)-4-methyl-1,3-pentadienyl)1,3-	$C_{10}H_{16}O_2$	0.557
17	6.251	Terpinolen	$C_{10}H_{16}$	0.509
18	14.241	ß-Linalool	$C_{10}H_{18}O$	0.410
19	4.039	α-Terpine	$C_{10}H_{16}$	0.338

20	5.408	γ-Terpine	$C_{10}H_{16}$	0.288
21	11.920	2-Cyclohexene-1-ol, 1-methyl-4-(1-methylethyl)	$C_{10}H_{18}O$	0.254
22	41.223	α-Himachalene	$C_{15}H_{24}$	0.239
23	15.343	(-)-Terpinene-4-ol	$C_{10}H_{18}O$	0.223
24	17.551	(+)-Ledene	$C_{15}H_{24}$	0.198
25	17.434	α-Terpineol	$C_{10}H_{18}O$	0.185
26	14.042	2-Cyclohexene-1-ol,1-methyl-4-(1-methyl)-trans	$C_{10}H_{18}O$	0.181
27	16.060	(-)-Alloaromadendrene	$C_{15}H_{24}$	0.123
28	26.252	12-Oxabicyclo(9,1,0)dodeca-3,7-diene, 1,5,5,8-tetramethyl-	$C_{15}H_{24}O$	0.115
29	5.922	p-Cimene	$C_{11}H_{18}O_2$	0.111
30	45.910	Unknown		0.108
31	35.395	Ocimene	$C_{10}H_{16}$	0.107
32	13.633	Dihydrocarveyl,acetate	$C_{12}H_{20}O_2$	0.105
33	15.001	Dihyrocrveol acetate	$C_{12}H_{20}O_2$	0.102
34	31.006	Thymol	$C_{10}H_{14}O$	0.096
35	19.927	Cyclohexanol, 2-methylene-3-(1-methylethenyl)-acetate,cis-	$C_{12}H_{18}O_2$	0.091
36	10.882	Linalool Oxide	$C_{10}H_{18}O$	0.088
37	30.448	Isothymol	$C_{10}H_{14}O$	0.086
38	16.252	Unknown		0.085
39	25.425	Spiro (5,6) dodecane	$C_{12}H_{22}$	0.084
40	32.324	α-Humulene	$C_{15}H_{24}$	0.083
41	11.452	Amyl vinyl carbinol	$C_{10}H_{14}O$	0.080
42	17.847	3,3,6-Trimetryl-1,5-heptadien-4-ol	$C_{10}H_{18}O$	0.080
43	23.228	p-metha-1(7),8(10) dien-9-ol,	$C_{10}H_{16}O$	0.076
44	18.810	Citral	$C_{10}H_{16}O$	0.072
45	23.946	Thujaketone	$C_9H_{16}O$	0.067
46	29.971	Bicyclo(7,2,0) andec-4-ene, 4,11,11-trimethyl-8-methylene-	$C_{15}H_{24}$	0.058

47	18.550	3-Hexen-1-ol, acetate, (e)	$C_8H_{14}O_2$	0.058
48	5.310	Trans-Ocimeme	$C_{10}H_{16}$	0.053
49	13.153	Isocamphopinone	$C_{10}H_{14}O$	0.052
50	19.747	Geraniol acetate	$C_{12}H_{20}O_2$	0.050
51	19.305	2-Cyclohexen-1-one, 3-(1,3-butadienyl)-	$C_{14}H_{20}O$	0.047
52	17.811	3,3,6-Trimethyl-1,5-heptadien-4-ol	$C_{10}H_{18}O$	0.045
53	12.848	d-Camphor	$C_{10}H_{16}O$	0.044
54	21.835	trans-Carveol	$C_{10}H_{16}O$	0.043
55	15.111	Camphene hydrate	$C_{10}H_{18}O$	0.041
56	24.846	6-(3-methyl-3-cyclohexenyl)-2-methyl-2,6,-Heptalenol	$C_{15}H_{24}O$	0.039
57	18.891	Geranyl, 2-methylbutyrate	$C_{15}H_{26}O$	0.037
58	22.253	Geranyl acetone	$C_{13}H_{22}O$	0.036
59	9.293	3-Hexen-1-ol, (e)	$C_6H_{12}O$	0.032
60	3.776	α-Phellandrene	$C_{10}H_{16}$	0.031
61	5.664	Cis-Ocimene	$C_{10}H_{16}$	0.031
62	9.100	Bicyclo(3,2,1) Octane, 2,3-bis(methylene)-	$C_{10}H_{14}$	0.031
63	19.487	(Bicyclopentyl)-1-ol	$C_{10}H_{18}O$	0.031
64	44.463	Peruviol	$C_{15}H_{26}O$	0.031
65	18.971	Trans-carveyl acetate	$C_{12}H_{18}O_2$	0.029
66	17.096	Isobornyl alcohol	$C_{10}H_{18}O$	0.027
67	43.960	Cyclopentaneaceta (dehyde, 2-formyl-3-methyl-a-methylene	$C_{10}H_{14}O$	0.025
68	43.408	γ-Palmitolactone	$C_{16}H_{30}O_2$	0.024
69	13.845	Bicyclo(2,2,1) heptan-7-one, 2-(1-methylethylethylidene)-	$C_{10}H_{14}O$	0.023
70	33.819	Isocaryophyllene	$C_{15}H_{24}$	0.022
71	29.860	Phenol, 2-methyl-5-(1-methylethyl)-, acetate	$C_{12}H_{16}O_2$	0.020
72	25.711	1,6-octadien-3-ol,3,7-dimethyl-,2-amino-	$C_{17}H_{23}NO_2$	0.019
73	47.130	Sandaracopimar-15-en-8-ß-yl-acetate	$C_{22}H_{36}O_2$	0.019

74	6.823	Cyclopentane, (1,2,4-trimethyl-(1α,2α,4ß) ---	C_8H_{16}	0.018
75	21.290	p-metha-1,8-dien-3-one, (+)	$C_{10}H_{14}O$	0.017
76	22.147	Benenmethanol, α,α,4,-trimethyl-	$C_{10}H_{14}O$	0.016
77	48.584	Caryophyllene oxide	$C_{15}H_{24}O$	0.014
78	7.294	4-Hexen-1-ol,acetate, (z)	$C_8H_{14}O_2$	0.012
79	17.018	Unknown		0.012
80	20.584	Myrtenol	$C_{10}H_{16}O$	0.012
81	31.928	Cis-(-)- 2,4α,5,6,9α,-Hexahydro-3,5,5,9-tetramethyl (1H) benzocycloheptene	$C_{15}H_{24}$	0.012
82	24.467	Cyclohexane,1-methyl-2,4, bis(1-methylthenyl)-	$C_{13}H_{22}$	0.011
83	29.622	Dipentene oxide	$C_{10}H_{16}O_2$	0.011
84	22.599	p-metha-6,8-dien-2-ol, cis	$C_{10}H_{16}O$	0.009
85	29.185	8α (2H) -Phenanthrenol, 7-ethenyl-	$C_{22}H_{36}O_2$	0.007
86	20.941	4(10)-Thijen-3-ol, (stereoisomer)	$C_{10}H_{16}O$	0.006
87	27.484	1,6,9-Tetradecatriene	$C_{14}H_{24}$	0.006
88	3.353	3-Hexenal,(z)-(z)	$C_6H_{10}O$	0.004
89	21.024	3-Octen-2-one, 7-methyl-,	$C_{10}H_{16}O$	0.004
90	27.255	1-Hydroxylinalool	$C_{10}H_{18}O$	0.001

Table A.2. The compounds of essential oil identified by GC/MS in thyme grown at 100% of full sunlight.

Pk#	Retention Time (Min)	Compounds of Essential Oil	Molecular Formula	% of Total Oil
1	30.646	Thymol	$C_{10}H_{14}O$	54.363
2	5.695	γ-Terpinene	$C_{10}H_{16}$	21.721
3	6.048	p-Cymene	$C_{10}H_{14}$	3.829
4	4.078	α-Terpinene	$C_{10}H_{16}$	2.602
5	31.083	Isothymol	$C_{10}H_{14}O$	2.588
6	14.87	Caryophyllene	$C_{15}H_{24}$	2.144
7	14.285	ß-Linalool	$C_{10}H_{18}O$	1.926
8	3.891	ß-Myrcene	$C_{10}H_{16}$	1.493
9	18.125	(-)-Borneol	$C_{10}H_{18}O$	1.354
10	11.746	2-Cyclohexen-1-ol, 1-methyl-4-(1-methethyl), trans,	$C_{10}H_{18}O$	1.091
11	4.836	1,8 Cineole	$C_{10}H_{18}O$	0.927
12	11.409	7-Octanen-4-ol	$C_{10}H_{16}O$	0.917
13	31.339	(+) Cedrol	$C_{15}H_{26}O$	0.586
14	18.217	Borneol	$C_{10}H_{18}O$	0.491
15	17.827	γ-Cadinene	$C_{15}H_{24}$	0.404
16	4.393	D-Limonene	$C_{10}H_{16}$	0.344
17	5.757	ß-cis-Ocimene	$C_{10}H_{16}$	0.328
18	14.998	α-Fenchyl acetate	$C_{12}H_{20}O$	0.290
19	22.213	Phenol, 2-methy-5-(1-methylethyl)-, acetate	$C_{12}H_{20}O$	0.236
20	6.268	z-Carene	$C_{10}H_{16}$	0.184
21	3.483	3-Carene	$C_{10}H_{16}$	0.159
22	19.46	γ-Cadinene	$C_{15}H_{24}$	0.149
23	4.599	ß-Thujene	$C_{10}H_{16}$	0.139
24	27.589	(-)-Globulol	$C_{15}H_{26}O$	0.131

25	22.003	Geranyl propionate	$C_{13}H_{12}O$	0.125
26	28.927	Champacol	$C_{15}H_{26}O$	0.122
27	17.618	Benzofuran, 4,7-dimethyl-	$C_{10}H_{10}O$	0.115
28	19.248	Citral	$C_{10}H_{16}O$	0.108
29	9.866	3-Octanol	$C_8H_{18}O$	0.098
30	12.957	(-)-Camphor	$C_{15}H_{26}O$	0.093
31	32.802	3-Tridencen-1-yne, (e)-	$C_{13}H_{22}$	0.084
32	45.284	Unknown		0.075
33	15.578	1-Terpinen-4-ol	$C_{10}H_{18}O$	0.071
34	6.197	Prenyl acetate	$C_7H_{12}O$	0.070
35	31.213	Bulnesol	$C_{15}H_{26}O$	0.058
36	17.418	α-Terpineol	$C_{10}H_{18}O$	0.054
37	33.125	(e,z)-Farnesol	$C_{16}H_{27}O$	0.051
38	8.028	Brenyl acetate	$C_7H_{12}O_2$	0.046
39	24.255	(+)-Nerolidol	$C_{15}H_{26}O$	0.046
40	16.32	ß-Cubebene	$C_{15}H_{24}$	0.046
41	18.991	(+)-Carvone	$C_{10}H_{14}O$	0.038
42	27.751	Acoradien	$C_{15}H_{24}$	0.035
43	15.25	1-Isopropyl-2-methoxy-4-methylbenzene	$C_{11}H_{16}O$	0.034
44	12.196	(+) Camphor	$C_{10}H_{16}O$	0.033
45	24.449	Cyclopentaneacetataldehyde, 2-formyl-3-methyl-α-methylene-	$C_{10}H_{14}O_2$	0.028
46	7.453	1-Pentene,5-butoxy-	$C_9H_{18}O$	0.025
47	16.756	α-Caryophyllene	$C_{15}H_{24}$	0.024
49	33.466	(e,e)-Farnesol	$C_{15}H_{26}O$	0.020
48	22.693	Lemonol	$C_{10}H_{18}O$	0.020
50	9.289	4-Hexen-1-ol, (z)	$C_6H_{12}O$	0.019
51	16.884	Chavical methyl ether	$C_{10}H_{12}O$	0.017
52	35.906	cis-z-Pinanol	$C_{11}H_{18}O$	0.014
53	15.363	(-)-4-Terpineol	$C_{11}H_{18}O$	0.013
54	24.1	Geranyl isobutyrate	$_C14H_{24}O_2$	0.011
55	35.055	1-Nonadecanol	$C_{19}H_{24}O$	0.010

APPENDIX B

ANALYSIS OF VARIANCE FOR YIELDS, GROWTH CHARACTERISTICS AND ESSENTIAL OIL

Table B.1. Analysis of variance for total fresh weight of sage.

Source	DF	SS	MS	F	P
Block (A)	2	92.1083	46.0542	0.69	0.5051
Treast (B)	3	1970.35	656.782	9.88	0.0000
A*B	6	255.192	42.5319	0.64	0.6978
Sample (C)					
A*B*C	48	3191.00	66.4792		
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Total	59	5508.65			
Grand Average	1	76862.6			

Table B.2. Analysis of variance for total dried weight of sage.

Source	DF	SS	MS	F	P
Block (A)	2	13.8502	6.92511	1.11	0.3364
Treast (B)	3	117.799	39.2662	6.32	0.0011
A*B	6	38.0176	6.33626	1.02	0.4240
Sample (C)					
A*B*C	48	298.275	6.21407		
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Total	59	467.942			
Grand Average	1	1848.37			

Table B.3. Analysis of variance for fresh leaf weight of sage.

Source	DF	SS	MS	F	P
Block (A)	2	17	8.82917	0.34	0.7147
Treast (B)	3	617.500	205.833	7.89	0.0002
A*B	6	83.2750	13.8792	0.53	0.7814
Sample (C)					
A*B*C	48	1253.00	26.1042		
Total	59	1971.43			
Grand Average	1	23920.1			

Table B.4. Analysis of variance for dried leaf weight of sage.

Source	DF	SS	MS	F	P
Block (A)	2	10.4046	5.20232	0.91	0.4102
Treast (B)	3	75.6405	25.2135	4.40	0.0082
A*B	6	37.2681	6.21136	1.08	0.3855
Sample (C)					
A*B*C	48	275.066	5.73054		
Total	59	398.379			
Grand Average	1	654.787			

Table B.5. Analysis of variance for plant height of sage.

Source	DF	SS	MS	F	P
Block (A)	2	59.4667	29.7333	0.49	0.6188
Treast (B)	3	5754.87	1918.29	31.70	0.0000
A*B	6	178.933	29.8222	0.49	0.8139
Sample (C)					
A*B*C	108	6534.60	60.5056		
Total	119	12527.9			
Grand Average	1	3.179E+05			

Table B.6. Analysis of variance for internode length of sage.

Source	DF	SS	MS	F	P
Block (A)	2	0.27248	0.13624	0.12	0.8903
Treast (B)	3	33.9866	11.3289	9.69	0.0000
A*B	6	8.23893	1.37315	1.17	0.3358
Sample (C)					
A*B*C	108	56.1320	1.16942		
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Total	119	98.6300			
Grand Average	1	1184.89			

Table B.7. Analysis of variance for numbers of internode of sage.

Source	DF	SS	MS	F	P
Block (A)	2	3.05000	1.52500	1.03	0.3630
Treast (B)	3	91.2333	30.4111	20.48	0.0000
A*B	6	6.61667	1.10278	0.74	0.6184
Sample (C)					
A*B*C	108	160.400	1.48519		
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Total	119	261.300			
Grand Average	1	11642.7			

Table B.8. Analysis of variance for numbers of branche of sage.

Source	DF	SS	MS	F	P
Block (A)	2	11.3167	5.65833	1.25	0.2904
Treast (B)	3	318.767	106.256	23.47	0.0000
A*B	6	36.8833	6.14722	1.36	0.2375
Sample (C)					
A*B*C	108	489.000	4.52778		
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Total	119	15008.0			
Grand Average	1				

Table B.9. Analysis of variance for numbers of leaves of sage.

Source	DF	SS	MS	F	P
Block (A)	2	431.717	215.858	0.89	0.4146
Treast (B)	3	9932.16	3310.72	13.71	0.0000
A*B	6	2877.42	479.569	1.99	0.0732
Sample (C)					
A*B*C	108	26071.7	241.405		
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Total	119	39313.0			
Grand Average	1	7.052E+05			

Table B.10. Analysis of variance for leaf length of sage.

Source	DF	SS	MS	F	P
Block (A)	2	7.63117	3.81558	1.11	0.3338
Treast (B)	3	189.865	63.2883	18.42	0.0000
A*B	6	16.7708	2.79514	0.81	0.5632
Sample (C)					
A*B*C	108	371.053	3.43568		
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Total	119	585.320			
Grand Average	1	21064.9			

Table B.11. Analysis of variance for leaf width of sage.

Source	DF	SS	MS	F	P
Block (A)	2	0.03317	0.01658	0.07	0.9249
Treast (B)	3	24.7770	8.25900	34.15	0.0000
A*B	6	0.81550	0.13592	0.56	0.7615
Sample (C)					
A*B*C	108	26.1180	0.24183		
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Total	119	51.7437			
Grand Average	1	867.256			

Table B.12. Analysis of variance for numbers of peltate hair / mm^2 of sage.

Source	DF	SS	MS	F	P
Treat (A)	3	1217.08	405.692	45.17	0.0000
Sample (B)	2				
A*B	6	323.300	8.98056		
Total	39	1540.38			
Grand Average	1	5880.63			

Table B.13. Analysis of variance for total fresh weight of thyme.

Source	DF	SS	MS	F	P
Block (A)	2	123.033	61.5167	1.20	0.3106
Treast (B)	3	176.850	58.9500	1.15	0.3392
A*B	6	462.600	77.1000	1.50	0.1978
Sample (C)					
A*B*C	48	2464.20	51.3375		
Total	59	3226.68			
Grand Average	1	93062.8			

Table B.14. Analysis of variance for total dried weight of thyme.

Source	DF	SS	MS	F	P
Block (A)	2	3.24424	1.62212	1.38	0.2605
Treast (B)	3	36.0702	12.0234	10.26	0.0000
A*B	6	20.3435	3.39059	2.89	0.0173
Sample (C)					
A*B*C	48	56.2700	1.17229		
Total	59	115.928			
Grand Average	1	1513.63			

Table B.15. Analysis of variance for height of thyme.

Source	DF	SS	MS	F	P
Block (A)	2	77.4000	38.7000	2.29	0.1038
Treast (B)	3	2099.67	699.889	41.44	0.0000
A*B	6	351.733	58.6222	3.47	0.0037
Sample (C)					
A*B*C	108	1824.00	16.8889		
Total	119	4352.80			
Grand Average	1	1.51E+05			

Table B.16. Analysis of variance for internode length of thyme.

Source	DF	SS	MS	F	P
Block (A)	2	0.13919	0.06959	0.08	0.9172
Treast (B)	3	13.4941	4.49803	5.03	0.0028
A*B	6	5.37526	0.89588	1.00	0.4287
Sample (C)					
A*B*C	108	96.5948	0.89440		
Total	119	115.603			
Grand Average	1	315.684			

Table B.17. Analysis of variance for leaf length of thyme.

Source	DF	SS	MS	F	P
Block (A)	2	0.01679	0.00840	0.28	0.7562
Treast (B)	3	2.65956	0.88652	30.07	0.0000
A*B	6	0.41938	0.06990	2.37	0.0342
Sample (C)					
A*B*C	108	3.18425	0.02948		
Total	119	6.27998			
Grand Average	1	81.7575			

Table B.18. Analysis of variance for leaf width of thyme.

Source	DF	SS	MS	F	P
Block (A)	2	0.03050	0.01525	1.57	0.2109
Treast (B)	3	0.73256	0.24419	25.15	0.0000
A*B	6	0.04350	0.00725	0.75	0.6152
Sample (C)					
A*B*C	108	1.04875	0.00971		
Total	119	1.85531			
Grand Average	1	22.3172			

Table B.19. Analysis of variance for number of peltate hairs / mm^2 of thyme.

Source	DF	SS	MS	F	P
Treat (A)	3	74.2750	24.7583	10.65	0.0000
Sample (B)					
A*B	36	83.7000	2.32500		
Total	39	157.975			
Grand Average	1	1199.03			

Table B.20. Analysis of variance for total oil concentration of sage.

Source	DF	SS	MS	F	P
Treat (A)	3	6.429E+06	2.143E+06	6.26	0.028
Sample (B)	2	1.166E+06	5.832E+05	1.70	0.2594
A*B	6	2.053E+06	3.422E+05		
Total	11	9.648E+06			
Grand Average	1	1.083E+08			

Table B.21. Analysis of variance for (+)-tujanone in sage oil.

Source	DF	SS	MS	F	P
Treat (A)	3	135.869	45.2898	6.04	0.0304
Sample (B)	2	61.9352	30.9676	4.13	0.0746
A*B	6	45.0231	7.50384		
Total	11	242.828			
Grand Average	1	366.639			

Table B.22. Analysis of variance for epimanol in sage oil.

Source	DF	SS	MS	F	P
Treat (A)	3	3.49380	1.16460	11.07	0.0074
Sample (B)	2	0.22002	0.11001	1.5	0.4079
A*B	6	0.63145	0.10524		
Total	11	4.34527			
Grand Average	1	155.232			

Table B.23. Analysis of variance for total oil concentration of thyme.

Source	DF	SS	MS	F	P
Treat (A)	3	2.886E+07	9.621E+06	25.89	0.0008
Sample (B)	2	2.322E+06	1.161E+06	3.12	0.1175
A*B	6	2.230E+06	3.716E+05		
Total	11	3.342E+07			
Grand Average	1	6.315E+07			

Table B.24. Analysis of variance for thymol in thyme oil.

Source	DF	SS	MS	F	P
Treat (A)	3	189.450	62.4834	13.61	0.0044
Sample (B)	2	22.7195	11.3598	2.47	0.1646
A*B	6	27.5484	4.59140		
Total	11	237.718			
Grand Average	1	25631.8			

Table B.25. Analysis of variance for isohymol in thyme oil.

Source	DF	SS	MS	F	P
Treat (A)	3	0.53007	0.17669	8.60	0.0136
Sample (B)	2	0.01832	0.00916	0.45	0.6600
A*B	6	0.12328	0.02055		
Total	11	0.67167			
Grand Average	1	58.7861			

Table B.26. Analysis of variance for myrcene in thyme oil.

Source	DF	SS	MS	F	P
Treat (A)	3	0.49882	0.16627	11.30	0.0070
Sample (B)	2	0.13982	0.06991	4.75	0.0579
A*B	6	0.08825	0.01471		
Total	11	0.72689			
Grand Average	1	15.8470			

Table B.27. Analysis of variance for p-cymene in thyme oil.

Source	DF	SS	MS	F	P
Treat (A)	3	156.064	52.0214	33.77	0.0004
Sample (B)	2	6.11252	3.05626	1.98	0.2181
A*B	6	9.24348	1.54058		
Total	11	171.420			
Grand Average	1	692.360			

Table B.28. Analysis of variance for γ-terpinene in thyme oil.

Source	DF	SS	MS	F	P
Treat (A)	3	102.419	34.1398	10.56	0.0083
Sample (B)	2	0.50180	0.25090	0.08	0.9262
A*B	6	19.3903	3.23071		
Total	11	122.311			
Grand Average	1	4369.70			

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