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THESIS

EFFECT OF ENVIRONMENT ON BIOLOGICAL ACTIVITY OF CASSUMUNAR GINGER (*Zingiber montanum* (Koenig) Link ex Dietr.)

BENYA MANOCHAI

A Thesis Submitted in Partial Fulfillment of
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Zingiber montanum (Koenig) Link ex Dietr. belongs to the Zingiberaceae locally called "Phlai" in Thailand. It is a perennial, rhizomatous herb. Volatile oils from rhizome contain terpinen-4-ol and (*E*)-1-(3', 4'- Dimethoxyphenyl) butadiene (DMPBD) are active ingredients. This oil has been used to treat sprains, contusions, muscular pain and inflammation related disorders. Various research articles on its biological activities have been published; however, there were insufficient data about environmental impacts on growth, yield and quality of Phlai. Therefore the objective of this study is to investigate the effect of the environmental factors on the antioxidant activity and volatile oil of *Zingiber montanum*. Three experiments were performed as follows: changes in concentration of biologically active components year round, and the effect of light intensity and water deficit on biologically active components. The results revealed that rhizome ages have positive relationships with fresh weight, dried weight, antioxidant activity and volatile oil content. Concentrations of sabinene, terpinen-4-ol and DMPBD were not affected by rhizome ages. Antioxidant activity was significantly affected by soil temperatures at 10 cm depth but not the volatile oil content and the active ingredients. Regarding percentage of light intensity, 50% and 25% light intensity promoted cassumunar ginger growth but decreased volatile oil content while antioxidant activity was not significantly affected. Water deficit at 120 days before harvest resulted in high volatile oil content but low fresh weight, while antioxidant activity was not affected by water deficit. Water deficit at 120 days resulted in an increase of sabinene content. The highest terpinen-4-ol content was obtained from the treatment with 60 days water deficit.

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Benya Manochai

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LIST OF ABBREVIATIONS

°C	=	degree celsius
cm	=	centimeter
Conc	=	concentration
et al.	=	et. alli (Latin), others
g	=	gram
hr	=	hour
l	=	litre
M	=	molar
µl	=	microlitre
µM	=	micromolar
µmol m^{-2} s^{-1}	=	micromole per square meter per second
m	=	meter
mm	=	millimeter
mg	=	milligram
min	=	minute
s	=	second
a.m.	=	ante meridiem
p.m.	=	post meridiem

EFFECT OF ENVIRONMENT ON BIOLOGICAL ACTIVITY OF CASSUMUNAR GINGER (*Zingiber montanum* (Koenig) Link ex Dietr.)

INTRODUCTION

Natural products from herbs have been increasingly popular in the past decade. Most herbs are naturally grown in Thailand with unique qualities as food and medicine. Therefore various research works have been focused on biological activities from these sources. In this regard, Thai herbs are being investigated and numerous species have potential for commercial production. Nowadays, some herbal products can substitute conventional medicine and are recommended for primary health care.

Zingiber montanum (Koenig) Link ex Dietr., Cassumunar ginger, is the one of the Thai herbs that is recommended for use in primary health care. It is locally known as "Phlai" in Thailand. This medicinal plant belongs to the Zingiberaceae family, and is distributed mainly in India and tropical Southeast Asia (Theilade and Maersk-Moller, 1991). All parts have been utilized to cure several diseases such as leaf- water extract for fever remedies (Farnsworth and Bunyapraphutsara, n.d.). Some local people consume flowers and young shoots as side dish vegetables for appetizers (Triboun *et al*., 2005). The most important part is the rhizome which exhibits various biological activities such as antioxidant, anti-inflammatory, antihistamine and antimicrobial activities.

Rhizome contains volatile oil which is composed of the active ingredients, terpinen-4-ol and (*E*)-1-(3', 4'- Dimethoxyphenyl) butadiene (DMPBD). These compounds are used to treat sprains, contusions, muscular pain and inflammation related disorders, and are used as the main indicator for Phlai oil quality (Thai Industrial Standard Institute, 1998). In general, the price of cassumunar ginger oil in Thai markets is approximately 3,000 baht per kilogram. The manufacturers pay lower prices to farmers for raw materials which do not meet their standard (Visuthipitakkul *et al*., 1997) which is specified by the Thai Industrial Standards Institute. The

specification of cassumunar ginger volatile oil should contain sabinene 31-48%, terpinen-4-ol 19-36%, α-pinene 1-3%, α-terpinene 3-8%, and γ-terpinene 2-12.66%. According to the specification set by the Thailand Institute of Scientific and Technological Research (TISTR), cassumunar volatile oil should contain 19-36% terpinen-4-ol and less than 5 % of DMPBD.

The anti-inflammatory drug from cassumunar ginger under the trade name Plygesal has been developed by TISTR and the Government Pharmaceutical Organization (GPO) since 1977 (Suntorntanasat *et al*., 1990). The effects of Plygesal cream were relatively close to the reference drug diclofenac in terms of their anti-inflammatory property. It has been since popular and the demand of this product has increased every year. At present, productivity is just 30,000 tubes (30 g/tube) per year. Cassumunar ginger was furthermore supported by the government's collaborative research with the private sector to develop a higher quality and standard. Thirty five active ingredients are identified structurally and numerous academic data support the efficacy of this herb. The commercial herbal extract, Plaitanoids, has been manufactured based on these academic data (Wanauppathamkul, 2003).

In this regard, there has been increasing demand for cassumunar ginger as a raw material while cultivation area is limited. Generally it is harvested from the wild or grown in home gardens or in small areas as an intercrop. Data on environmental impacts on growth, yield and quality of cassumunar ginger is rarely available. Therefore it is necessary to find the environmental factors that influence the yield and quality of this plant.

OBJECTIVE

To find out the relationship between the environmental factors, antioxidant activity and volatile oil of *Zingiber montanum* (Koenig) Link ex Dietr.

LITERATURE REVIEW

1. Botanical character of cassumunar ginger

Cassumunar ginger (*Zingiber montanum* (Koenig) Link ex Dietr. (syn. *Z. cassumuna* Roxb., *Z. purpureum* Roscoe) belongs to the Zingiberaceae (ginger family), locally called "Phlai" in Thailand. It is a perennial rhizomatous herb. Rhizome is greenish yellow internally (Figure 1C) with a strong aroma. The leafy stem (pseudostem) is covered with leaf-sheaths and is 1.2-1.8 meters tall (Theilade and Maersk-Moller, 1991). Leaves are simple, alternate and distichous, lanceolate-oblong, 3.5-5.5 cm wide and 18-35 cm long (Figure1A). Inflorescence is a spike (Figure 1B), arising from the apex of the rhizome. Flowers are white or yellowish white, bracts greenish purple. Fruit is a globose capsule (Saralamp *et al.* 1996).

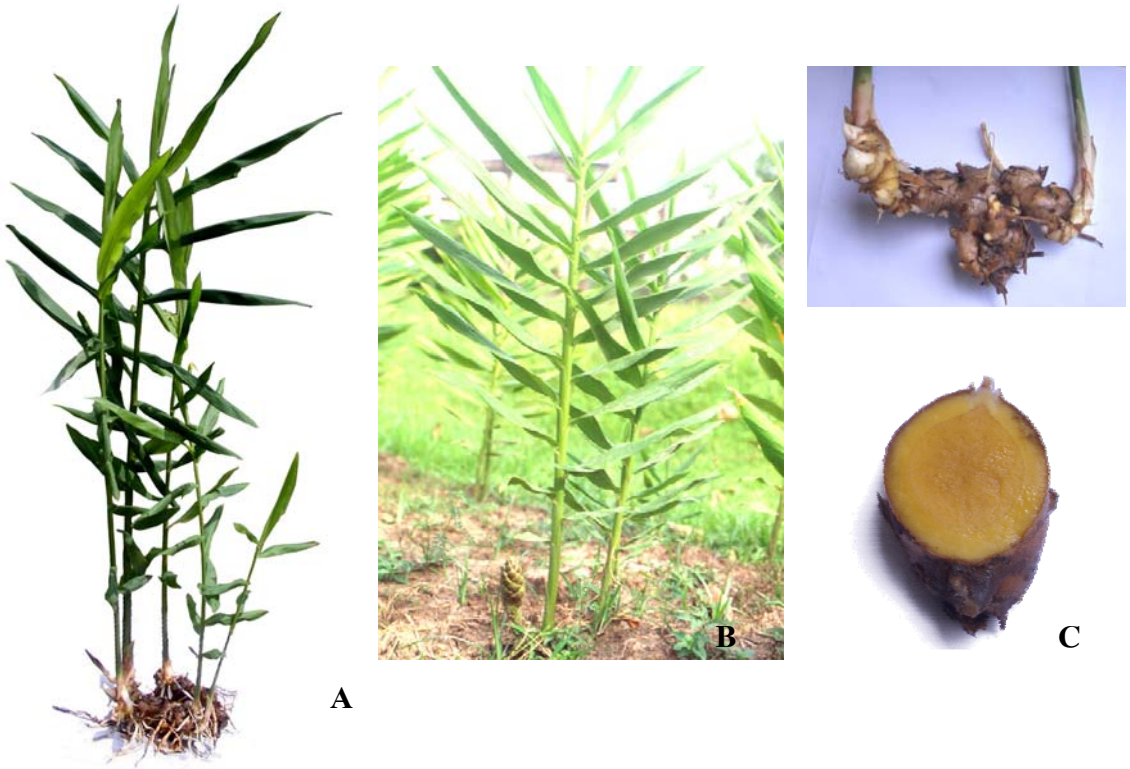

Figure 1 Morphology of *Zingiber montanum* A, whole plant; B, inflorescence; and C, rhizome.

The rhizome consists of thin epidermis, scattered vascular bundles, starch-bearing parenchyma cells and special secretive oil and resin cell (Figure 2). The yellowish oil cells contain a very aromatic, volatile oil that is referred to as a flavor (Dickison, 2000).

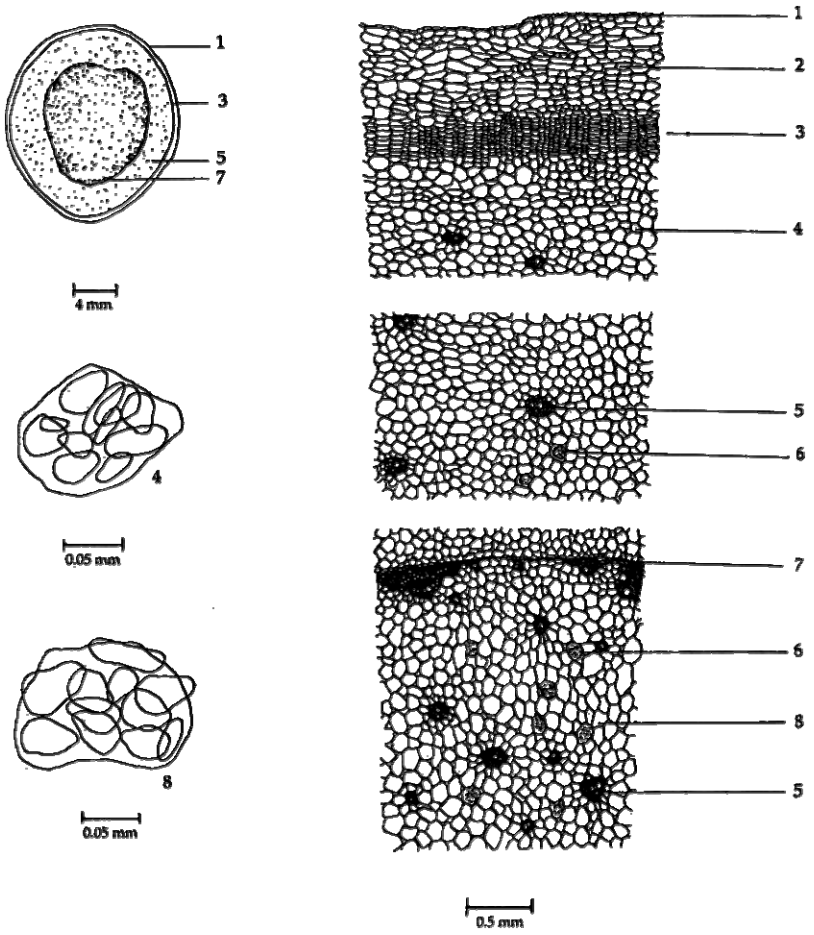

Figure 2 Anatomical diagram of *Zingiber montanum* (Koenig) Link ex Dietr. rhizome.

1. epidermis
2. outer cortical parenchyma
3. cork
4. inner cortical parenchyma containing starch granule
5. vascular bundles
6. oleoresins
7. endodermis
8. stele parenchyma containing starch granule

Source: Department of Medical Sciences (1998)

2. Cassumunar ginger cultivation in Thailand

Cassumunar ginger can be cultivated throughout Thailand. The cultivating period is limited by the climate. The farmers usually grow cassumunar ginger at the early rainy season (April - June) and the crop is ready for harvesting in about eight to ten months when mature leaves turn yellow and start drying gradually during the dry season (January – April). The plant is normally propagated by rhizome division, twenty to thirty grams with 2-3 buds rhizome segments are used for planting at 2-3 cm. below the soil surface.

Visuthipitakkul *et al*. (1997) reported that narrow spacing gave the maximum yield. They suggested that the suitable spacing for cassumunar ginger is 75x25 cm.

The plant prefers rich, well-drained, sandy loam or clay soil (Vamanon and Subcharoen, 1995). Recommended planting conditions by The Department of Agricultural Extension of Thailand are under indirect sunlight. Generally it prefers 75% shade, but it will tolerate full sun if adequate water is provided. Average temperature between 25-30^{o}C and 60-80% relative humidity are suitable for growth of cassumunar ginger. The plant was harvested for medicinal use at least 10 months after planting.

Cassumunar ginger is harvested by digging out rhizomes when the tops have died down. The rhizomes are cleared of all adhering matter and roots are removed, washing and then sun dried to help preserve them. If left undisturbed, the rhizomes will sprout new buds and the plant will repeat the growth cycle. Harvesting after the second growing season (21 months) gave 4 times higher yield (10,196 kg/rai) than the first growing season (9 months), 2,010 kg/rai (Visuttipitakul *et al*, 1997).

3. Utilization

Zingiber montanum (Koenig) Link ex Dietr. is a medicinal plant used widely in Southeast Asia especially in Thailand and Indonesia (Phongbunrod, 1965). The

rhizome is extensively used in Thai traditional medicine for local treatment of sprains, contusions, joint inflammations, muscular pain, abscess and similar inflammation-related disorders (Farnsworth and Bunyapraphutsara, n.d.)

Chamratpan and Homchuen (2005) reported that the natives of upper northeastern of Thailand use cassumunar ginger to cure paralysis symptoms. Flowers and young shoots are eaten as side dish vegetables for appetizers. It is used in Indonesian traditional medicine as a vermifuge, an analeptic for the uterus and to relieve pain, colic, diarrhea and rheumatism (Ozaki *et al*., 1991).

A well-known product of this plant is Luk Prakop which contains cassumunar ginger as a major ingredient. It is a Thai herbal combination which has been traditionally used to treat a variety of illnesses such as muscle-skeletal pain, inflammation and fungal skin infection. A cotton bag containing the Phlai rhizome mixed with other three to fourteen kinds of herbs, depending upon each formula, is placed on a hot steam pot, and then pressed onto painful spots to relieve the symptoms (Nandhasri and Pawa, 2005).

TISTR and the Government Pharmaceutical Organization (GPO) have developed Cassumunar ginger oil cream for medical purpose under trade name Plygesal (Suntorntanasat *et al*., 1990). This cream is developed to relieve muscular pain, bruises, sprains and swelling. Nowadays they are sold in drugstores as anti-inflammatory drug which has various forms such as cream, gel, balm and oil.

It has been successful in developing Plaitanoids, an extract from cassumunar ginger plants and rhizomes, which comes in the form of essential oil and powder to be used as a raw material for more than 30 products such as toothpaste, shampoo, skin-whitening, massage oils and essential oils for spas. For example, Twin Lotus has added cassumunar ginger extract to its toothpaste, while Aesthetic Clinic uses it as a major component in soaps, shampoos and lotions. Recently it has been used as skin-whitening and anti-ageing agent.

4. Biochemical constituents

Thirty five active ingredients have been found in cassumunar ginger. The chemical structures were elucidated and the efficacy of this herb has been supported academically (Wanauppathamkul, 2003). It contains cyclohexane derivative, naphthoquinone derivatives, arylbutanoids derivatives, vanillin, curcumin, ß-sitosterol and volatile oil which contained many biological active ingredients including α-pinene, ß-pinene, sabinene, myrcene, α-terpinene, limonene, γ-terpinene, ρ-cymene, terpinolene, and terpinen-4-ol (Department of Medical Science, 1998).

The chemical constituents in volatile oil which are extracted from this plant can be divided into three main categories. Those are terpene, oxygenated derivatives and benzene derivatives. Terpenes hydrocarbon are main plant constituents of essential oil and most of them are monoterpenes. It exhibits the highest volatility among all constituents. The examples of monoterpenes are α-pinene, ß-pinene, sabinene, α-terpinene and γ-terpinene. The second group is oxygenate derivatives which present the characteristic scent of essential oil. A noticeable oxygenate compounds contained in this plant is terpinen-4-ol. The last group is benzene derivatives which are utilized as aroma. Most medicinal compounds are classified into this group such as (*E*)-1-(3', 4'-Dimethoxyphenyl) butadiene (or DMPBD). The minor compounds found in the essential oil include ρ-cymene, ß –phellandrene, terpinolene, curcumin, α-thujone, sabinene hydrate and terpineneol. Only terpinen-4-ol and DMPBD (Figure3) are major active compounds of cassumunar ginger because they exhibited strong anti-inflammatory activity (Poonsukcharoen, 2004).

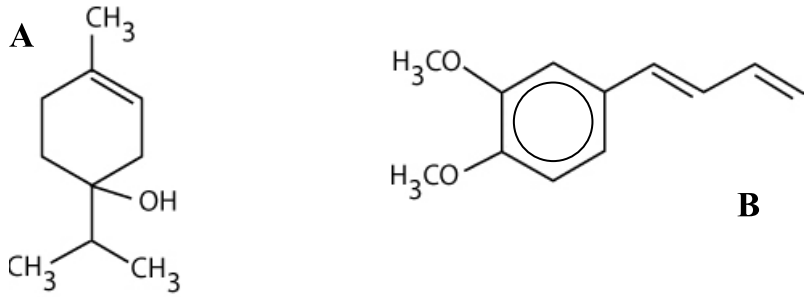

Figure 3 Chemical structure of terpinen-4-ol (A) and (*E*)-1-(3',4'-dimethoxyphenyl) butadiene: DMPBD (B).

Source: Bernard *et al*. (1966); Jeenapongsa *et al*. (2003) with modification.

5. Antioxidant activity

Antioxidant plays an important role against oxidation by giving electrons to free radicals to stop chain reaction. When a free radical gains the electron from an antioxidant it no longer needs to attack the cell and the chain reaction of oxidation is terminated. After donating an electron an antioxidant becomes a free radical by definition. Antioxidants in this state are not harmful because they have the ability to accommodate the change in electrons without becoming reactive. There are two lines of antioxidant defense within the cell. The first line, found in the fat-soluble cellular membrane consists of vitamin E, beta-carotene, and coenzyme Q (10). Of these, vitamin E is considered to be the most potent chain breaking antioxidant within the membrane of the cell. Inside the cell water soluble antioxidant scavengers are present. These include vitamin C, glutathione peroxidase, superoxide dismutase (SOD), and catalase (Dekkers *et al*., 1996). Only Vitamin A, C, E and the mineral selenium are commercially available antioxidants these days.

Antioxidant activity is found in crude extract (Trakultivakorn, 1999: Chirangini *et al*., 2004) and volatile oil (Lertsatitthanakorn *et al*., 2006) of Cassumunar ginger.

Vankar *et al*. (2006) reported that methanol extract of cassumunar ginger gave moderate antioxidant activity while dichloromethanol extract gave highest antioxidant activity when evaluated with DPPH method. It was observed that antioxidant activity of cassumunar ginger is in water soluble and fat soluble.

Jitoe *et al.* (1992) revealed that curcuminoids in *Z. cassumunar* exhibited antioxidant activity. The extraction was achieved by soaking the rhizome for 18 days in acetone. The residue was evaluated for its antioxidant activity by thiocyanate and thiobarbituric acid (TBA) method. Cassumunar ginger contains large quantities of curcuminoids and have strong activity which can replace a tocopherol as a naturally occuring antioxidant. Three curcuminoids, diferuloylmethane, (p-hydroxycinnamoyl)-feruloymethane, p,p'-dihydroxyldioinna-moyldethane were obtained after purification by silica gel TLC.

Masuda and Jitoe (1994) isolated cassumunins A, B and C from *Zingiber cassumunar*. The fresh rhizome was soaked in acetone and then evaporated. The crude acetone extract was dissolved with water and re-extracted with hexane. The aqueous layer was partitioned by ethyl acetate using silica gel column chromatography. This separation produced thirteen fractions. The last fraction containing cassumunins A, B and C was further purified. Cassumunins A-C were tested for antioxidant (TLC and thiocyanate method) and anti-inflammatory activities (TPA induced). The result revealed that cassumunins A-C activities were stronger than that of curcumin in both anti-inflammatory and antioxidant activity.

6. Volatile oil of cassumunar ginger

Cassumunar ginger rhizomes contain 80% volatile oil (Ministry of Public Health, 1990) with distinct aroma. Volatile oils are mostly located in secretive oil of parenchyma layer. It has a pale amber color. The scent is a cool, green peppery one with a touch of a bite. Active chemicals are sabinene (25-45%), γ-terpinene (5-10%), α-terpinene (2-5%), terpinen-4-ol (25-45%), and (*E*)-1-(3',4'- dimethoxyphenyl)-butadiene:DMPBD (1-10%) (Wanauppathamkul, 2003).

In Thailand, the general extraction method of volatile oil is steam distillation. It is the oldest technique used in industrial preparation of essential oil (Chaintreau, 1999). The main advantage of the steam distillation technique is that the extracts do not contain any non volatiles. In a laboratory, using simple steam distillation, the sample is dispersed in water and placed in a round- bottom flask which is heated directly or indirectly by steam. Vapor is condensed and the sample is collected (Da Costa and Eri, 2005). By this method one ton of cassumunar ginger rhizome can produce approximately 0.8 to 1 liter of volatile oil. However the percentage of volatile oil at 2 percent v/w based on fresh weight was specified by the Department of Medical Sciences (1998)

7. Environment and active ingredients

Plants require sunlight, temperature, moisture, air and nutrients for their growth. These are provided by an environment in which plants live (Schroeder *et al*., 1997). The optimum environmental condition will provide the essential compounds such as sugar, polysaccharide, nucleotide, amino acids, proteins, common fatty acids and lipids. These are necessary for plant survival and plant growth (Mann, 1987). In contrast, changes in their environment such as extreme temperature, water storage, insufficient or excessive light and nutrition deficit, plant growth will be limited. Various environmental factors are associated with metabolic process (Smirnoff, 1995) and cause change in the composition and concentration of plant chemical compounds.

Hornok (1986) reported that the same plant species cultivated at different locations or in different seasons provided different quantities of secondary metabolite. Similar results were reported by Chavalittumrong and Jirawattanapong (1992) where turmeric grown in difference places differed in curcuminoids and volatile oil content.

Owens *et al*. (1998) revealed that the total monoterpenoid concentration was significantly affected by the season and by the plant population. Mean monoterpenoid concentration of a population from each central Texas was 9.16 mg/g fresh weight of juniper needles while the mean concentration of a west central Texas population was

11.62 mg/g of fresh weight. Monoterpenoid concentrations were typically lowest during the summer and highest during the spring and winter in the western populations, but there was no seasonal pattern in the eastern populations. The eastern populations were slightly (4.8%) more flammable than the western populations, and male trees were slightly (3.8%) more flammable than female trees. The concentration of limonene was positively related to plant flammability and could increase flammability by 30% over the range of concentrations found in this species. Secondary metabolite, usually considered as antiherbivore mechanisms, may also serve an important role in determining the likelihood of a plant being consumed by fire.

Anderson *et al*. (1992) found that the antioxidant metabolite of eastern white pine (*Pinus strobus* L.) needles increased 2-4 fold from the summer to the winter season. Antioxidant enzymes in needle tissue increased between 2- and 122-fold during this same period. Levels of antioxidant metabolites and enzymes were always lowest during the summer or active growing season and highest during the winter, or dormant season. They indicated that needle temperatures exceeding 25°C may result in impairment of antioxidant metabolism.

Visuthipitakkul *et al*. (1997) reported that cassumunar ginger grown at Kanchanaburi and harvested at 10 months after planting contained 1.10% volatile oil based on fresh weight, and major components in volatile oil are sabinene (45.22-47.86%), terpinen-4-ol (20.51-21.43%) and (E)-1(3', 4'-dimethylphenyl) butadiene or DMPBD (10.12-11.68%). Aengwanich (2002) reported that cassumunar ginger was grown in Khon Kaen province and harvested at 18 months after planting contained 3.49% volatile oil based on fresh weight and 24.23% terpinen-4-ol.

Other environmental factors such as water, temperature and light can also significantly affect the concentration and composition of phytochemicals in plants.

Baher *et al*. (2002) reported that water stress decreased plant height and total fresh and dry weight of cultivated *Satureja hortensis* L. The accumulation of oil increased significantly under severe water stress at the flowering stage, when the mean

leaf water potential decreased from -0.5 to -1.6 MPa. That stress has more effect on the quantity of the essential oils than moderate water stress during the vegetative and flowering stages.

Dunford and Vazquez (2005) reported that the crop yield of Mexican oregano increased significantly with increasing moisture and age of the plants. Although on average the older plants contained less oil than the younger plants, the differences were not statistically significant. Total thymol and carvacrol content of oregano oils obtained from younger plants was higher than that of the mature plants. The amount of water received by the plant did not have a significant effect on the thymol and carvacrol content of the oil extracted from Mexican oregano.

Loreto *et al*. (1998) indicated that, monoterpenes may help plants cope with heat stress by enhancing membrane stability, thus providing a rather non-specific protection of photosynthetic and respiratory processes. Monoterpene emission was maximal at a temperature of 35 °C and was inhibited at higher temperatures. This is likely that high temperatures inhibit enzyme activity involved in monoterpene synthesis.

Wang and Lincoln (2004) revealed that the constitutive leaf monoterpene content of *Myrica cerifera* is higher in a sunny habitat than in an adjacent shady habitat at a southeastern USA coastal site. A significant negative correlation of monoterpene content and leaf area loss suggests that monoterpenes may play toxic or deterrent roles in these plants. The plants treated in high light intensity had significantly higher monoterpene content, higher growth rate, and denser glandular trichomes than the plants treated in low light intensity.

In conclusion various environmental conditions play an important role in the production of active compounds in many plants. Therefore the active ingredient in cassumunar ginger may also change due to the environment.

MATERIALS AND METHODS

Materials

1. Rhizome of cassumunar ginger (*Zingiber montanum* (Koenig) Link ex Dietr.) from Nakhon Pathom province.
2. Thermometer and Hygrometer
3. Digital illumination meter (Hioki 3234 Lux HiTester Digital Illumination Meter)
4. Nets
5. 95% Ethanol
6. Diphenyl-2-picrylhydrazyl (DPPH)
7. Cleventure apparatus for volatile oil extraction
8. Mantle heater
9. Spectrophotometer (Spectronic 20D)
10. Gas chromatography (Fisons instrument model 8000 series)

Methods

Three experiments were undertaken as follow:

1. The year round fluctuation in the quantity of biological active components.
2. The effect of light intensity on biologically active components.
3. The effect of water deficit on biologically active components.

Biologically active component include antioxidant activity, sabinene, terpinen-4-ol and (*E*)-1(3', 4'-dimethylphenyl) butadiene or DMPBD.

1. Field experiment

1.1 Year round fluctuation in the quantity of biologically active components.

Clonally propagated clone of *Zingiber montanum* was grown under field condition at the experimental field of the Department of Horticulture, Kasetsart University, Kampangsean campus. The experiment was conducted from June 2003 to June 2005. Twenty to thirty grams with 2-3 buds of rhizome segments were used for propagation. Rhizomes were harvested at 2 month interval and were subjected to analyze antioxidant activity and volatile oil extraction.

Climatic data was taken from Nakhonpathom Meteorological Station at Kasetsart University, Kampang Sean campus, Nakhon Pathom province. Correlation between growth, major components and climate were performed using SPSS 13.0 for window computer program.

A complete randomized block design (RCBD) was used in this experiment with 3 replications. Rhizomes were collected at an interval of 2 months until 24 months. Rhizomes were washed carefully and the roots were discarded. Fresh rhizomes were sliced and kept in plastic bags at -20^{o}C for extraction. The objective of this work was to find relationships between growth and the biologically active components of cassumunar ginger.

1.2 The effect of light intensity on biologically active components

Zingiber montanum is propagated by rhizome division. Each rhizome segment, 20-30 grams with 2-3 buds, was planted in 10-inch pot at the experimental field of the Department of Horticulture, Kasetsart University Bang Khen campus. The experiment was conducted from August 2004 to May 2005. A completely randomized design (CRD) was used. There are 3 treatments and 10 replications consisting of 100 % (full sunlight), 50% and 25% light intensity. Cassumunar ginger was grown under field condition until 4 months then treated with 3 levels of light intensity. For light intensity

at 50% and 25%, the plants were grown under 50% and 75% plastic shaded cloth respectively. Light intensity was measured by digital illumination meter (Hioki 3234 Lux HiTester Digital Illumination Meter). Data on light intensity were collected at 7 day intervals and were recorded every hour from 6.00 am to 6.00 pm during field experiment.

Rhizomes were harvested at 10 months, washed carefully and the roots were discarded. . Fresh rhizome was sliced and kept in plastic bags at -20^{o}C until extraction. Growth and light intensity were recorded every week. Climatic data were obtained from the meteorology station in the Bang Khen area. The major components and antioxidant activity were evaluated after harvest. The objective of this work is to find the effect of light intensity level on the change of major components.

1.3 The effect of water deficit on biologically active components.

Zingiber montanum was propagated by rhizome division. Each segment, 20-30 grams with 2-3 buds, was planted in 10-inch pots at the greenhouse of the Department of Horticulture, Kasetsart University Bang Khen campus. The experiment was conducted from August 2005 to May 2006. Completely randomized design (CRD) was used with 5 treatments and 5 replications. The treatments consist of watering (control) and stop watering at 30, 60, 90 and 120 days before harvested.

Water consumption of cassumunar ginger was estimated from evapotranspiration of containerized cassumunar ginger. The growing media moisture was set at field capacity by adding water into containers for 3 hours. The containers with 100% water saturated media were weighed after 24 hrs. When the extra water was stopped and drained off, it was weighed again after 2 days. Evapotranspiration of each container was determined by monitoring the daily weight decrease (Southwick and Davenport, 1986), one gram of decreased weight equals to 1 ml of water loss. From the observation we found that water consumption of cassumunar ginger which was used in this experiment is 500 ml/ plant/day.

Rhizomes were harvested at 10 months, washed carefully and the roots were discarded. Fresh rhizome was sliced and kept in plastic bags at -20^{o}C until extraction. Growth was recorded every week. Climatic data was obtained from the meteorology station in Bang Khen area. The major components and antioxidant activity was evaluated. The objective of this work is to find the effect of water deficit on the major components.

2. Analytical methods

2.1 Ethanol extraction

Biologically active components were extracted using the method of Trakultivakorn (1999) with modification for year round fluctuating evaluation. A frozen rhizome of each treatment (50g) was homogenized and was extracted twice with 150 ml of 95% ethanol at room temperature for 4 days. The extracts were kept in cap bottles at -20^{o}C until further use for antioxidant activity assay.

The extraction method of Chirangini *et al*. (2004) with modification was used in the experiments on the effect of light intensity and the effect of water deficit on the biologically active components. One gram of frozen rhizome of each treatment was ground using a mortar and pestle with liquid nitrogen, 6 ml of 95% ethanol solution was added and homogenized. The homogenate was collected and centrifuged at 8,000 rpm for 10 minutes, 3-4 cycles of centrifugation, until a clear supernatant was obtained. Finally, the supernatant was decanted from the centrifuge tube. The supernatant was kept in a capped bottle at -20^{o}C until further use for antioxidant activity assay.

2.2 DPPH radical scavenging activity

The evaluation of radical scavenging activity (antioxidant activity) was conducted by the method of Blois (1958) with modifications. A stock solution of the sample (167 mg/ml) was diluted to make a dilution series of 1x, 2x, 4x, 10x, 20x, 30x, 40x and 50x. Proper dilution was done if the absorbance value measured was in the

range of the cassumunar ginger standard curve. The reaction mixtures contain 3 ml of 0.1 mM Diphenyl-2-pierlyhydrazyl (DPPH, in 95% ethanol) and 0.5 ml of the dilutions series of cassumunar extract. After allowing the mixtures to stand at room temperature for 20 minutes under light protection, the absorbance was recorded at 517nm. Deionized water or 95% aqueous ethanol was used as a control. The scavenging activity of DPPH radicals (%) was calculated by the following equation:

$$[(A_{517\ blank} - A_{517\ sample}) \div A_{517\ blank}.] \times 100\%.$$

The proper dilution of cassumunar extract is 4 x dilutions. This dilution was used to evaluate the antioxidant activity of cassumunar ginger with the same method as described above.

2.3 Extraction of the volatile oil

Steam distillation was employed to extract volatile oil from rhizomes. A hundred grams of chopped rhizome was homogenized and put into a one liter round bottom flask and filled with 500 ml of distilled water. When the water boils, the steam passes through the condenser and condenses into the reservoir flask. Volatile oil was separated from water by collecting the upper layer and placing it in 5 ml glass bottles and kept at -20 ^{o}C for analysis of the major components.

2.4 Gas chromatography analysis

Volatile oil constituent was analyzed by the Thailand Institute of Scientific and Technological Research using gas chromatography with a flame ionization detector (Fisons instrument model 8000 series) for quantitative analysis. The separation was performed on a 30m x 0.25mm i.d. x 0.25μm film thickness fused silica capillary DB-5 column (J&W Scientific, USA). Two micro liters of each extract was injected into the column. The inlet temperature was set at 230 ^{o}C and the oven temperature programmed from 50 ^{o}C to 220 ^{o}C at 4 ^{o}C/ minute. A completion time of

one chromatogram is therefore 42.5 minute per one injection. Helium gas (99.995%) was used as a carrier gas with a flow rate of 1.2 ml/min and with split ratio at 1:10.

3. Statistical analyses

The results were presented as mean. Mean differences between treatments were examined with Duncan's new multiple range test (DMRT) by Sirichai statistics 6.00 computer program. Correlation between ages and major components was performed with the Pearson product moment correlation coefficient by SPSS 13.00 for Windows computer program.

RESULTS AND DISCUSSION

1. The year round fluctuation in the quantity of biological active components.

This experiment was conducted from June 2003 to June 2005 at an experimental field of Kasetsart University, Kampangsean campus. The changes in plant growth, antioxidant activity, volatile oil content, volatile constituents and their correlation with the environment are described as follows.

1.1 Plant growth

1.1.1 Plant height

The results showed that at 6 months after planting the aerial stem reached its maximum average height (124.44 cm.) for the first growth cycle (June-April) and decreased gradually till 10 months when the aerial stem of cassumunar ginger dried down. This pattern is similar with the second growth cycle (June-February) with the maximum height at 18 month ages (146.63 cm.) and gradually decreased until 20 months when the aerial stem dried down again. The aerial stem height of the second growth cycle is higher than the first growth cycle and it tends to be higher in the third growth cycle. (Figure 4)

1.1.2 The number of plants per clump

At 6 months after planting, the highest number of plants per clump (4.11plants/clump) was obtained. The number of plants per clump of the second crop (5.56 plants/clump) is slightly higher than the first crop at 14 months. However during the third growth cycle at 24 months the number of plants per clump (10.67 plants per clump) increased sharply, almost 2 times more than the second cycle of plant growth. (Figure 5)

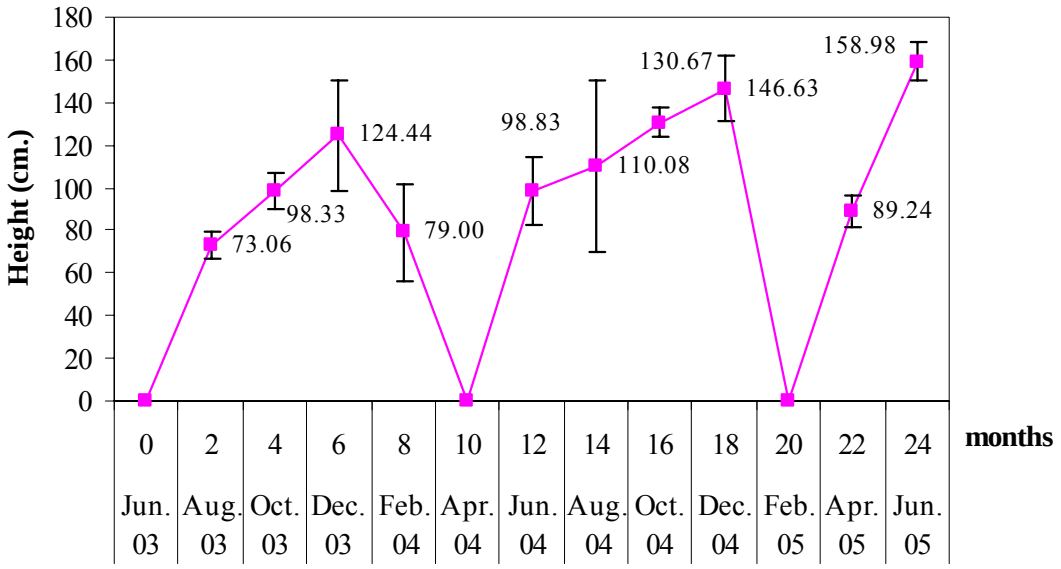

Figure 4 The height of cassumunar ginger (*Zingiber montanum*) at various rhizome ages.

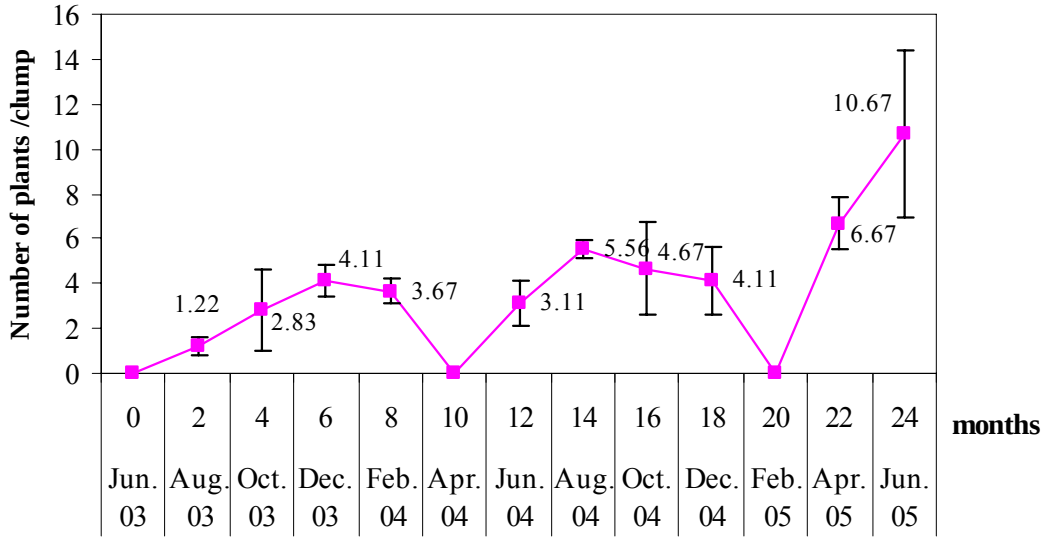

Figure 5 The number of plants per clump of cassumunar ginger (*Zingiber montanum*) at various rhizome ages.

1.1.3 The number of leaves per plant

The number of leaves per plant of the first growth cycle gradually increased with rhizome ages until 6 months after planting (32.75) the highest number of leaves per plant was obtained. During the second growth cycle the highest number of leaves per clump (39.23) was reached. (Figure 6)

1.1.4 Fresh weight and dry weight of rhizome

The yield of rhizome based on fresh weight is shown as kilograms per clump. At 12 months after planting rhizome yield (0.78 kg/clump) reached it highest weight. However at 24 months, the highest fresh weight (2.20 kg/clump) of the second growth cycle was obtained and is significantly higher than the first crops (Figure7). Fresh weights tend to increase with rhizome ages and the productivity of the second crop was higher than the first crop by approximately 3 times.

Dry weight was shown as kilogram per clump. The pattern of growth is similar to fresh weight but the highest weight (0.128 kg/clump) is reached for the first cycle at 8 months and at 20 months (0.496 gram/clump) for the second cycle.

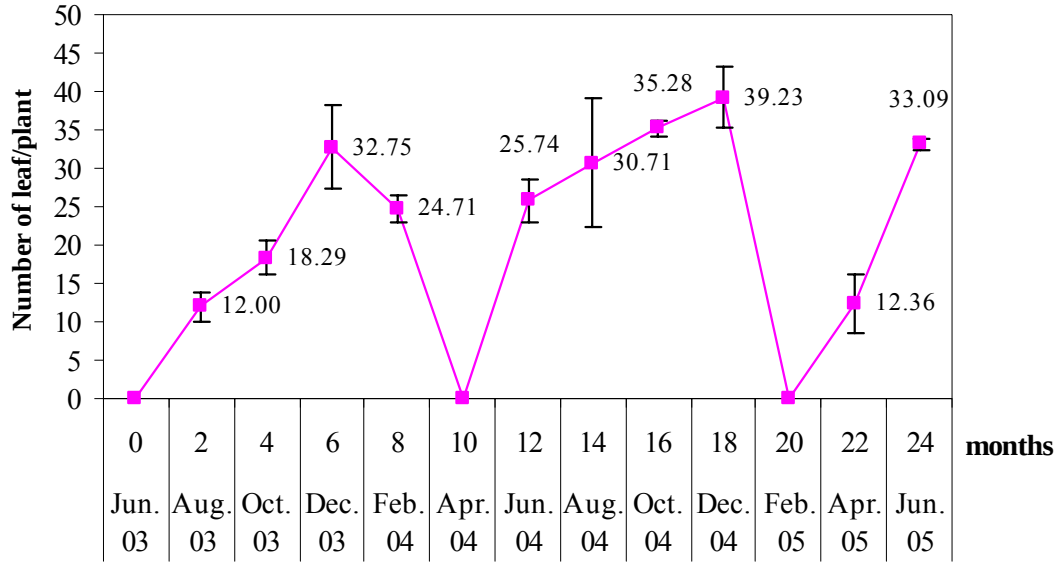

Figure 6 The number of leaves per plant of cassumunar ginger (*Zingiber montanum*) at various rhizome ages.

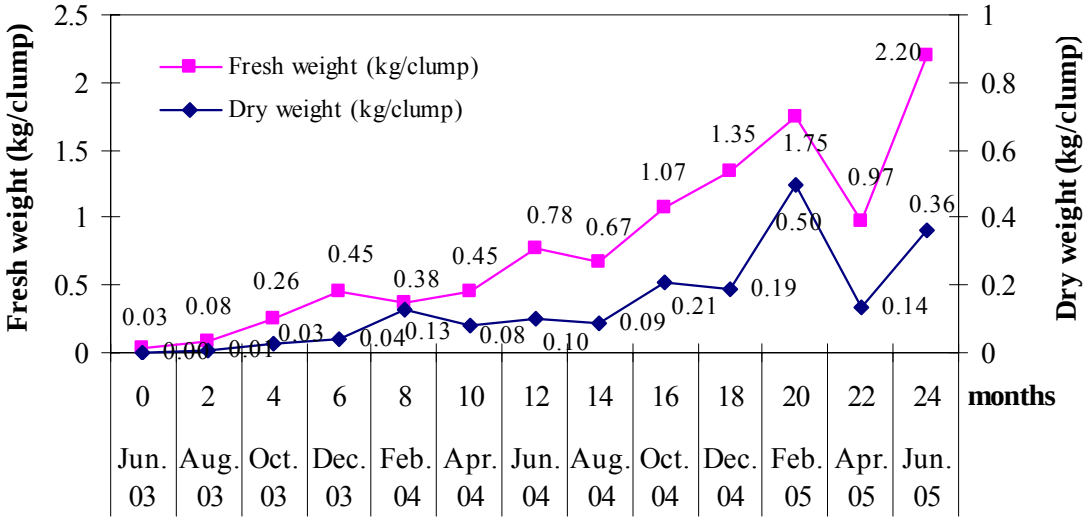

Figure 7 Fresh weight and dry weight of cassumunar ginger (*Zingiber montanum*) at various rhizome ages.

According to the cassumunar ginger growth of the above ground part, the height, the number of plants per clump and the number of leaves per plant were similarly and positively increasing. The plant took 6 months to reach maximum growth during the first growth cycle and the aerial parts completely dried and collapsed at 10 months. However the underground rhizomes continue their growth which is noticeable from the increase of fresh weight and dry weight of rhizome (Figure 7). The plants went to the dormant stage for 2 months before the aerial part started sprouting again. The growth pattern of the second growth cycle is similar to the first growth cycle. The growth of the second growth cycle increases over two times compared with the first growth cycle. The same growth pattern has been found in several other rhizomatous species such as *Zingiber officinale* (Whiley, 1980) and *Zingiber mioga* Roscoe. (Gracie *et al*., 2000).

Fresh weight and dried weight dropped sharply at 22 months after planting which resulted from an unusually low precipitation period during the later period of the second growth cycle (Figure 8B). Therefore growth and development of rhizome and the accumulation of fresh and dried mass increase slowly. The same growth pattern was also observed in *Anemone nemorosa* when severe drought in the spring had a negative effect on rhizome growth (Philipp and Peterson, 2007). Moreover, they also observed that at the time when leaves and flowers emerged above ground, which was taken 2-4 weeks before the beginning of growth of rhizome, the dry weight decreased abruptly about 2.02 mg per one mm of rhizome internodes. In the cause of following weeks, dry weight rhizome segments approximately 4.23 mg/mm. This agrees with our results, both fresh and dry weight decreased abruptly at 22 months while shoots and leaves start sprouting a few weeks before. The stored food in rhizome has been used up for the next growth cycle.

At 22 months, the aerial part cassumunar ginger started sprouting and photosynthesis functioned to accumulate food for their growth. Xu *et al*., (2004a) reported that at the seedling stage, the aerial stem is the growth center of ginger growth and 80.7% of carbon assimilation from rhizome was transferred to this part. But the precipitation was low during that month as Xu and Zhou (2005) reported that severe and extreme water stress limited carbon translocation from above ground into

below ground sink organ. Therefore translocation of photosynthetic substance to rhizome was limited and lead to the decreasing accumulation of rhizome weight at 22 months.

Not only did drought affect decreased weight at 22 months but high temperature during that time is also one of the reasons. The highest temperature at 22 months (Figure 8c) may affect the decreased weight. The increasing temperature increased the respiration rate. This is supported by Lunáčková *et al*. (2000) who reported that the respiration rates of the root of *Karwinskia humboldtiana* Zucc. and *Karwinskia parvifolia* Rose. were higher in the plants that were cultivated under 35/20 °C (the summer temperature) than in those under 20/5 °C (the winter temperature). Respiration is a major cause of carbohydrates loss which can account for up to 50% of the daily carbon gain by photosynthesis (Poorter *et al*., 1995). A reasonable explanation for this result is that drought and high temperature during that period caused the decreasing weight at 22 months.

The growth of the second growth cycle was greater than the first growth cycle because the rhizome segments at the first growth cycle were a small size which took more time to accumulate stored food. Therefore, the cassumunar ginger rhizome increased in terms of size and the number of buds which later support the formation of new shoots and the growth rate of the following crop. This result is supported by the work of Visutipitakul *et al.* (1997) who reported that the abundant bud seed- rhizomes of cassumunar ginger promote plant growth and gave the maximum yield compared with less bud seed- rhizomes because the abundant bud seed- rhizomes were usually larger and have more stored food. Klimes *et al*. (1999) reported that glucose and starch concentrations increases with rhizome age in *Phragmites australis*. The increased stored food was the result of increasing photosynthesis efficiency in response to greater demand for assimilates (Whiley, 1980).

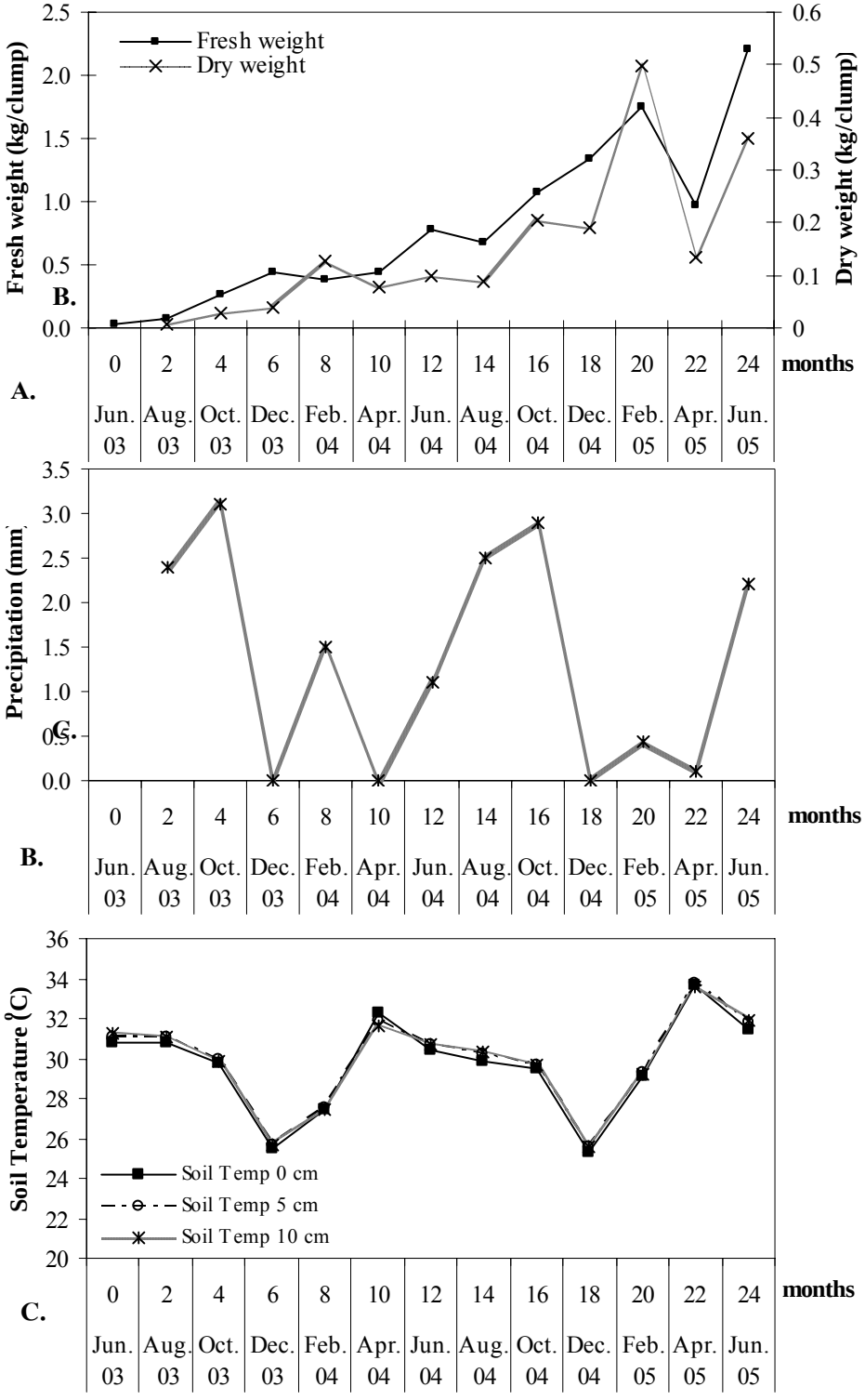

Figure 8 Fresh weight and dry weight of cassumunar ginger (*Zingiber montanum*) (A) compare with the precipitation (B) and soil temperature (C).

1.2 Plant quality

1.2.1 Antioxidant activity

DPPH radicals are widely used in the model system to investigate the scavenging activities of several compounds. When a DPPH radical is scavenged, the color of the reaction mixture changes from purple to yellow with decreasing absorbance at the wavelength 517 nm. The antioxidant activity of cassumunar ginger significantly differs at different rhizome ages. At 24 months and 22 months after planting there was no significant different, but the 22 months old rhizome gave the highest antioxidant activity (79.19%). In contrast with 6 and 10 months age the antioxidant activity was rather low but was lowest (47.20 %) at 6 months after planting (Table1). The activity tends to increase with rhizome age (Figure 9). This agrees with Shi *et al*. (2006) who reported that the content of ginsenoside, antioxidant compound, in root and root-hair increase with ages of *Panax ginseng* from one to five years. Other similar results, 3 year-old *Aloe vera* provide a higher antioxidant activity than 2 year-old (Hu *et al*., 2003), glutathione (antioxidant) increase with age of potato tuber (Kumar and Knowles,1996). These results support our finding that the antioxidant activity was positively associated with rhizome age because of at development stages the rhizome may contain different active components and exhibit antioxidant activity to different degrees.

The possible explanation is that in relatively young tuber and under normal growing conditions, the free radical scavenging system effectively quenches free radicals produced as a consequence of normal metabolism. In contrast, during rhizome development, oxidative stress increases, and increases the ability to quench free radicals, the equilibrium between free radicals production and removal gradually shifts in favor of production. That is why the percentage of DPPH scavenging of the second growth cycle is higher than the first growth cycle.

Table 1 Average antioxidant activity and volatile oil content of cassumunar ginger (*Zingiber montanum*) at various rhizome ages.

Ages (months)	**Antioxidant activity** (%)	**Volatile oil content** (ml/kg)
4	$57.12^{ab} \pm 9.24$	$5.17^{d} \pm 1.02$
6	$47.20^{b} \pm 5.92$	$9.52^{bc} \pm 1.51$
8	$62.44^{ab} \pm 6.61$	$6.32^{cd} \pm 1.20$
10	$51.49^{b} \pm 5.75$	$9.30^{bc} \pm 1.73$
12	$60.14^{ab} \pm 2.27$	$8.29^{bcd} \pm 1.30$
14	$58.46^{ab} \pm 3.22$	$6.22^{cd} \pm 2.50$
16	$68.04^{ab} \pm 14.89$	$13.02^{a} \pm 1.11$
18	$57.30^{ab} \pm 12.25$	$8.16^{bcd} \pm 0.34$
20	$67.94^{ab} \pm 10.38$	$9.48^{bc} \pm 2.85$
22	$79.19^{a} \pm 8.14$	$11.18^{ab} \pm 1.98$
24	$78.72^{a} \pm 5.29$	$11.43^{ab} \pm 1.40$
F-test	**	**
CV	13.86%	15.39%

** Mean within each column followed by the same letter is not significantly different at 99% level of confidence based on Duncan' New Multiple Range Test.

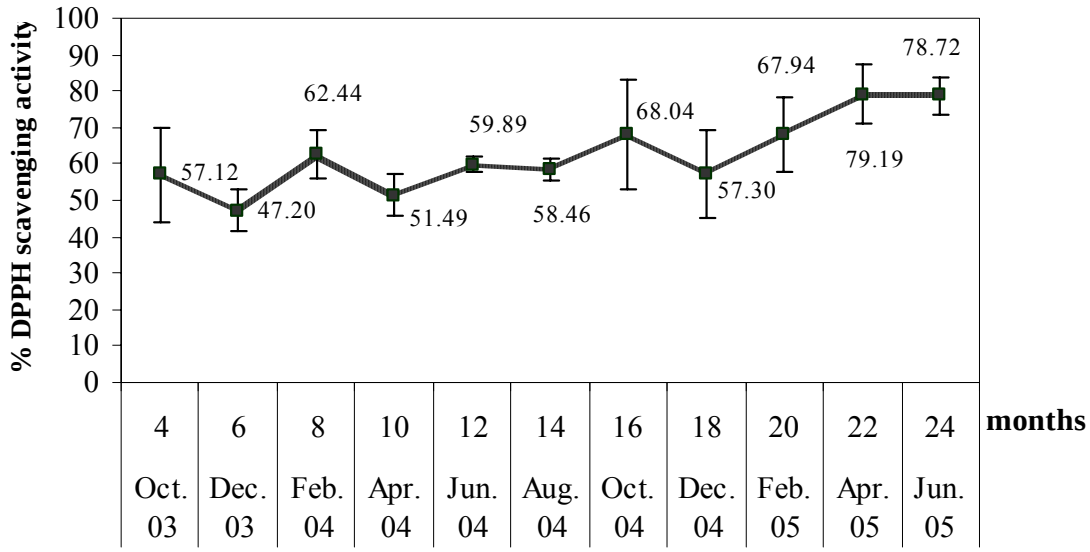

Figure 9 Antioxidant activity of cassumunar ginger (*Zingiber montanum*) at various rhizome ages.

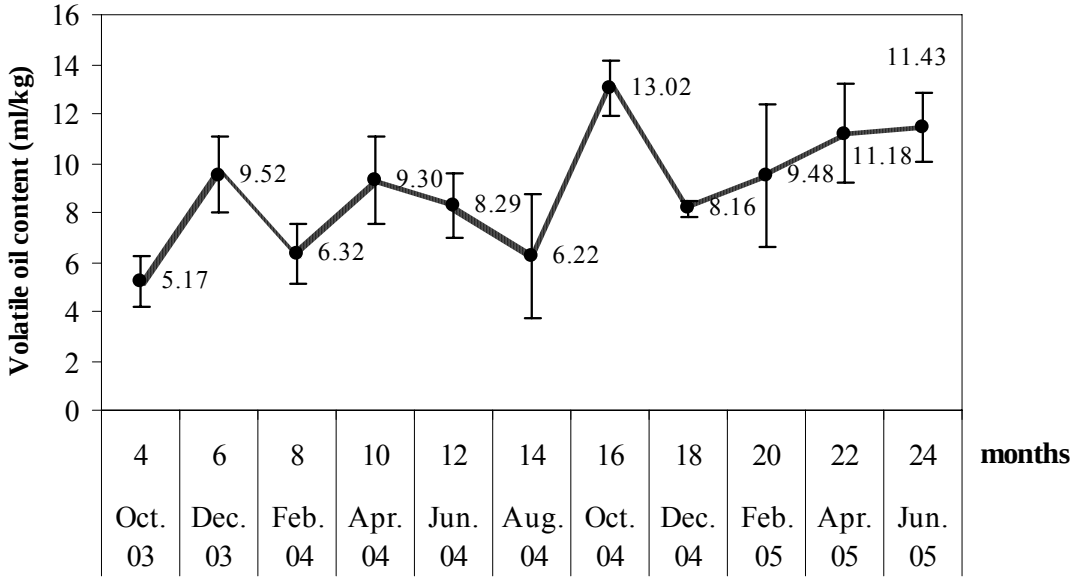

Figure 10 Volatile oil yield (ml/kg based on fresh weight) of cassumunar ginger (*Zingiber montanum*) at various rhizome ages.

1.2.2 Volatile oil content

Volatile oil was obtained as a pale amber color. The yield based on fresh weight was shown as milliliter per kilogram. The highest volatile oil volume (13.02 ml/kg) was obtained from 16 month old rhizome and the lowest volatile oil volume (3.58 ml/kg) from 4 months (Table1). The volatile oil content varies with the growth stage. Cassumunar ginger enters a dormant stage at 10 months and it was observed that at 2 months before dormancy, 8^{th} months of the first growth cycle and at 18^{th} months of the second growth cycle, volatile oil content decreased. While at 6^{th} months of the first growth cycle and 16^{th} months of the second growth cycle was the highest volatile oil content in each growth cycle. This result may indicate that cassumunar ginger takes 4 months after planting to reach the highest growth and development. During that stage, cassumunar ginger produced the highest volatile oil. The reason for this result is supported by Dixit and Srivastava (2000) who reported that the youngest turmeric leaves were most active in fixing carbon. Fixation capacity into primary metabolite (sugar and organic acid) and then translocation of carbon in primary metabolite form from the terminal part to the underground part is follwed by conversion into secondary metabolites (essential oil and curcumin) through biosynthesis.

The decreasing volatile oil content of cassumunar ginger before dormancy was similar tp the pattern of essential oil content of turmeric which is dormant at 8^{th} months after planting and essential oil decreased at 7^{th} months and during plant growth and development from 3 to 8 months indicating that essential oil of turmeric shows wide fluctuations in its percentage during development (Mathai, 1978). This result is associated with the stage of plant growth and development of this plant. The growth of the above ground part at two months before dormancy was declining as the result of the decreasing efficiency of photosynthesis which affected volatile oil accumulation. Apart from the decreasing efficiency of photosynthesis causing low volatile oil productivity during that stage, before dormancy, the above ground part enters senescence which is generally known to be controlled by ethylene. Therefore the decreasing volatile oil content may be due to the senescence of

cassumunar ginger. Cassumunar ginger may convert volatile oil content to ethylene form because DMPBD, one of the major volatile oil constituents, consisting of butadiene or diethylene conjugate with the aromatic ring (Figure 3). This is supported by a decrease of DMPBD during that stage. (Figure 12) However for clear explanation this relation should be investigated further.

In comparisons between the first and the second growth cycle, the results showed that the volatile oil content of the second was greater than the first growth cycle. With regard to the volatile oil of cassumunar ginger, Aengwanich (2002) reported that harvested rhizome of cassumunar ginger at 18 months gave volatile oil 3.49% and Visuthipitakkul *et al*. (1997) reported that at 10 months after planting of cassumunar ginger yielded 1.10% volatile oil. Both reports agree with our result which indicates that rhizome age and volatile oil volume are related.

1.2.3 Volatile oil constituents

A gas chromatogram of volatile oil constituents from fresh rhizome is shown in Figure 11. The components of volatile oil are summarized in Table 2. The volatile oil of cassumunar ginger consists of at least 14 compounds (Table 2). Monoterpene compounds are expressed at the beginning of chromatogram, include α thujene, α-pinene, sabinene,β-myrcene, α-terpinene, *p*-cymene, β-phellandrene, γ-terpinene, sabinene hydrate, terpinolene, terpinen-4-ol, terpinyl acetate and β-sesquiphellandrene corresponding to their mean retention times at 7.87, 8.12, 9.47, 9.91, 10.83, 11.11, 11.26, 12.34, 12.88, 13.40, 16.93, 17.08 and 28.67 min, respectively. Another peak with the mean retention at 32.28 min corresponds to a benzene derivative which is (*E*)-1-(3,4-dimethoxyphenyl)-butadiene (or DMPBD).

The quantitative change of the volatile oil constituent over two years, the total percentage of completely identified volatile oil constituents (VOC) is shown in Table 2. The percentage of volatile oil constituent was calculated from the peak area of the gas chromatogram. Total volatile constituents fluctuate during plant growth and

development. We observed that during the decrease of the total percentage of VOC, some components disappeared while others increased.

Cassumunar ginger harvested at 4, 6, 8 and 10 months of the first growth cycle yielded 90.07, 94.65, 94.13 and 94.53 % total VOC respectively. At the 4^{th}, 6^{th}, 8^{th} and 10^{th} months of the second growth cycle total VOC are 93.91, 97.40, 98.38 and 94.59 % respectively.

The major active components in the volatile oil of cassumunar ginger are sabinene, terpinen-4-ol and DMPBD. The results revealed that sabinene significantly differs between rhizome ages (Table 3). Six months old rhizome gave the highest percentage (57.22% sabinene) in contrast to the lowest (26.14%) at 4 months age. Terpinen-4-ol and DMPBD were not significantly varied with rhizome age. However, it has been noticed that the percentages of terpinen-4-ol (Figure 12) increase at two months before entering dormancy in both growth cycles in contrast to the DMPBD which decreased in the same periods.

Terpinen-4-ol and DMPBD were not significant, resulting from the percentage coefficient of variation (% CV) which is a rather high value. (Table 3) The CV is a relative measure of variation of the experimental data. The lower magnitude of CV is a reflection of the reliability (precision) of the experimental results. However, the CV of field experiments varied with the situation. The acceptable range of the CV for field experiments is 23% (Patel *et al*., 2001). Therefore this result indicated that terpinen-4-ol and DMPBD were highly varied due to the efficiency of the anabolism of volatile oil content. Sabinene was found in volatile oil content in a large proportion while the other monoterpenes fluctuated among them. (Table 2) The fluctuation of terpinen-4-ol and DMPBD may result in a high CV. However the factor that had an effect on the variation of terpinen-4-ol and DMPBD should be investigated further.

Table 2 Volatile oil constituents of cassumunar ginger (*Zingiber montanum*) at two month intervals from October 2003 to June 2005.

Compounds	Retention time (min)	(%) Area ratio of volatile oil constituents									
		4 months	6 months	8 months	10 months	14 months	16 months	18 months	20 months	22 months	24 months
α-thujene	7.87	tr	0.29	0.49	0.41	0.52	0.50	tr	tr	tr	tr
α-pinene	8.12	0.84	1.30	1.21	1.17	1.25	1.35	1.50	0.89	1.30	1.44
sabinene	9.47	26.14	57.22	49.07	48.06	32.36	41.30	44.40	34.25	38.15	49.69
β-myrcene	9.91	0.99	1.70	1.62	1.50	1.52	1.67	1.75	1.33	1.50	1.73
α-terpinene	10.83	1.91	0.98	2.29	1.53	3.54	3.03	2.80	1.65	2.43	2.12
ρ-cymene	11.11	0.88	0.22	0.87	0.73	0.60	0.66	0.81	0.88	0.97	0.99
β-phellandrene	11.26	1.125	0.92	0.95	0.71	1.29	1.19	1.25	0.81	0.94	1.23
γ-terpinene	12.34	3.71	1.92	4.08	2.99	6.46	5.35	5.40	3.85	5.16	4.15
sabinene hydrate	12.88	tr	0.47	0.34	0.41	tr	tr	tr	tr	tr	tr
terpinolene	13.40	0.71	0.37	0.85	0.53	1.30	1.04	0.99	0.71	0.86	0.76
terpinen-4-ol	16.93	14.40	6.88	17.25	11.61	17.28	15.10	18.79	17.46	18.15	11.87
α-terpinene	17.08	tr	0.30	0.41	0.29	tr	tr	tr	tr	tr	tr
terpinyl acetate	28.67	6.18	2.57	1.50	2.14	3.25	2.37	3.80	3.00	2.92	2.70
β-sesquiphellandrene	32.28	33.20	19.52	13.23	22.48	24.56	23.86	16.91	30.78	24.17	19.56
Total		90.07	94.65	94.13	94.53	93.91	97.40	98.38	95.59	96.53	96.21

tr = trace (< 0.1 %); components are listed in order of elution on DB-5 column (J&W Scientific, USA)

Table 3 Means of percentage area ratio of major components in volatile oil extracts.

Ages (months)	Percentage of area ratio		
	Sabinene	Terpinen-4-ol	DMPBD
4	$26.14^{d} \pm 14.61$	14.40 ± 0.18	33.20 ± 10.67
6	$57.22^{a} \pm 5.70$	6.88 ± 1.97	19.52 ± 5.49
8	$49.07^{ab} \pm 3.72$	17.25 ± 1.04	13.23 ± 7.36
10	$48.06^{ab} \pm 8.10$	11.61 ± 2.83	22.48 ± 4.36
12	$42.14^{bc} \pm 6.79$	14.40 ± 0.07	22.89 ± 5.29
14	$32.36^{cd} \pm 10.13$	17.28 ± 2.91	24.56 ± 3.59
16	$41.30^{bc} \pm 0.86$	15.10 ± 3.79	23.86 ± 5.08
18	$44.40^{abc} \pm 5.24$	18.79 ± 5.13	16.91 ± 1.77
20	$34.25^{cd} \pm 2.56$	17.46 ± 12.66	30.78 ± 15.29
22	$38.15^{bcd} \pm 6.27$	18.15 ± 0.04	24.17 ± 5.39
24	$49.69^{ab} \pm 4.25$	11.87 ± 2.56	19.56 ± 4.76
F-test	**	ns	ns
CV	8.99%	32.17%	23.76%

** Mean within each column followed by the same letter is not significantly different at 99% level of confidence based on Duncan' New Multiple Range Test.

ns = not significant

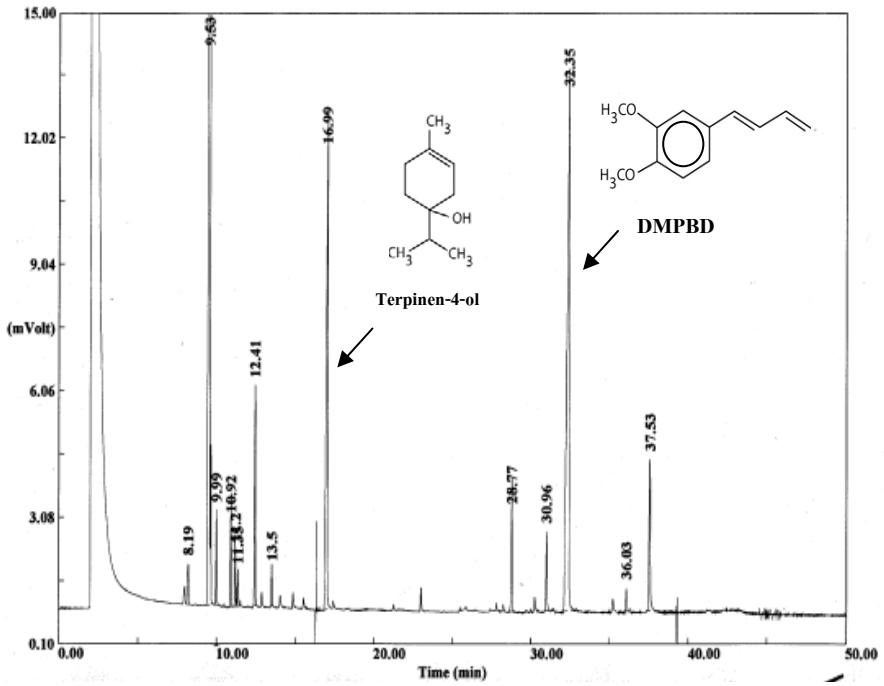

Figure 11 GC chromatogram obtained from hydro-distillation oil of cassumunar ginger (*Zingiber montanum*).

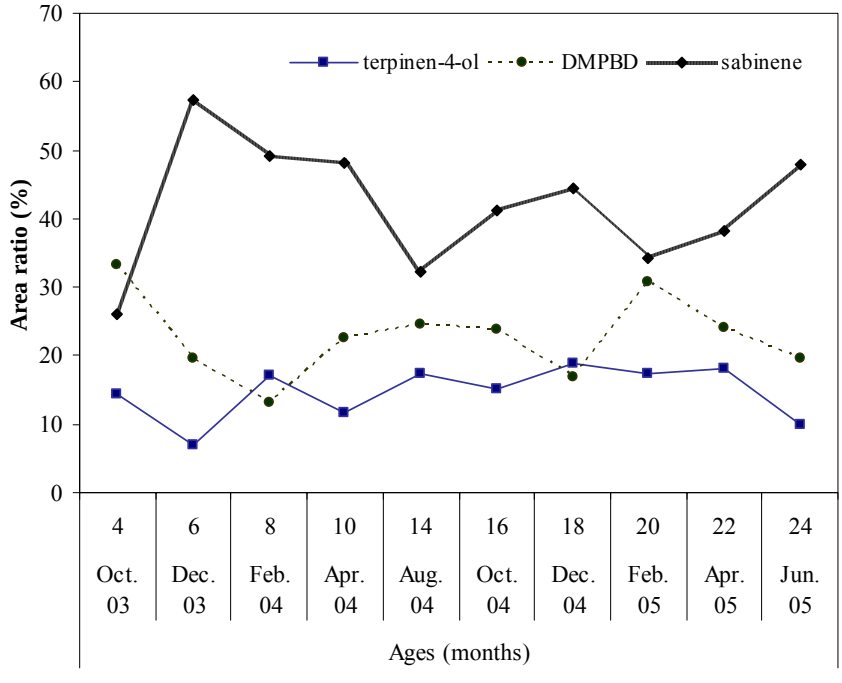

Figure 12 Percentage of sabinene, terpinen-4-ol and DMPBD in volatile oil of cassumunar ginger (*Zingiber montanum*) at 2 growth cycles.

1.3 Correlation between growth, major components, and climate

The results revealed that the age of rhizome is positively correlated with the fresh and dry weight of rhizome, antioxidant activity and volatile oil volume but is not correlated with the percentage of sabinene, terpinen-4-ol and DMPBD (Table4). There is no correlation among most major components except between sabinene and DMPBD which is negatively correlated. Negative correlation between sabinene and DMPBD indicates that these compounds might share the same metabolic pathway. This relationship should be investigated further.

With regard to correlation between climate and antioxidant activity, volatile oil volume and major component in volatile oil, the results reveal that only antioxidant activity was significantly correlated with soil temperature at 10 cm depth (Table 5) while the other parameters are not correlated (Table 6). This result indicates that soil temperature at the depth of rhizome layer has a positive effect on antioxidant activity. Tomaino *et al*. (2004) reported that antioxidant activity of clove, basil, oregano and thyme increased when incubated at temperature 80^{o}C compared with room temperature but the activity decreased at temperatures higher than 100 ^{o}C.

Table 4 shows antioxidant activity highly correlated with fresh weight but not correlated with dry weight. Similar results were indicated by Al-Farsi *et al*. (2005), reported that antioxidant activity of *Phoenix dactylifera* L. was significantly lost by sun-drying. Toor and Savage (2006) also reported that antioxidants (flavonoid, lycopene and ascorbic acid) and antioxidant activity of fresh tomato are higher than dried tomatoes. They indicated that the loss activity of antioxidants could be due to the decomposition of natural antioxidants after drying.

There is no significant correlation between the growth of cassumunar ginger and the environment during the first and the second growth cycle (Table 7 and Table 8). This result may indicate that the climatic conditions during that growth period are suitable for cassumunar ginger growth. The climatic fluctuation is not high enough to inhibit or to promote plant growth.

Table 4 Correlation between major components and growth of cassumunar ginger (*Zingiber montanum*).

	Age	Height	Shoot number	Leave number	Fresh weight	Dry weight	Volatile oil content	sabinene	terpinen-4-ol	DMPBD	Antioxidant activity
Age	1										
Height	.154	1									
Shoot number	.463	.772**	1								
Leave number	.003	.932**	.610*	1							
Fresh weight	.888**	.202	.450	.107	1						
Dry weight	.649*	-.172	.062	-.232	.833**	1					
Volatile oil content	.643*	.121	.380	.078	.574	.315	1				
sabinene	-.038	.206	.278	.324	.076	-.202	.435	1			
terpinen-4-ol	.405	-.094	-.112	-.106	.180	.275	-.180	-.582	1		
DMPBD	-.038	-.377	-.349	-.509	-.029	.288	-.183	-799**	.077	1	
Antioxidant activity	.801**	.166	.601	-.043	.665**	.583	.493	-.200	.421	.079	1

*,** Correlations are significant at the 0.05 and 0.01 level , respectively.

Table 5 Correlation between air temperature, soil temperature and major components in cassumunar ginger (*Zingiber montanum*).

	Air Temperature (^{o}C)			Soil temperature (^{o}C)				Antioxidant activity	Volatile oil content	sabinene	terpinen-4-ol	DMPBD
	Max	Min	Dif	0 cm	5 cm	10 cm	20 cm					
Air Temperature (oC)												
Max	1											
Min	.812**	1										
Dif	-.072	-.641*	1									
Soil temperature (^{o}C)												
0 cm	.878**	.946**	-.460	1								
5 cm	.860**	.957**	-.498	.997**	1							
10 cm	.847**	.959**	-.524	.993**	.999**	1						
20 cm	.830**	.957**	-.544	.988**	.996**	.999**	1					
Antioxidant activity	.444	.514	-.294	.552	.587	.603*	.598	1				
Volatile oil content	.295	.144	.141	.271	.271	.270	.277	.493	1			
sabinene	-.157	-.451	.565	-.322	-.341	-.351	-.338	-.200	.435	1		
terpinen-4-ol	.169	.093	.063	.094	.106	.116	.088	.421	-.180	-.582	1	
DMPBD	.213	.498	-.570	.383	.392	.390	.383	.079	-.183	-799**	.077	1

*,** Correlations are significant at the 0.05 and 0.01 level , respectively.

Table 6 Correlation between relative humidity, precipitation, light duration and major components in cassumunar ginger (*Zingiber montanum*).

	Relative humidity (%)		Precipitation (mm)	Light duration (hrs/day)	Antioxidant activity	Volatile oil content	sabinene	terpinen-4-ol	DMPBD
	Max	Min							
Relative humidity (%)									
Max	1								
Min	-.506	1							
Precipitation (mm)	-.389	.778**	1						
Light duration (hrs/day)	.315	-.878**	-.602	1					
Antioxidant activity	.212	.232	.211	-.093	1				
Volatile oil content	.377	-.162	-.232	.236	.493	1			
sabinene	.227	-.491	-.472	.281	-.200	.435	1		
terpinen-4-ol	.514	-.053	.082	.084	.421	-.180	-.582	1	
DMPBD	-.451	.446	.304	-.176	.079	-.183	-799**	.077	1

** Correlations are significant at the 0.01 level.

Table 7 Correlation between air temperature, soil temperature and cassumunar ginger (*Zingiber montanum*) growth.

	Air Temperature (oC)			Soil temperature (°C)				Age	Height	Shoot number	Leave number	Fresh weight	Dry weight
	Max	Min	Dif	0 cm	5 cm	10 cm	20 cm						
Air Temperature (°C)													
Max	1												
Min	.812**	1											
Dif	-.072	-.641*	1										
Soil temperature (°C)													
0 cm	.878**	.946**	-.460	1									
5 cm	.860**	.957**	-.498	.997**	1								
10 cm	.847**	.959**	-.524	.993**	.999**	1							
20 cm	.830**	.957**	-.544	.988**	.996**	.999**	1						
Age	.429	.290	.069	.361	.391	.406	.408	1					
Height	-.581	-.293	-.263	-.305	-.259	-.225	-.184	.154	1				
Shoot number	-.049	.153	-.325	.195	.243	.270	.303	.463	.772**	1			
Leave number	-.692*	-.468	-.110	-.512	-.474	-.443	-.406	.003	.932**	.610*	1		
Fresh weight	.220	.128	.071	.114	.151	.168	.179	.888**	.202	.450	.107	1	
Dry weight	.218	.162	.010	.074	.100	.107	.097	.649*	-.172	.062	-.232	.833**	1

*,** Correlations are significant at the 0.05 and 0.01 level , respectively.

Table 8 Correlation between relative humidity, precipitation, light duration and cassumunar ginger (*Zingiber montanum*) growth.

	Relative humidity (%)		Precipitation (mm)	Light duration (hrs/day)	Age	Height	Shoot number	Leave number	Fresh weight	Dry weight
	Max	Min								
Relative humidity (%)										
Max	1									
Min	-.506	1								
Precipitation (mm)	-.389	.778**	1							
Light duration (hrs/day)	.315	-.878**	-.602	1						
Age	.569	-.035	-.133	-.005	1					
Height	.071	.500	-.554	.361	.154	1				
Shoot number	.016	.453	.237	-.510	.463	.772**	1			
Leave number	.219	.374	.300	-.500	.003	.932**	.610*	1		
Fresh weight	.556	-.010	-.084	-.052	.888**	.202	.450	.107	1	
Dry weight	.405	-.059	.062	.103	.649*	-.172	.062	-.232	.833**	1

*,** Correlations are significant at the 0.05 and 0.01 level , respectively.

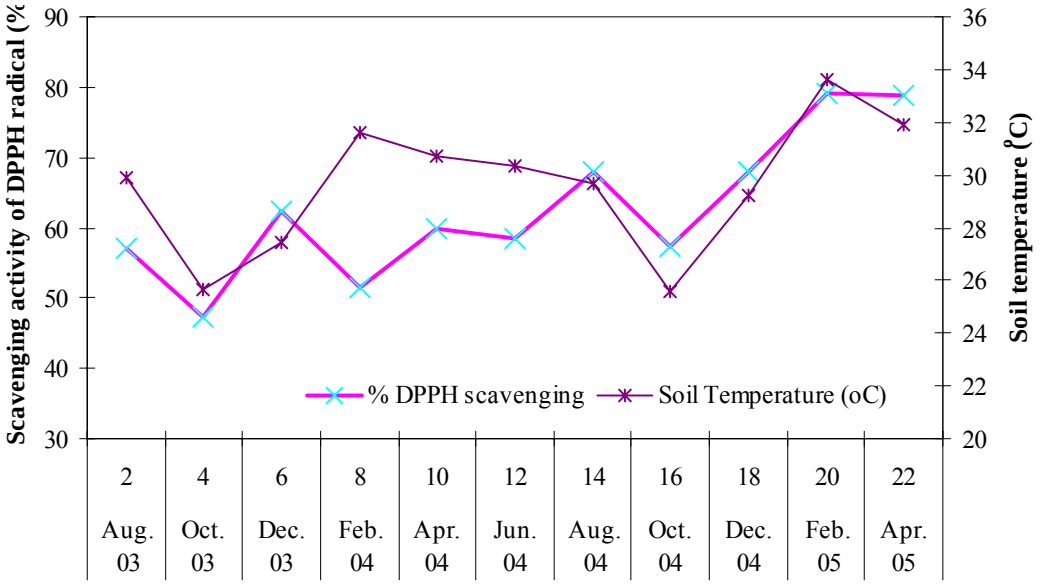

Figure 13 Correlation between percentage DPPH scavenging activity and soil temperature.

2. The effect of light intensity on biologically active components

Mean light intensity of each month and light intensity of each hour are shown in Figure 14 and 15 respectively. Light intensity during November, December, January, February, March, April and May were 46,366, 40,950, 52,216, 35130, 54,504, 51,008 and 42,098 respectively. This data revealed that light intensity fluctuated during the experimental period. According to light intensity during the day, the highest light intensity was at 11.00 am.

The experimental data provide information on controlled (treatment effect) and uncontrolled variation. The uncontrolled variation is expressed as experimental error called the 'coefficient of variation'. Besides, regarding fertility variation among experimental units, the factors contributing towards uncontrolled variation are climatic and experimental. Therefore, the CV of field experiments varies with the situation (Patel *et al*., 2001). This supported our results that the CVs of this experiment are rather high such as the fresh weight of rhizome, antioxidant activity, the volatile oil content and terpinen-4-ol. (Table 9 and Table 10) It is noticeable that high %CVs are from the underground part. The uncontrolled environment of this trial may be associated with precipitation which caused growth media compaction then affected plant growth and development. Therefore, the variation of this experiment is caused by performance in an open system which gives rise to uncontrolled variation.

2.1 Plant growth

The plant height of cassumunar ginger grown under full sun, 50% and 25% light intensity were 58.83, 86.82 and 90.72 cm, respectively; and rhizome fresh weight were 35.50, 73.00 and 82.50 gram/pot. Plant height and fresh weight of rhizome were significantly increased in contrast with the percentage of light intensity. In particular, fresh weight of rhizome increased almost 2 times when grown under low light intensity (Table 9).

The percentage of light intensity affected the growth of cassumunar ginger (Figure 16). Decreasing light intensity gradually promoted the plant height and fresh

weight of rhizome. Similar result was observed in *Antirrhinum majus* L. when gradually increasing shading ranged 0-68 percentage had positive effects on plant height, fresh weight and dry weight (Munir *et al*., 2004). Suh *et al*. (2005) reported that oxalis plant height and the number of leaves increased with shading levels of 35, 55, and 75 % as compared to open shade. Moreover, total chlorophyll and chlorophyll (a) content of leaf significantly increased when grown under 55 and 75% shading percentages.

Low light intensity promoted plant growth of cassumunar ginger greater than high light intensity (full sunlight) because of light transmission from a shaded net can prevent the critical sunlight. Xu *et al*. (2004b) reported that optimum photon flux density (PFD) photosynthesis, for a single leaf of ginger was about 1,290 μmol m^{-2} s^{-1}. Our observation on light intensity during the day indicated that at 10.00 am, 11.00 am, 12.00 pm, 1.00 pm and 2.00 pm mean light intensitoes are 103,300 116,800 95,800 69,325 and 55,807 lux which are equivalent to1,461, 1,612, 1,517, 1,438 and 1,304 μmol m^{-2} s^{-1}, respectively (data not shown). These values are higher than the optimum value. This result indicated that full sunlight had a direct negative effect on the growth of cassumunar ginger. High light intensity photon flux density can induce photoinhibition and even photodamage of photosynthetic apparatus in plants as reported by Kudairi (1970) that plenty of light directly damaged the chlorophyll.

Light intensity at 50% and 25% of full sunlight promotes the aerial stem height of cassumunar ginger. This result indicated that cassumunar ginger responds to low light intensity by a shade avoidance mechanism. The mechanism of shade avoidance operates via the differential absorption of red (R) and far red (FR) light by leaves. R light is absorbed strongly and FR light is transmitted. Thus, in dense canopies where R is absorbed in the lower portion of the canopy FR, leading to low differentiation and the competitive signal for stem elongation to capture more light (Khattak *et al*., 2004). Smith (1982) reported that light that passes through a canopy of leaves has a reduced red to far-red ratio (R: FR). In response to low R: FR ratio signals, many plants display a rapid and pronounced increase in the elongation growth rate of stem and petioles, often at the expense of leaf and storage organ development

(Franklin and Whitelam, 2005). Vandenbussche *et al*. (2003) reported that decreased light intensities coincided with increased ethylene production in *Arabidopsis rosettes*. Therefore R: FR ratio signals may act as an intermediary to hormones .Pierik et al. (2004) reported that phytochrome-mediated shade avoidance responses involve ethylene action, at least partly by modulating GA action in tobacco (*Nicotiana tabacum*). The elongation responses to low R:FR are harmonized by increased endogenous levels of active gibberellins (GA). The resulting increased GA action then loosen the cell walls then causes cell elongation.

However in field study, temperature is a cofactor in activating the light intensity effect. Shaded net can prevent extreme temperature. The temperature at 50% light intensity was lower than 75% and outside (full sunlight), respectively (Figure 17). Therefore heat and light are cofactors that affect plant growth when grown under full sunlight.

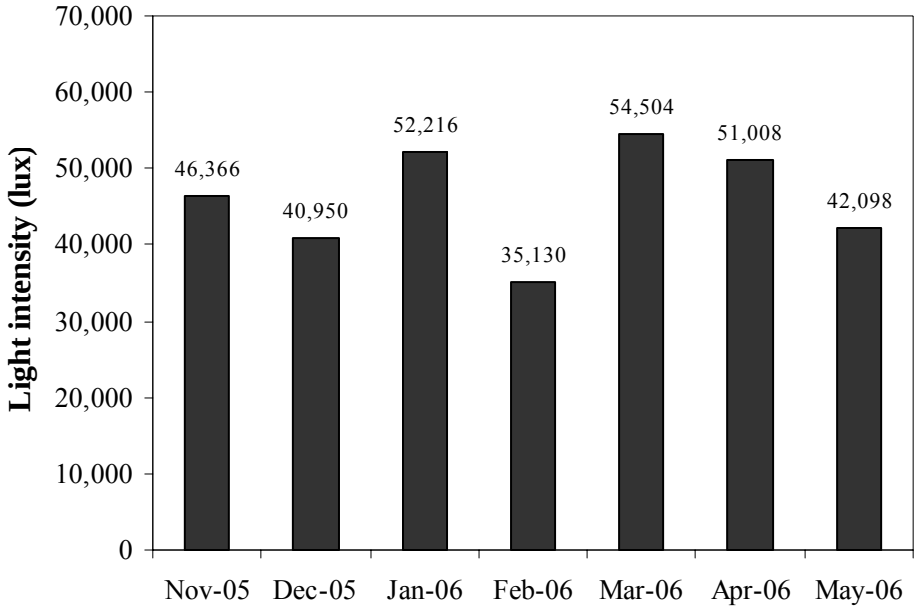

Figure 14 Mean light intensity under full sun for each month since November 2005 to May 2006.

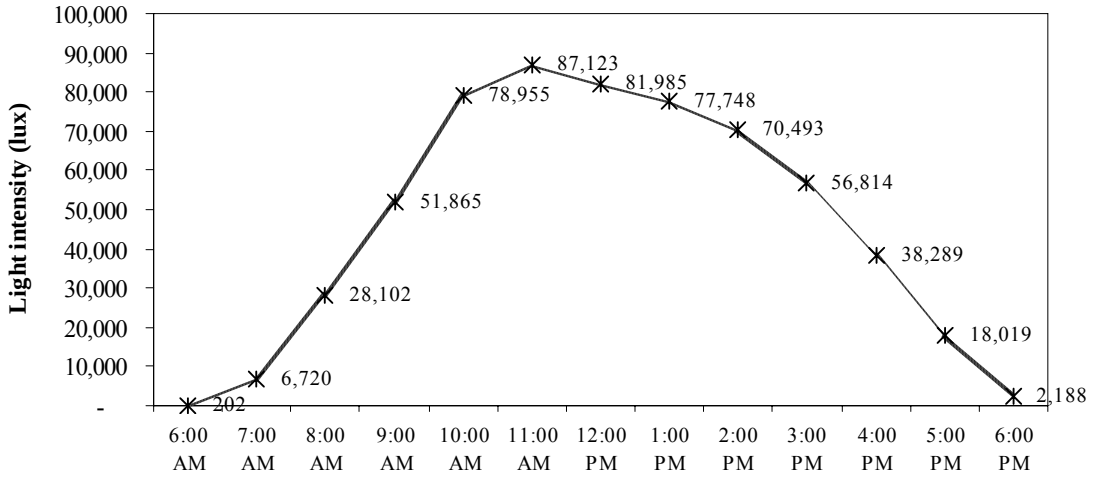

Figure 15 Mean light intensity under full sun of each hour during 6.00 am to 6.00 pm since November 2005 to May 2006.

Table 9 Height and fresh weight at various percentage of light intensity.

Light intensity	Height (cm)	Fresh weight (g/pot)
(full sunlight)	53.83[b]±7.28	35.50[b]±14.80
50%	86.82[a]±9.96	73.00[a]±25.08
25%	90.72[a]±15.31	82.50[a]±29.74
F-test	**	**
CV	14.72%	37.75%

** Mean within each column followed by the same letter is not significantly different at 99% level of confidence based on Duncan' New Multiple Range Test.

Table 10 The effect of light intensity on antioxidant activity, volatile oil content and volatile oil constituents.

Light intensity	Antioxidant activity	Volatile oil Content (ml/kg)	Volatile oil constituent		
			sabinene	Terpinen-4-ol	DMPBD
100%	52.98±14.28	15.34[a]±4.53	56.34±1.48	10.17±1.94	10.88±2.96
50%	41.75±12.00	10.13[b]±3.33	55.29±4.29	7.16±3.58	14.7±2.51
25%	42.93±12.72	7.87[b]±2.07	52.64±3.87	10.15±3.12	13.43±0.90
F-test	ns	*	ns	ns	ns
CV	27.94%	31.11%	6.66%	32.32%	17.70%

* Mean within each column followed by the same letter is not significantly different at 95% level of confidence based on Duncan' New Multiple Range Test.
ns = not significant

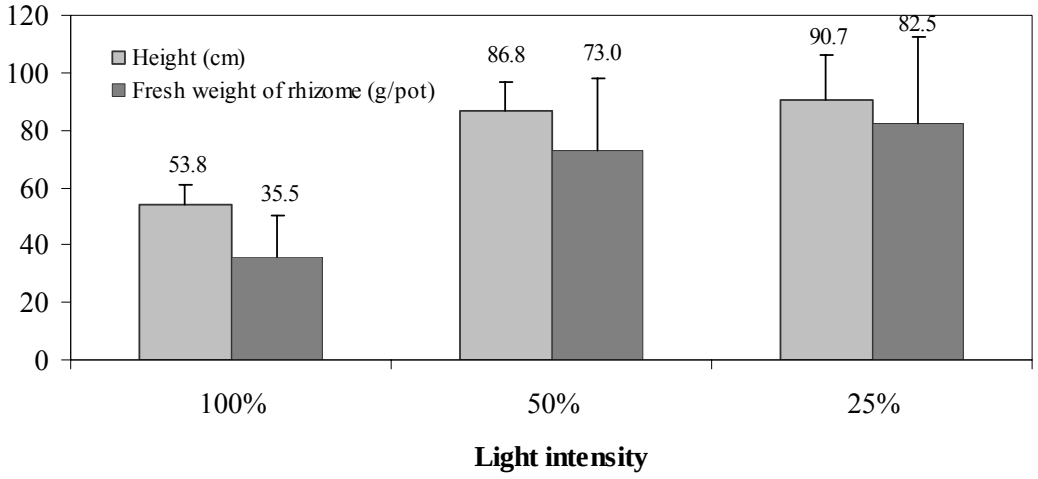

Figure 16 The effect of light intensity on plant height and rhizome fresh weight of cassumunar ginger (*Zingiber montanum*).

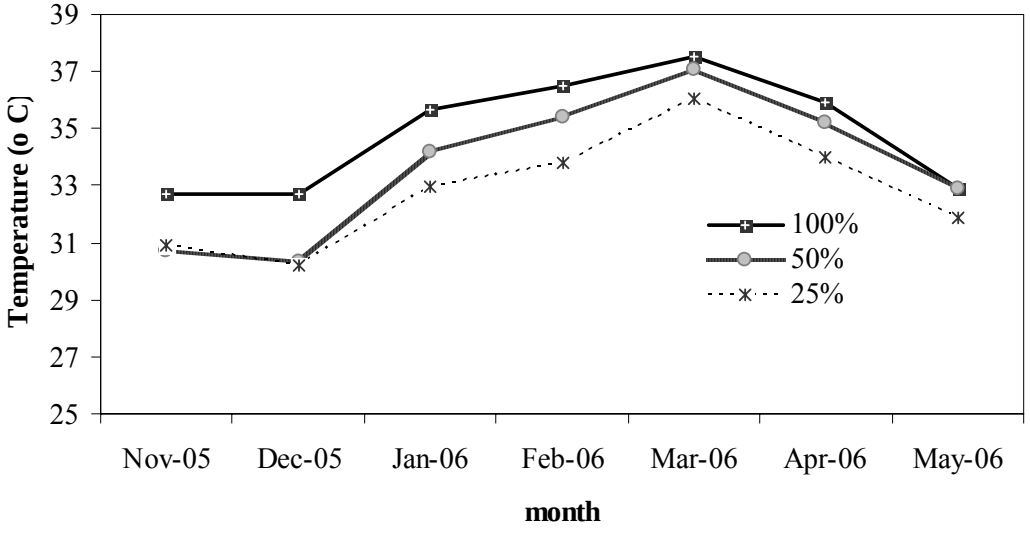

Figure 17 Air temperature in full sun and under 50% and 25% light intensity between November 2005 and May 2006.

2.2 Plant quality

2.2.1 Antioxidant activity

Oxidative stress occurs when there is a serious imbalance in any cell compartment between the production of reactive oxygen species (ROS) and antioxidant defense, leading to damage. The production of ROS, such as super oxide anion (O_2^-), hydrogen peroxide (H_2O_2), singlet oxygen $^1O_2$, is an unavoidable consequence of aerobic metabolism. In plants, ROS are produced in mitochondria, chloroplasts, and nitrogen-fixing nodules as unwanted by products. ROS production also occurs in the course of major metabolic pathways, especially those in the peroxisomes, and ROS are used as a weapon against invading pathogens in the oxidative (Møller, 2001).

From our results, the antioxidant activity of cassumunar ginger is not significantly different when grown under different light intensities. Antioxidant activity of 52.98%, 41.75% and 42.93% were obtained from the plants grown under 100% (full sunlight), 50% and 25%, respectively (Table 10). However, antioxidant activity tends to decrease with decreasing light intensity (Figure 18). It is clear that cassumunar gingers did not suffered from oxidative stress at different light intensities because of the equilibrium between ROS and antioxidants from cassumunar ginger such as cucuminoid, cassumunin A,B and C and volatile oil.

Cassumunar ginger grown under high light intensity has the highest antioxidant activity. This resulted from the loss of rhizome moisture content. Therefore, based on fresh weight, antioxidant activity of the plant grown under full sunlight is higher than that grown under low light intensity.

2.2.2 Volatile oil content

Volatile oil content significantly decreased while grown under decreased light intensity at 50% and 25% respectively. The plant grown under full sun

light gave maximum yield of volatile oil (15.34 ml/kg) compared with those grown under low light intensity at 50% (10.13 ml/kg) and 25% (7.87 ml/kg). Hälvä *et al*. (1992) similarly reported that dill essential oil accumulation increased with increases in the light level and was greatest under full sunlight. Wang and Lincoln (2004) noted that *Myrica cerifera* treated in high light intensity had significantly higher monoterpene content, higher growth rate, and denser glandular trichomes than the plants treated in low light intensity.

Volatile oil content is gradually decreased with decreased light intensity (Figure 19) while fresh weight increased (Figure 16). This may be explained by cassumunar ginger that is grown under sunlight having a photosynthetic rate greater than that grown under low light intensity. This is supported by Nagamatsu-Lopez *et al*. (2003)who reported that *Amaranthus hypochondriacus* plants were grown under three photosynthetic photon flux densities (PPFD). Mature plants grown at full sunlight (38.8 mol m^2 d^{-1}) had higher maximum net photosynthetic rate (PN) than plants that developed under lower PPFD (19.4 and 12.8 mol m^2 d^{-1}). The ultimate partitioning of the photosynthetically fixed carbon is an important component of the physiological mechanism of essential oil production. Therefore, photosynthetics are the centre stage in making a carbon skeleton for the anabolism of the oil components (Sangwan *et al*., 2001)

The low R:FR may be associated with the low volatile oil content of cassumunar ginger that is grown under low light intensity. The low R:Pfr have an increased endogenous level of active GA. It belongs to terpreniods which are built from five carbon isoprene units; the immediate precursor to GA is a diterpenene. The mevalonic acid pathway is used for the biosynthesis of GA (Arteca, 1996). GA and volatile oil share metabolic pathways; therefore, cassumunar ginger under low light intensity convert melvalonic to GA more than producing volatile oil. This resulted in a low volatile oil content of cassumunar ginger that is grown under low light intensity.

Moreover, cassumunar ginger that is grown under full sunlight which is activated by high temperature may increase lost rhizome moisture content

than that grown under light intensities of 50% and 25%. Thus with the same weight of rhizome the amounts of volatile oil are not equal.

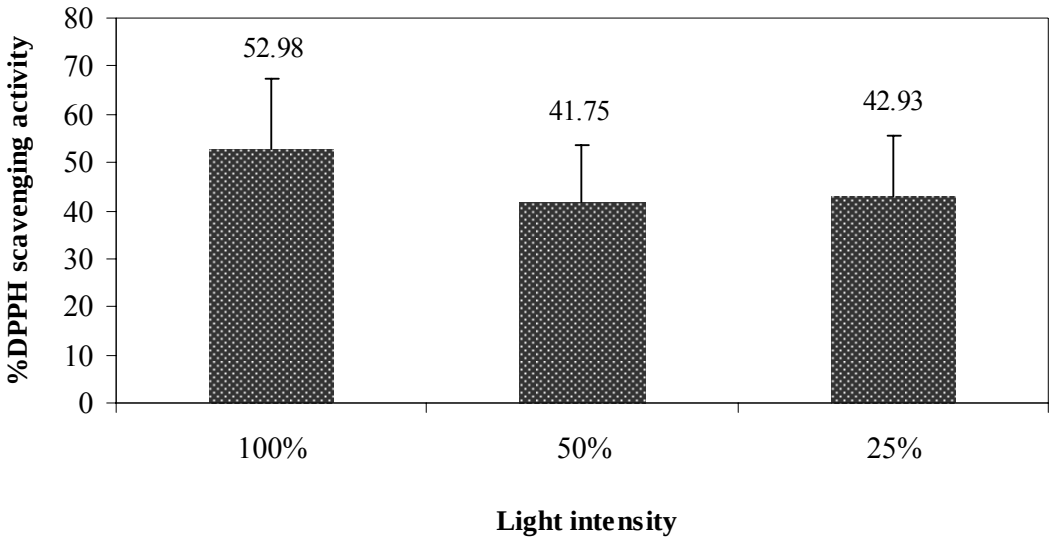

Figure 18 The effect of the percentage of light intensity on the antioxidant activity of the cassumunar ginger (*Zingiber montanum*) rhizome.

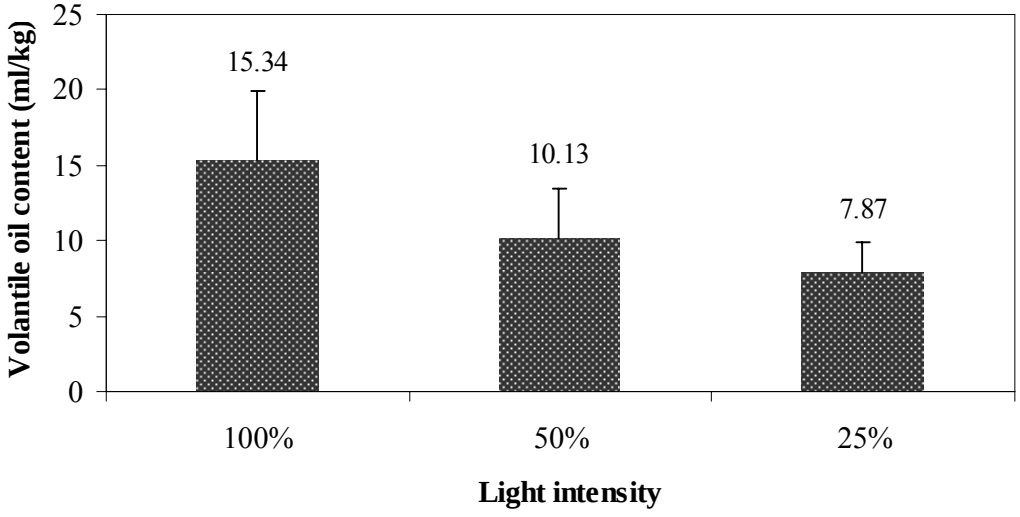

Figure 19 The effect of the percentage of light intensity on volatile oil contents of the cassumunar ginger (*Zingiber montanum*) rhizome.

2.2.3 Volatile oil constituent

The volatile oil of cassumunar ginger contains at least 15 identified compounds, Fourteen compounds of monoterpene expresses at the early part of chromatogram as follows: α thujene, α-pinene, sabinene,β-myrcene, α-terpinene, *p*-cymene,. β-phellandrene, γ-terpinene, sabinene hydrate, terpinolene, terpinen-4-ol, terpinyl acetate and β-sesquiphellandrene with corresponding mean retention times at 9.18, 9.46, 11.05, 11.34, 12.34, 12.62, 12.76, 13.92, 14.26, 14.99, 18.65, 18.92, 24.59 and 30.55 min, respectively. The peak of benzene derivative which is (*E*)-1-(3,4-dimethoxyphenyl)-butadiene (or DMPBD) came out later with the mean retention time at 34.25 min (Table 11). With regard to the major active components in volatile oil, light intensity has no effect on sabinene, terpinen-4-ol and DMPBD. However, sabinene tends to be decreased by a decreasing percentage of light intensity (Table 10).

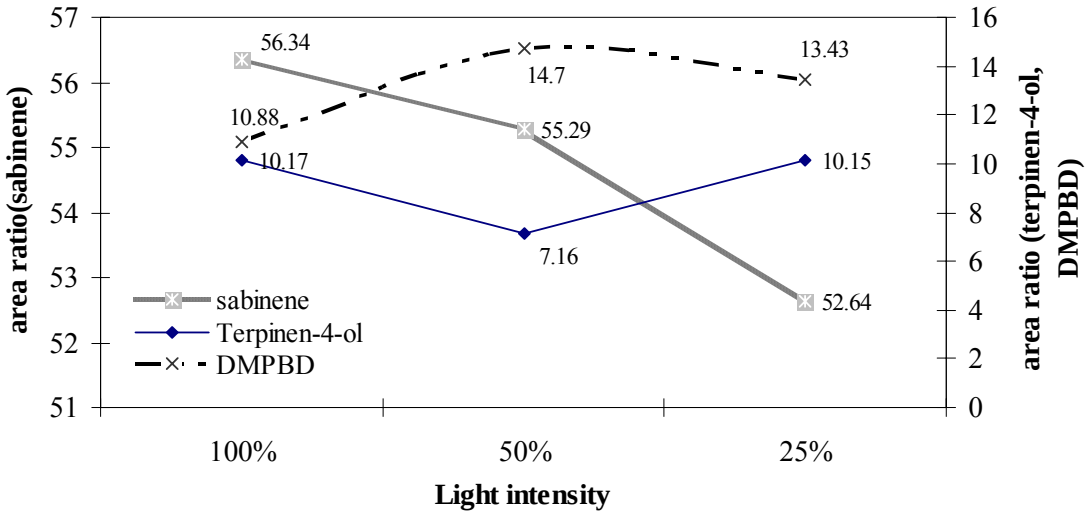

Figure 20 The effect of the percentage of light intensity on volatile oil constituents of cassumunar ginger (*Zingiber montanum*).

Table 11 The volatile oil constituents of cassumunar ginger (*Zingiber montanum*) under various light intensities from August 2005 to May 2006.

Volatile oil constituent	Retention time	Light intensity		
		100%	50%	25%
α-thujene	9.18	0.47	0.46	0.44
α-pinene	9.46	1.61	1.55	1.53
sabinene	11.05	56.34	55.29	52.64
β-myrcene	11.34	1.90	2.03	2.03
α-terpinene	12.34	2.08	2.49	2.98
ρ-cymene	12.62	0.58	0.68	0.65
β-phellandrene	12.76	1.10	1.05	1.19
γ-terpinene	13.92	4.25	5.08	6.20
sabinene hydrate	14.26	0.29	tr	0.09
terpinolene	14.99	0.76	0.95	1.08
terpinen-4-ol	18.65	10.17	7.16	10.15
α-terpinene	18.92	0.14	0.16	0.13
terpinyl acetate	24.59	0.32	0.27	0.27
β-sesquiphellandrene	30.55	3.75	2.76	2.83
DMPBD	34.25	10.88	14.70	13.43

tr = trace (< 0.1 %); components are listed in order of elution on DB-5 column (J&W Scientific, USA)

3. The effect of water deficit on plant growth and biologically active components

The coefficients of variations of all parameters in this experiment were in an acceptable range because this experiment was performed in a greenhouse where the environment can be controlled.

3.1 Plant growth

Our results show that water deficit did not affect plant height but did affect fresh weight (Table 12). Plant height was not affected by water deficit because the application time was applied after maximum plant growth (Figure 21). However, even water deficit does not affect plant height but after watering was stopped at 120 days before harvesting, cassumunar ginger entered the dormancy stage as can be seen from the yellowing, wilting and collapse of the aerial parts within one week after watering was stopped. Water deficit at 90 and 60 days exhibit the same growing pattern (Figure 22). With regard to control and water deficit at 30 days, the aerial parts were dried together, in the same week. Our result shows that cassumunar ginger needs a dormancy period because even when watering until harvested time, the aerial part still dried and collapsed. Watering may prolong vegetative plant growth but cannot prevent cassumunar ginger entering dormancy stage (Figure 22).

Fresh weight was affected by water deficit. The lowest fresh weight of rhizome (40 g/pot) was obtained from the 120 days water deficit treatment while the other treatments were not significantly different. Fresh weights tend to decrease with the increased number of days of water deficit (Table 10). This may result from the accumulation of food storage in rhizome which has not yet reached its peak when the aerial parts start yellowing and collapse soon after watering is stopped. With this condition rhizome has insufficient storage of food for use during a dry period until harvest. Besides this treatment was subjected to a long period of water deficit; therefore, water potential in the growing media decreased leading to the loss of rhizome moisture content. This result indicates that water deficit induces early dormancy in cassumunar ginger.

Table 12 The effect of water deficit on plant height and rhizome fresh weight of cassumunar ginger (*Zingiber montanum*).

Water deficit	Height (cm.)	Fresh weight (g)
Control	81.29±6.79	104[a]±11.94
30 days	80.77±5.76	90[a]±10.00
60 days	78.37±3.15	84.[a]±19.84
90 days	76.44±7.14	73[a]±15.65
120 days	74.23±7.18	40[b]±21.51
F-test	ns	**
CV	7.92%	20.95%

** Mean within each column followed by the same letter is not significantly different at 99% level of confidence based on Duncan' New Multiple Range Test.
ns = not significant

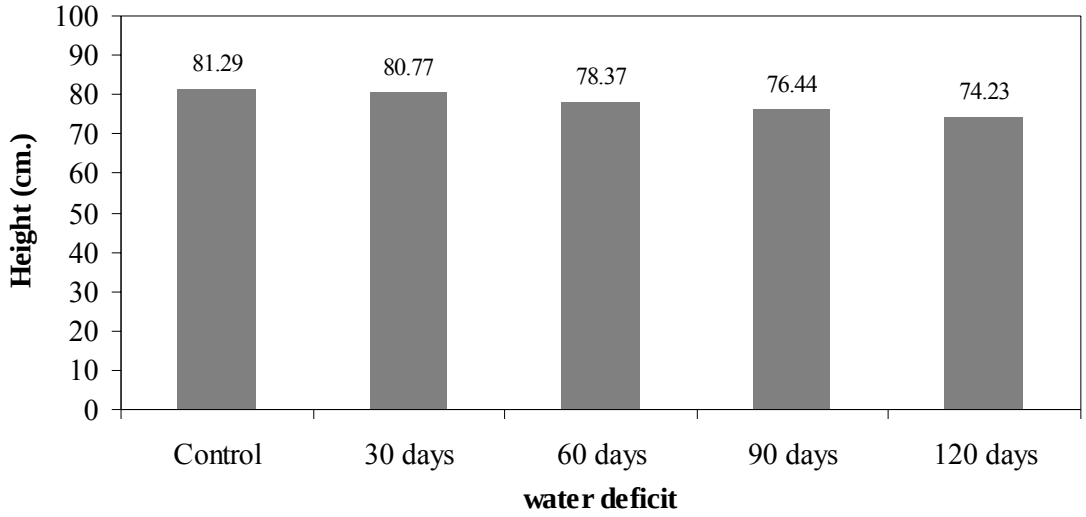

Figure 21 The effect of water deficit on plant height of cassumunar ginger (*Zingiber montanum*).

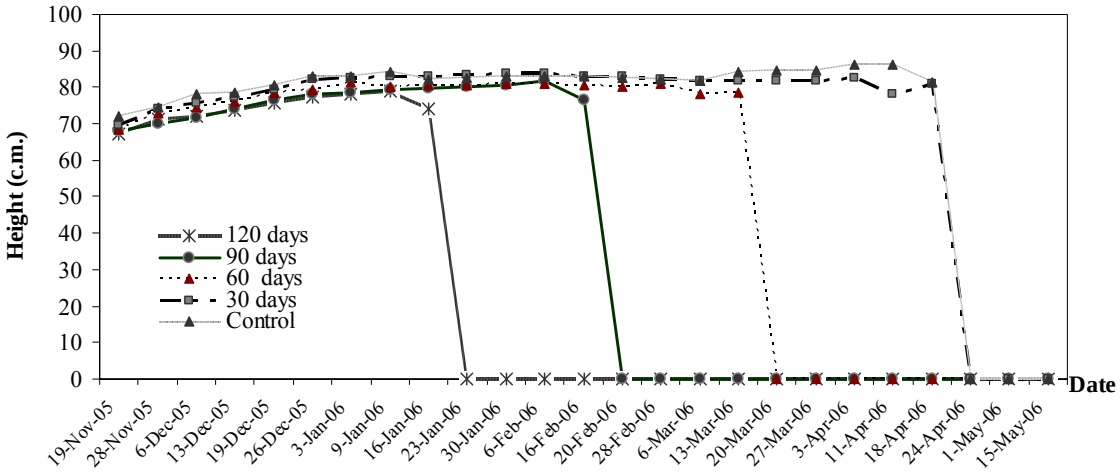

Figure 22 The effect of water deficit on the dormancy of cassumunar ginger (*Zingiber montanum*).

3.2 Plant quality

Water deficit has an effect on volatile oil content, sabinene and terpinen-4-ol but not on antioxidant activity and DMPBD (Table 13).

Table 13 The effect of water deficit on antioxidant activity, volatile oil content and volatile oil constituents of cassumunar ginger.

Water deficit (day)	Antioxidant activity %	Volatile oil Content (ml/kg)	Volatile oil constituent		
			Sabinene	Terpinen-4-ol	DMPBD
0	48.94±12.31	12.32^{b}±2.20	44.23^{b}±4.58	12.84^{ab}±1.08	21.03±3.96
30	47.61±3.97	12.06^{b}±3.65	45.59^{ab}±6.65	10.64^{b}±1.44	21.09±4.44
60	49.49±4.12	14.05^{b}±2.25	45.50^{ab}±4.67	14.4^{a}±1.90	17.97±3.82
90	53.78±9.16	14.47^{b}±1.79	46.06^{ab}±2.11	13.11^{ab}±3.24	20.61±4.47
120	54.85±13.99	23.80^{a}±0.74	52.89^{a}±2.04	10.41^{b}±1.18	15.58±2.93
F-test	ns	**	*	*	ns
CV	18.91%	15.15	9.34%	15.77%	20.59%

*, ** Mean within each column followed by the same letter is not significantly different at the 95% level of confidence and the 99% level of confidence, respectively based on Duncan' New Multiple Range Test.
ns = not significant

3.2.1 Antioxidant activity

Antioxidant activity is shown as % DPPH scavenging activity. Water deficit does not significantly affect antioxidant activity (Table13) but there is a tendency to increase activity with the prolonged water deficit period (Figure 23). The high antioxidant activity found at 120 days of water deficit may result from the

increase in volatile oil content in that treatment in accordance with Lertsatitthanakorn *et al*. (2006) who reported that volatile oil also exhibited antioxidant activity.

Water deficit does not significantly affect antioxidant activity because of the response mechanism of this plant to stress. Cassumunar ginger survives the dry season by the underground rhizome entering at dormancy stage. Myking (1998) reported that during the dormancy stage, respiration is decreased compared with the vegetative stage. This research was observed in buds of three hard woods (*Betula pendula* Roth., *Prunus padus* L. and *Alnus glutinosa* L.). During respiration, reactive oxygen species can be formed (ROS), such as superoxide anions, hydrogen peroxide and hydroxyl radical. Boveris and Chance (1973) reported that mitochondria convert 1–2% of the oxygen consumed into the superoxide anion. This free radical can cause significant cell stress and damage; thus, antioxidant protection is essential for survival under an aerobic environment. Therefore, cassumunar ginger at resting stage with low respiration does not suffer from oxidative stress.

However, antioxidant activity of cassumunar ginger tends to increase with the level of water deficit. The explanation for this result is the increase in volatile oil contents by almost 2 times under stress conditions. The amount of antioxidant in volatile oil is also increased

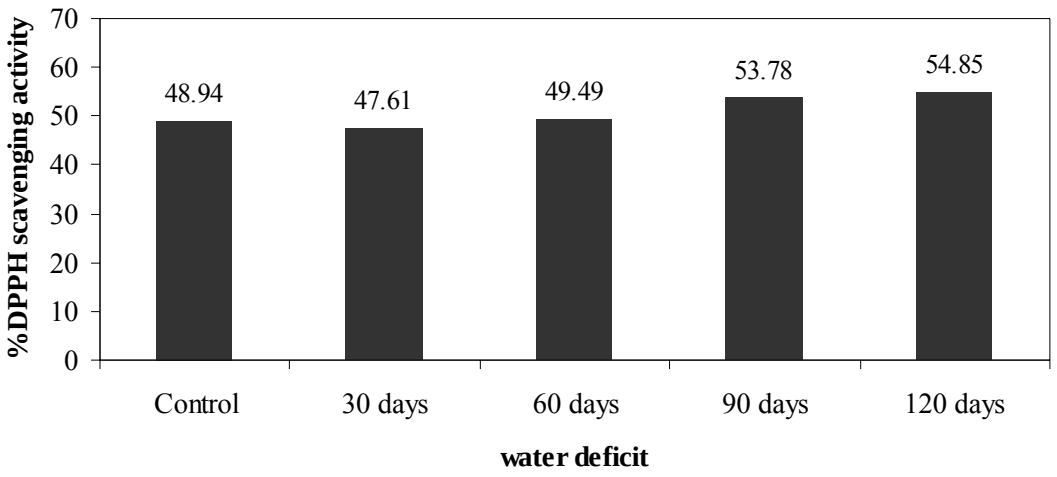

Figure 23 The effect of water deficit on the antioxidant activity of cassumunar ginger (*Zingiber montanum*).

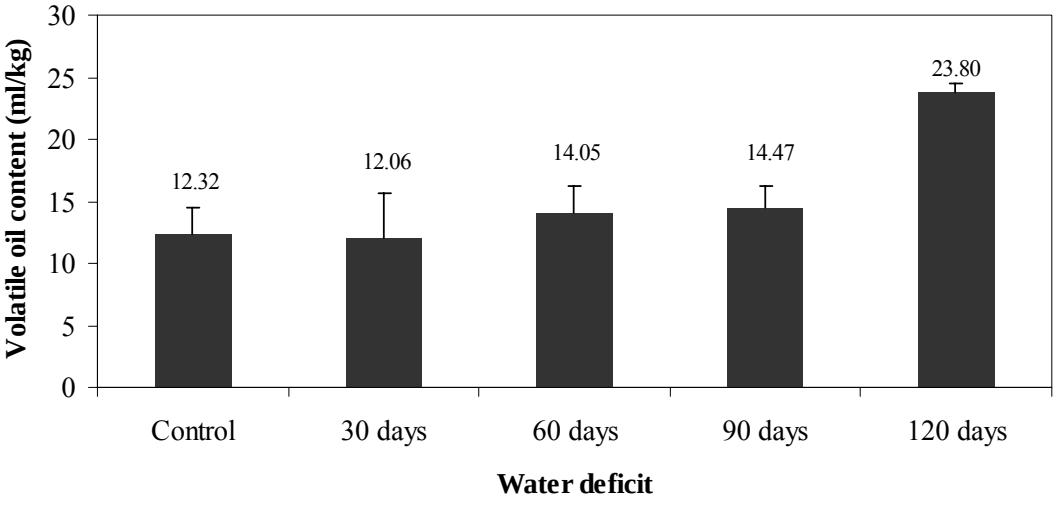

Figure 24 The effect of water deficit on the volatile oil content of cassumunar ginger (*Zingiber montanum*).

3.2.2 Volatile oil content

Volatile oil content was reported as the volatile oil volume (milliliter) based on fresh weight (kilogram). Water deficits significantly affected volatile oil content. Water deficit at 120 days gave maximum volatile oil content (23.80 ml/kg). However water deficit at 0 day (control), 30, 60 and 90 days were not significant in volatile oil 12.32, 12.06, 14.05 and 14.47 ml/kg, respectively (Table 13). The quantity of volatile oil of water deficit 120 days treatment was higher than the other treatments by almost two times (Figure 24).

This experiment has clearly shown that the duration of water deficit plays an important role on volatile oil content in cassumunar ginger. Similar results were reported by Sigh-Sangwan *et al* (1994). *Cymbopogon pendulus* (Steud.) Wats. was grown under mild and moderate water stress, watering second and third day, respectively for 45 and 90 days. The volatile oil content increased by 22% when the plants grown under moderate stress for 45 days and increased approximately 37.9% while grown under mild and moderate stress of 90 days. Baher *et al*. (2002) reported that the accumulation of *Satureja hortensis* L. oil increased significantly under severe water stress (when the mean leaf water potential decreased from -0.5 to -1.6 MPa). Charls *et al*., (1990) and Simon *et al*., (1992) had suggested that , under stress, due to the reduction in leaf area, a higher density of the oil glands results in an elevated amount of oil accumulation. This supported our result that volatile oil content under stress is higher.

3.2.3 Volatile oil constituent

The volatile oil of cassumunar ginger is composed of at least 14 monoterpene compounds which were expressed at the beginning of the chromatogram as follows: α thujene, α-pinene, sabinene,β-myrcene, α-terpinene, *p*-cymene, β-phellandrene, γ-terpinene, sabinene hydrate, terpinolene, terpinen-4-ol, α-terpinene, terpinyl acetate and β-sesquiphellandrene corresponding to their mean retention times at 8.49, 8.71, 10.21, 10.57, 11.52, 11.82, 11.96, 13.08, 13.61, 14.14,17.76, 18.35,

23.81 and 29.55 min, respectively. The peak of benzene derivative which is (*E*)-1-(3,4-dimethoxyphenyl)-butadiene (or DMPBD) which appears later on the chromatogram, gave the mean retention at 33.42 min. (Table 14, Appendix $C_{14\text{-}18}$).

Water deficits significantly affected the sabinene and terpinen-4-ol content but not the DMPBD. Water deficit at 120 days gave maximum sabinene (52.89%) while control gave the lowest sabinene (44.23%). With regard to the percentage of sabinene content, there is a tendency to increase with increasing days of water deficit. Consideration on percentage of terpinen-4-ol, water deficit at 60 days gave the highest percentage of terpinen-4-ol (14.4 %) and water deficit at 120 days gave the lowest percentage of terpinen-4-ol (10.41%). Both terpinen-4-ol and DMPBD have a tendency to decrease with a prolonged water deficit period (Figure 25).

Water stress affected cassumunar ginger volatile oil constituents. This finding was supported by Singh-Sangwan (1994) who reported that geraniol and citral, major constituents of essential oil of lemongrass, are changing due to mild and moderate water stress; and water stress also causes change in the oil composition in mint (Charles *et al*., 1990) and sweet basil (Simon *et al*., 1992).

The volatile oil constituent of cassumunar ginger contains a high percentage of sabinene and it was increased with increasing days of water deficit. This is in line with Turtola *et al*. (2003), who reported that sabinene concentration increased when Norway spruce (*Picea abies (*L.*)* Karst.*)* grown under control, medium drought and severe drought, respectively. These results indicate that water stress may affect the monoterpene enzyme. Adam and Croteau (1998) noted that sabinene comes from sabinene synthase which catalyzes the cyclization of geranyl diphosphate. Therefore, water deficit may promote the activity of sabinene synthase which is a key enzyme in sabinene synthesis.

Water deficit at 120 days gave the lowest percentage of terpinen-4-ol while the highest percentage is found in the treatment with a 60 day water deficit.. Terpinen-4-ol in essential oil of tea tree is not an immediate product of monoterpene

synthase but is derived by rearrangement from another oxygenated monoterpene, sabinene-hydrate (Southwell and Stiff, 1989). Cornwell *et al*. (1995) also reported that ρ-menthanes terpinen-4-ol, α- and γ-terpinene, terpinolene and ρ-cymene are unlikely to be enzyme products but rather artifacts derived from cis-sabinene hydrate, trans-sabinene hydrate and sabinene. Therefore terpinen-4-ol in cassumunar ginger may be a result of the skeletal rearrangement of sabinene hydrate.

Table 14 Volatile oil constituent of cassumunar ginger (*Zingiber montanum*) at various water deficits from August 2005 to May 2006.

Volatile oil constituent	Retention time	Water deficit				
		0 days	30 days	60 days	90 days	120 days
α-thujene	8.49	0.47	0.41	0.43	0.50	0.44
α-pinene	8.71	1.31	1.26	1.42	1.44	1.56
sabinene	10.21	44.23	45.58	45.50	46.06	53.46
β-myrcene	10.57	1.59	1.65	1.67	1.38	1.83
α-terpinene	11.52	2.36	2.34	2.36	2.31	2.10
ρ-cymene	11.82	0.59	0.30	0.45	0.67	0.34
β-phellandrene	11.96	1.01	0.91	0.99	1.09	1.11
γ-terpinene	13.08	4.79	4.81	4.34	4.99	4.40
sabinene hydrate	13.61	0.21	0.19	0.27	0.20	tr
terpinolene	14.14	0.85	0.87	0.86	0.86	0.60
terpinen-4-ol	17.76	12.84	10.64	14.40	13.11	10.14
α-terpinene	18.35	0.23	0.14	0.23	0.18	0.14
terpinyl acetate	23.81	0.29	0.28	0.27	0.25	0.26
β-sesquiphellandrene	29.55	2.11	2.25	1.88	1.78	3.01
DMPBD	33.42	21.03	21.09	17.97	20.61	14.96

tr = trace (< 0.1 %); components are listed in order of elution on DB-5 column (J&W Scientific, USA)

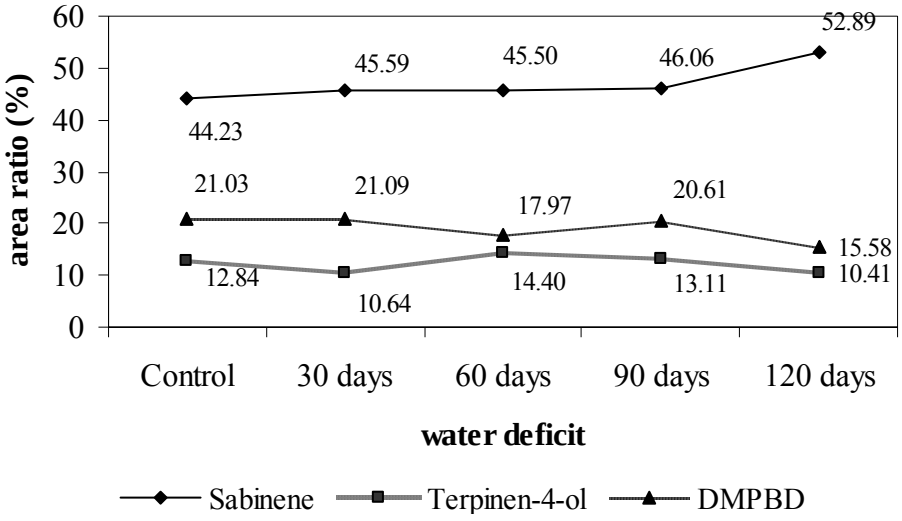

Figure 25 The effect of water deficits on volatile constituents of cassumunar ginger (*Zingiber montanum*).

CONCLUSION AND RECOMMENDATION

Conclusion

From our experiment, the conclusions are:

1. Rhizome age had positive correlation with fresh weight, dried weight, antioxidant activity and volatile oil content but was not correlated with sabinene, terpinen-4-ol and DMPBD. Soil temperature at 10 cm. depth had an effect on antioxidant activity but not on volatile oil content and the active ingredient.

2. Light intensity, at 50% and 25% promotes cassumunar ginger growth but lower volatile oil content. Light intensity has no effect on antioxidant activity

3. Water deficit for 120 days before harvest gave the highest volatile oil content but the lowest fresh weight. Antioxidant activity was not affected by water deficit. However, water deficit for 120 days promoted an increase in sabinene but water deficit at 60 days gave the highest terpinen-4-ol.

Recommendation

The fresh weight of cassumunar ginger of the first experiment was shown as kilogram per clump. But generally the productivity of cassumunar ginger should be presented as weight per area. The spacing of cassumunar ginger is 75 cm between rows and 25 cm between plants thus the productivity was 8,533.33 clump per rai (rai = 1,600 m^2). Therefore, the productivity of cassumunar ginger at different rhizome age is calculated using this number multiplied by the rhizome fresh weight of cassumunar ginger (Appendix D1). Volatile oil productivity of cassumunar ginger at various rhizome ages is also calculated the same way and the result is presented in appendix D2.

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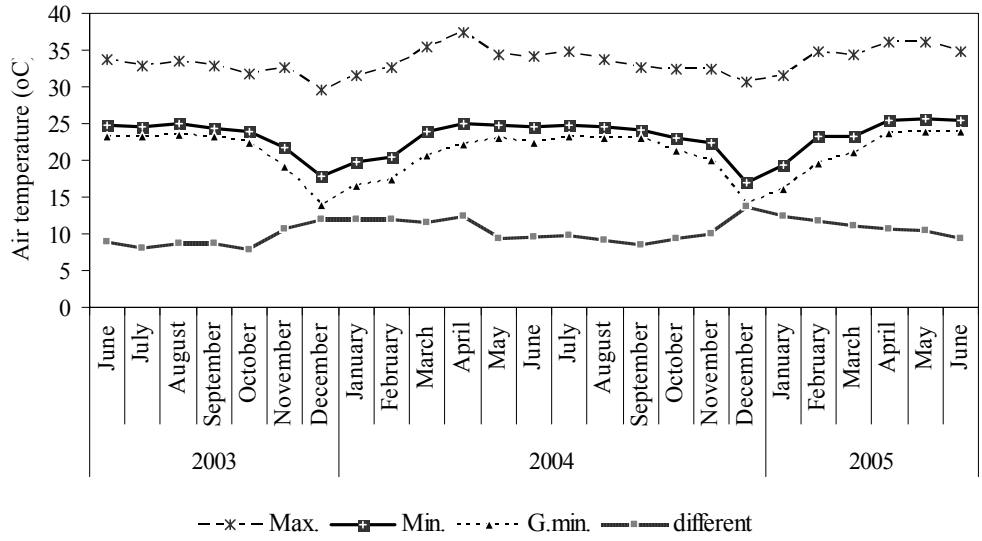
APPENDICES

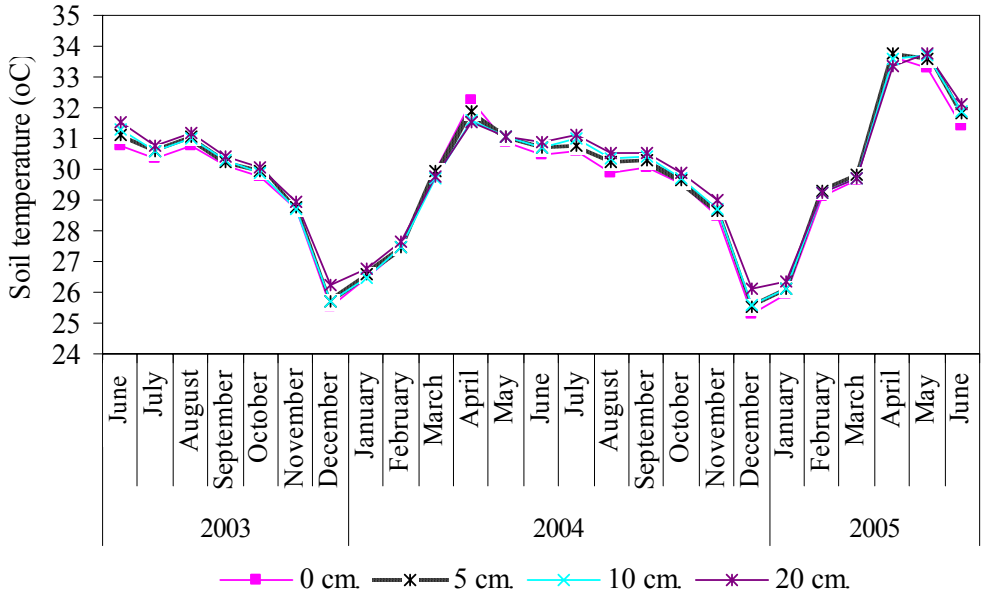
Appendix A

Meterological data

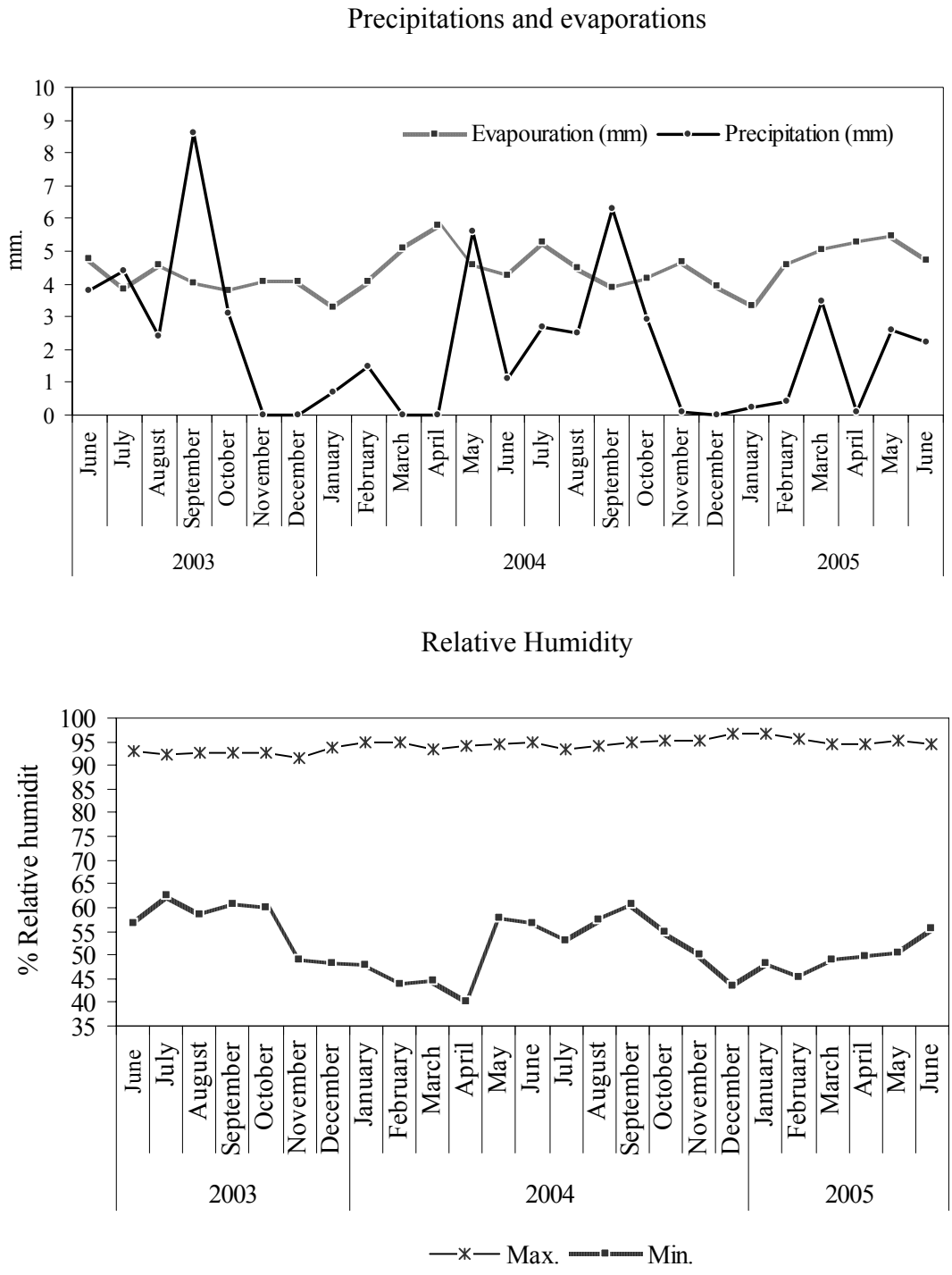
Air temperature



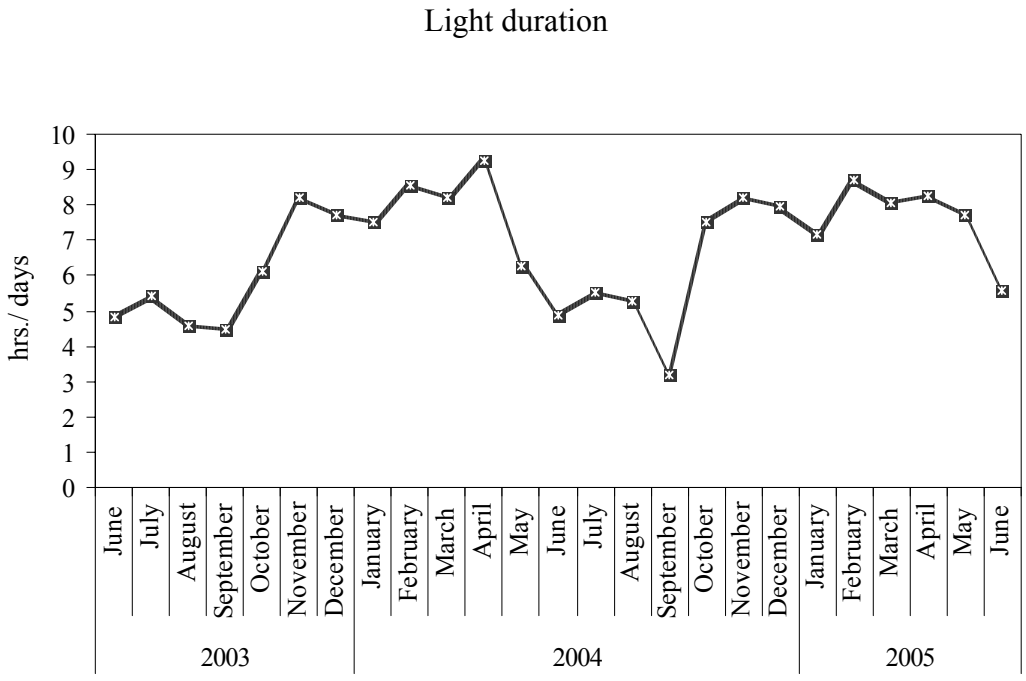
Soil temperature

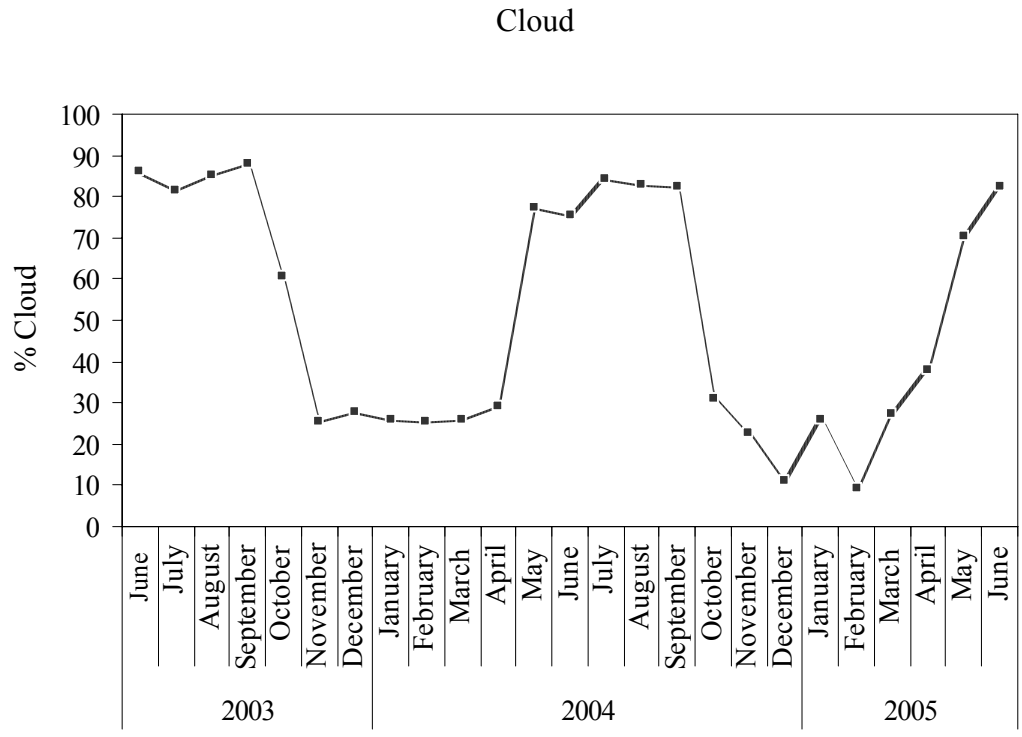


Appendix Figure A1 Mean air temperature and soil temperature since June 2003 to June 2005 at Nakhonpathom Meteorological Station at Kasetsart University, Kampang Sean campus, Nakhon Pathom province.

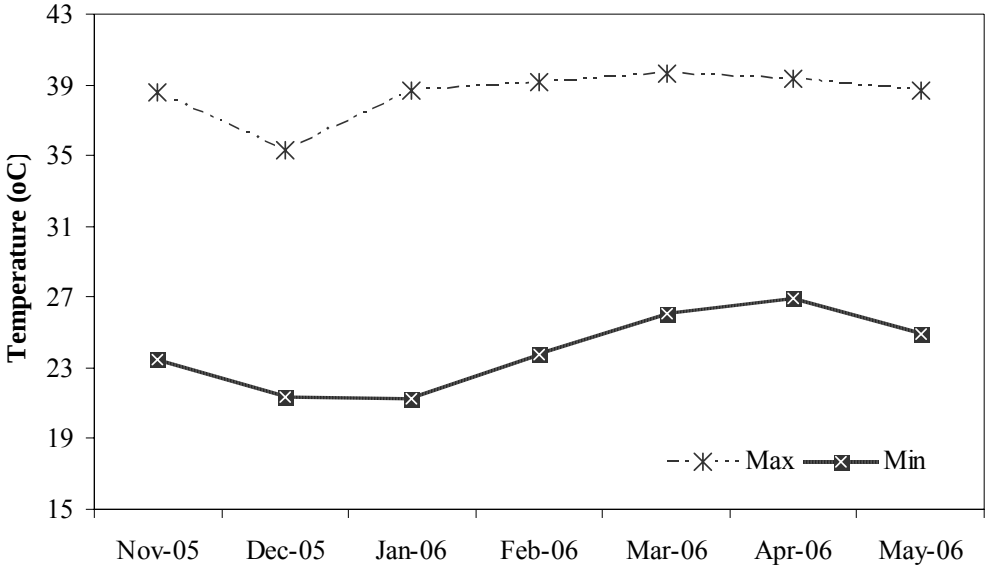


Appendix Figure A2 Mean precipitation, evaporations and relative humidity, since June 2003 to June 2005 at Nakhonpathom Meteorological Station at Kasetsart University, Kampang Sean campus, Nakhon Pathom province.

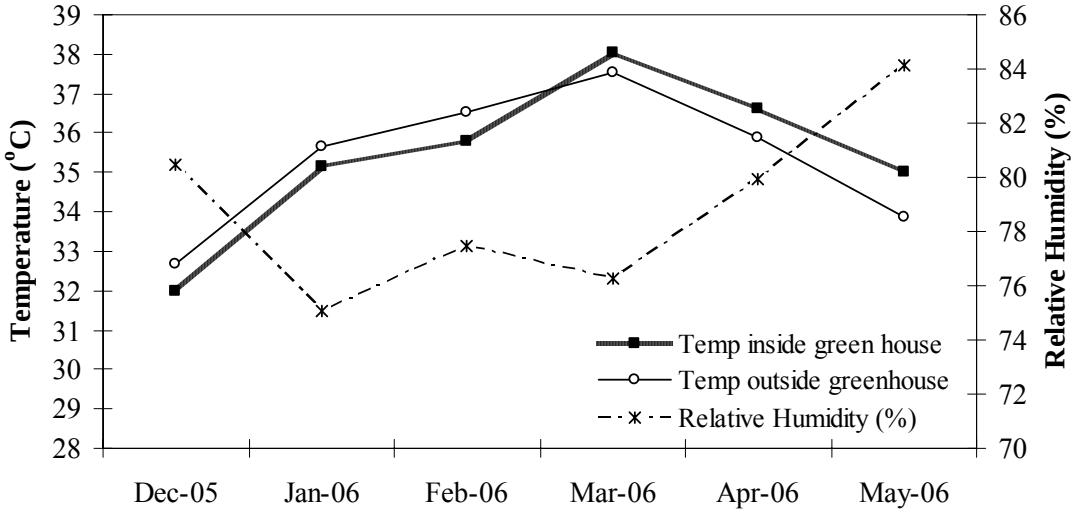




Appendix Figure A3 Mean photo periods and cloud, since June 2003 to June 2005 at Nakhonpathom Meteorological Station at Kasetsart University, Kampang Sean campus, Nakhon Pathom province.



Appendix Figure A4 Mean temperature, since Novenber 2005 to May 2006 at experimental field of experimental field of the Department of Horticulture, Kasetsart University Bang Khen campus.



Appendix Figure A5 Mean temperature inside and outside greenhouse and relative humidity (%) inside greenhouse, since December 2005 to May 2006 at experimental field of experimental field of the Department of Horticulture, Kasetsart University Bang Khen campus.

Appendix B

Tables

Appendix Table B1 Analysis of variance for fresh weight of rhizome of investigating changes in the quantitative of biological active components year round.

SV	df	SS	MS	F
Block	2	427215.6930	213607.8465	1.40ns
TR	10	11337336.4963	1133733.6496	7.42**
Error	20	3057443.0559	152872.1528	
Total	32	14821995.2452	463187.3514	

% C.V. = 41.71

** Significant at 99% level of confidence

ns not significant

Appendix Table B2 Analysis of variance for dried weight of rhizome of investigating changes in the quantitative of biological active components year round.

SV	df	SS	MS	F
Block	2	14734.9360	7367.4680	0.55ns
TR	10	684168.5833	62197.1439	4.64 **
Error	20	294616.6006	13391.6637	
Total	32	993520.1199	28386.2891	

% C.V. = 73.43

** Significant at 99% level of confidence

ns not significant

Appendix Table B3 Analysis of variance for antioxidant activity of investigating changes in the quantitative of biological active components year round.

SV	df	SS	MS	F
Block	2	70.1788	35.0894	0.47ns
TR	10	3108.8015	310.8801	4.14**
Error	20	1502.9001	75.1450	
Total	32	4681.8803	146.3088	

% C.V. = 13.86

** Significant at 99% level of confidence

ns not significant

Appendix Table B4 Analysis of variance for volatile oil content of investigating changes in the quantitative of biological active components year round.

SV	df	SS	MS	F
Block	2	25.1747	12.5873	6.69**
TR	10	173.0165	17.3016	9.20**
Error	20	37.6124	1.8806	
Total	32	235.8036	7.3689	

% C.V. = 15.39

** Significant at 99% level of confidence

Appendix Table B5 Analysis of variance for sabinene of investigating changes in the quantitative of biological active components year round.

SV	df	SS	MS	F
Block	2	423.0210	423.0210	29.58**
TR	10	1605.9083	160.5908	11.23**
Error	20	143.0117	14.3012	
Total	32	2171.9410	103.4258	

% C.V. = 8.99

** Significant at 99% level of confidence

Appendix Table B6 Analysis of variance for terpinen-4-ol of investigating changes in the quantitative of biological active components year round.

SV	df	SS	MS	F
Block	2	1.2962	1.2962	0.06ns
TR	10	256.2981	25.6298	1.13ns
Error	20	227.7139	22.7714	
Total	32	485.3082	23.1099	

% C.V. = 32.17

ns not significant

Appendix Table B7 Analysis of variance for DMPBD of investigating changes in the quantitative of biological active components year round.

SV	df	SS	MS	F
Block	2	278.5345	278.5345	9.47*
TR	10	650.9850	65.0985	2.21ns
Error	20	294.1296	29.4130	
Total	32	1223.6490	58.2690	

% C.V. = 23.76

* Significant at 95% level of confidence

ns not significant

Appendix Table B8 Analysis of variance for height of effect of light intensity on biologically active components.

SV	df	SS	MS	F
TR	2	8214.7406	4107.3703	31.85**
Error	27	3481.3928	128.9405	
Total	29	11696.1334	403.3149	

% C.V. = 14.72

** Significant at 99% level of confidence

Appendix Table B9 Analysis of variance for fresh weight of effect of light intensity on biologically active components.

SV	df	SS	MS	F
TR	2	12351.6667	6175.8333	10.69**
Error	27	15595.0000	577.5926	
Total	29	27946.6667	963.6782	

% C.V. = 37.75

** Significant at 99% level of confidence

Appendix Table B10 Analysis of variance for antioxidant activity of effect of light intensity on biologically active components.

SV	df	SS	MS	F
TR	2	609.5196	304.7598	1.85ns
Error	27	3453.0523	164.4311	
Total	29	4062.5719	176.6336	

% C.V. = 27.94

ns not significant

Appendix Table B11 Analysis of variance for volatile oil content of effect of light intensity on biologically active components.

SV	df	SS	MS	F
TR	2	146.6698	73.3349	6.13*
Error	27	143.5162	11.9597	
Total	29	290.1860	20.7276	

% C.V. = 31.11

* Significant at 95% level of confidence

Appendix Table B12 Analysis of variance for sabinene of effect of light intensity on biologically active components.

SV	df	SS	MS	F
TR	2	36.2951	18.1475	1.53ns
Error	27	142.5005	11.8750	
Total	29	178.7955	12.7711	

% C.V. = 6.66

ns not significant

Appendix Table B13 Analysis of variance for terpinen-4-ol of effect of light intensity on biologically active components.

SV	df	SS	MS	F
TR	2	30.1063	15.0531	1.72ns
Error	27	105.1371	8.7614	
Total	29	135.2434	9.6602	

% C.V. = 32.32

ns not significant

Appendix Table B14 Analysis of variance for DMPBD of effect of light intensity on biologically active components.

SV	df	SS	MS	F
TR	2	37.9399	18.9699	3.58ns
Error	27	63.6159	5.3013	
Total	29	101.5558	7.2540	

% C.V. = 17.70

ns not significant

Appendix Table B15 Analysis of variance for height of Effect of water deficit on biologically active components.

SV	df	SS	MS	F
TR	4	175.3431	43.8358	1.14 ns
Error	20	767.1693	38.3585	
Total	24	942.5124	39.2713	

% C.V. = 7.92

ns not significant

Appendix Table B16 Analysis of variance for fresh weight of effect of water deficit on biologically active components.

SV	df	SS	MS	F
TR	4	11638.7500	2909.6875	10.83**
Error	20	5373.7500	268.6875	
Total	24	17012.5000	708.8542	

% C.V. = 20.95

** Significant at 99% level of confidence

Appendix Table B17 Analysis of variance for antioxidant activity of effect of water deficit on biologically active components.

SV	df	SS	MS	F
TR	4	202.8332	50.7083	0.55ns
Error	20	1855.5795	92.7790	
Total	24	2058.4128	85.7672	

% C.V. = 18.91

ns not significant

Appendix Table B18 Analysis of variance for volatile oil content of effect of water deficit on biologically active components.

SV	df	SS	MS	F
TR	4	469.3486	117.3372	21.74**
Error	20	107.9668	5.3983	
Total	24	577.3154	24.0548	

% C.V. = 15.15

** Significant at 99% level of confidence

Appendix Table B19 Analysis of variance for sabinene of effect of water deficit on biologically active components.

SV	df	SS	MS	F
TR	4	237.2602	59.3150	3.10*
Error	20	382.6019	19.1301	
Total	24	619.8621	25.8276	

% C.V. = 9.34

* Significant at 95% level of confidence

Appendix Table B20 Analysis of variance for terpinen-4-ol of effect of water deficit on biologically active components.

SV	df	SS	MS	F
TR	4	58.3896	14.5974	3.89*
Error	20	74.9896	3.7495	
Total	24	133.3792	5.5575	

% C.V. = 15.77

* Significant at 95% level of confidence

Appendix Table B21 Analysis of variance for DMPBD of effect of water deficit on biologically active components.

SV	df	SS	MS	F
TR	4	117.6407	29.4102	1.87ns
Error	20	314.4309	15.7215	
Total	24	432.0716	18.0030	

% C.V. = 20.59

ns not significant

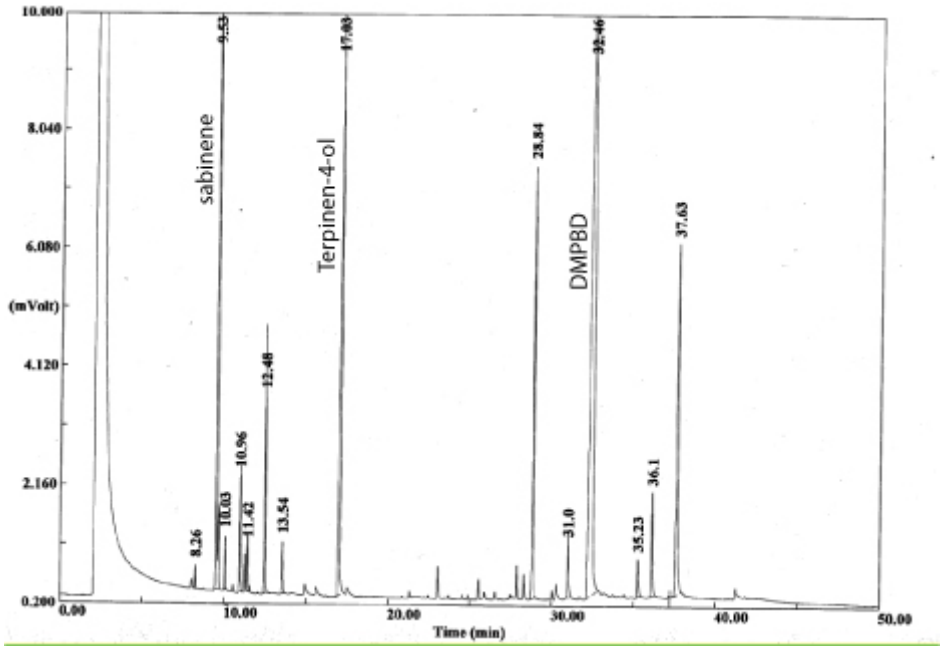
Appendix Table B22 Volatile oil Standard of cassumunar ginger (*Zingiber montanum*).

Ingredients	**TISI**[1]	**TISTR**[2]
Sabinene	31.00-48.00	25.70-40.31
Terpinen-4-ol	19.00-36.00	25.40-41.50
α-pinene	1.00-3.00	0.9-4.16
α-terpinene	3.00-8.00	6.25-16.50
γ-terpinene	6.00-10.00	2.00-12.66
DMPBD	-	0.5-5.88

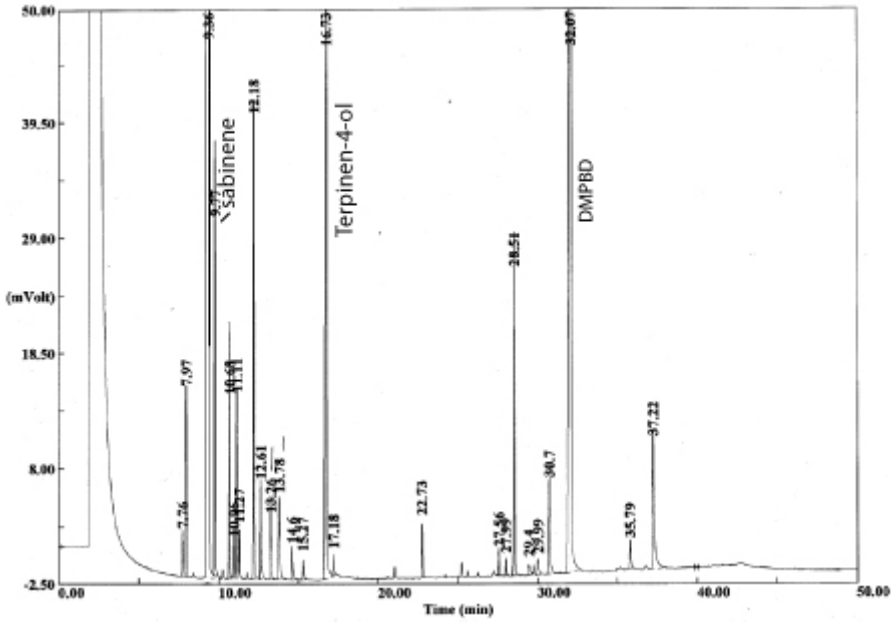
Source: [1] Thai Industrial Standards Institute and Thailand Institute of Scientific and [2] Technological Research

Appendix C

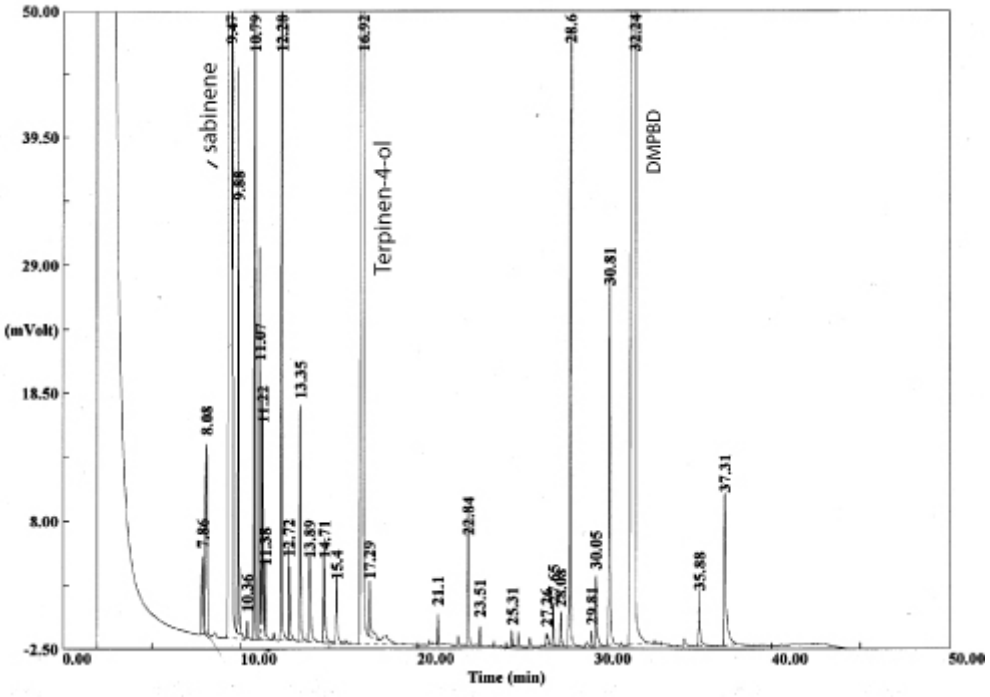
GC chromatogram



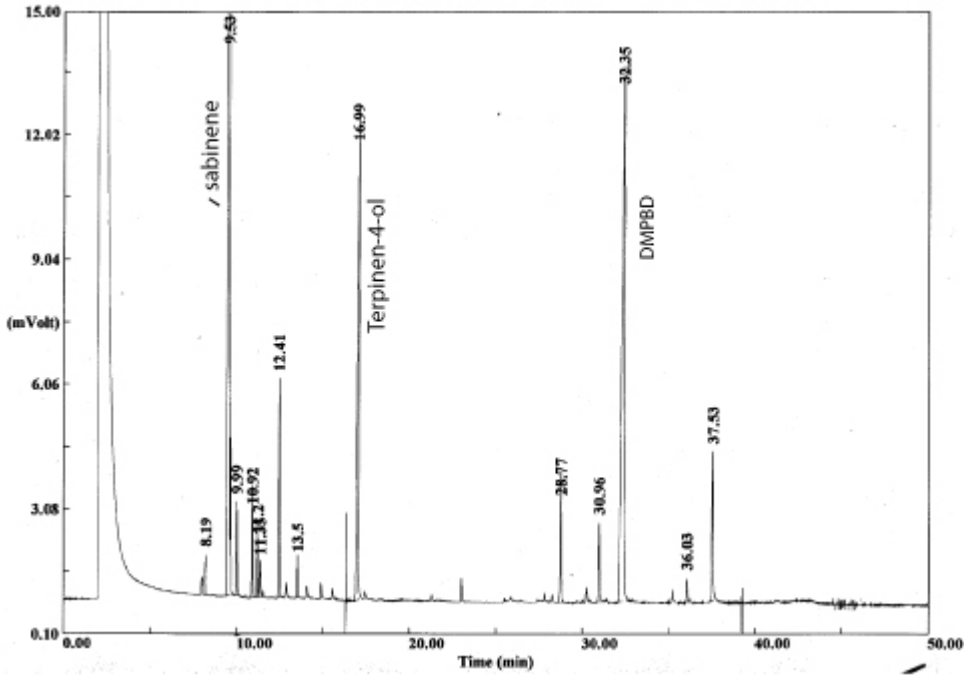
Appendix Figure C1 GC chromatogram of cassumunar ginger (*Zingiber montanum*) at 4 months.



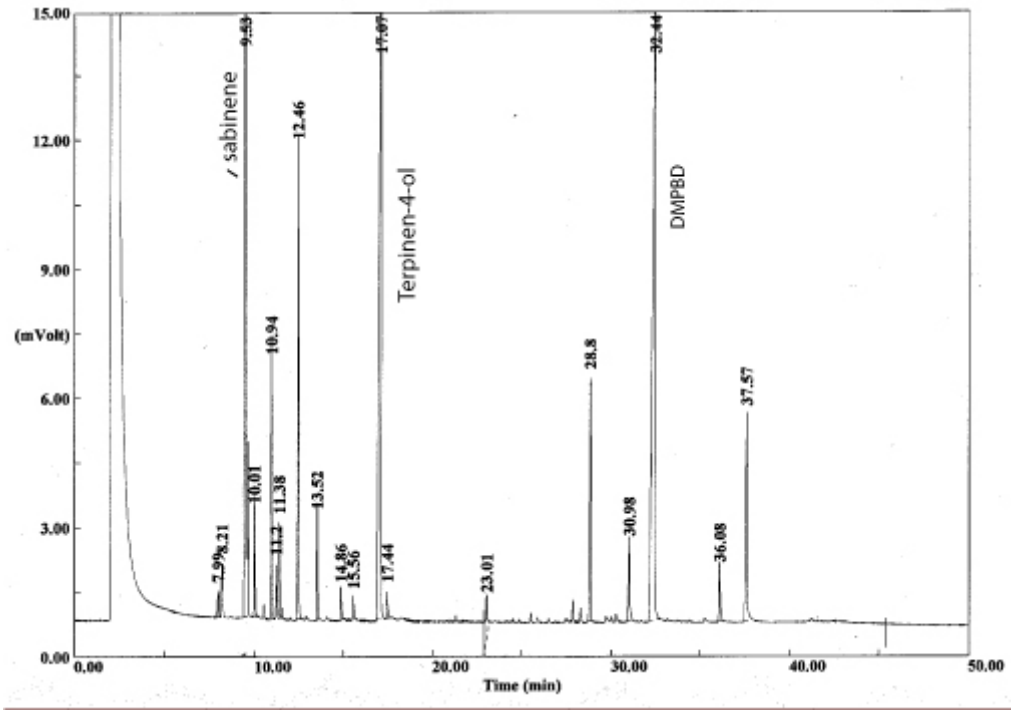
Appendix Figure C2 GC chromatogram of cassumunar ginger (*Zingiber montanum*) at 6 months.



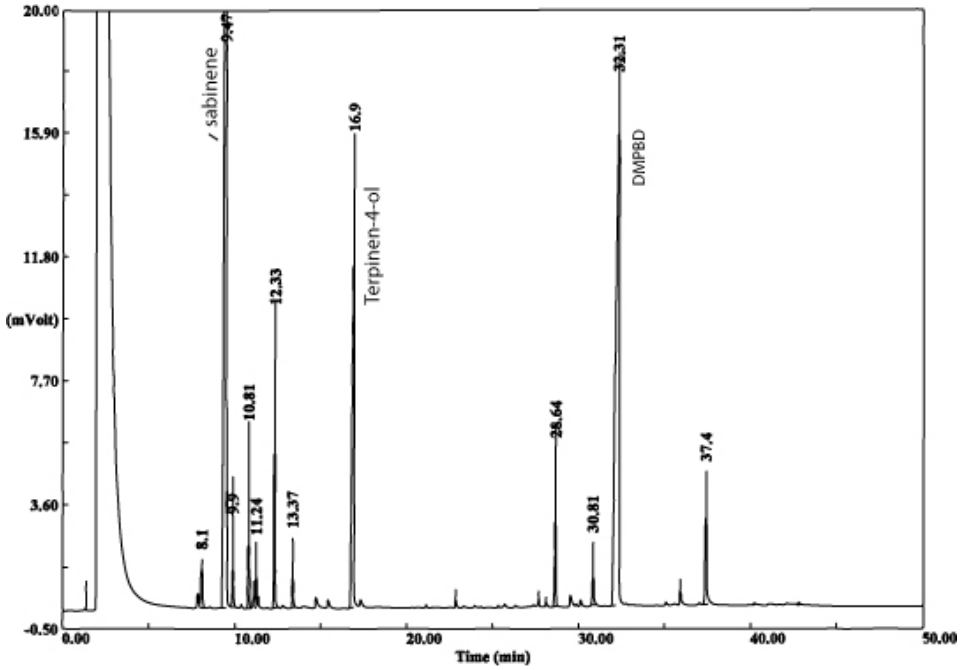
Appendix Figure C3 GC chromatogram of cassumunar ginger (*Zingiber montanum*) at 8 months.



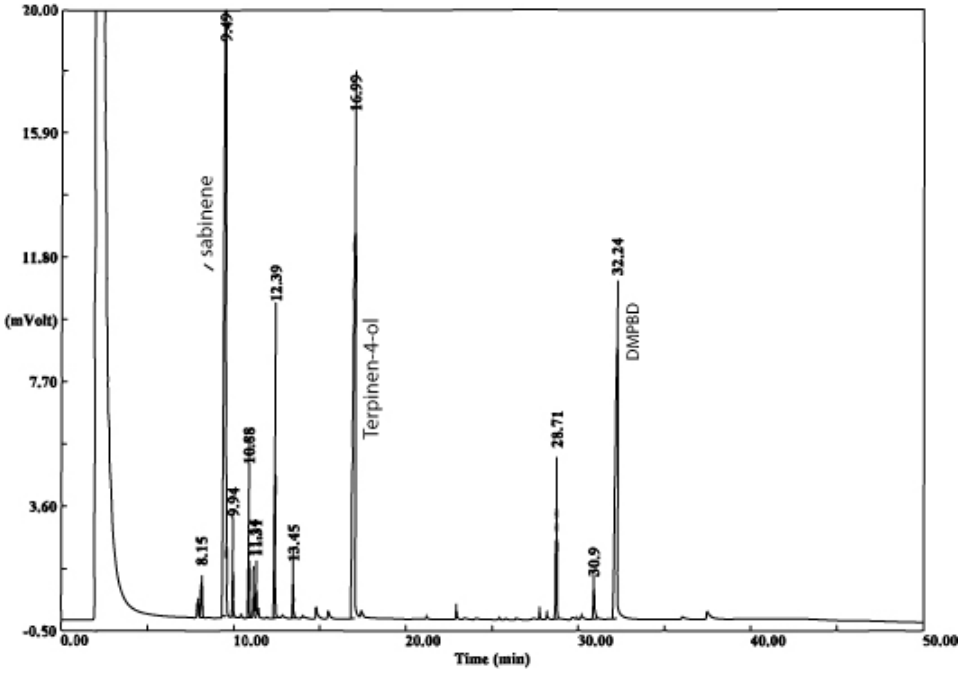
Appendix Figure C4 GC chromatogram of cassumunar ginger (*Zingiber montanum*) at 10 months.



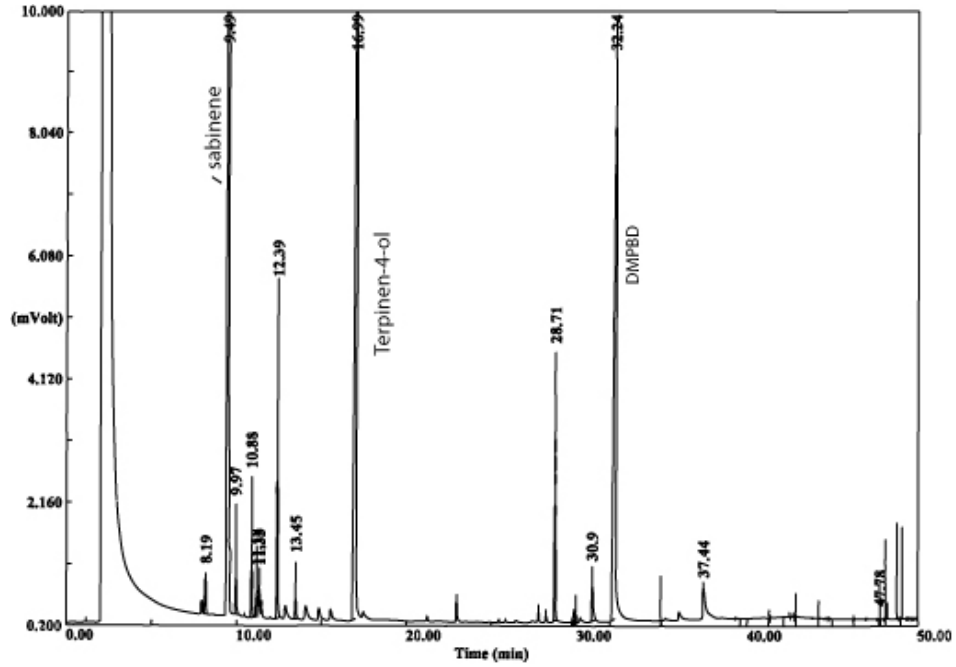
Appendix Figure C5 GC chromatogram of cassumunar ginger (*Zingiber montanum*) at 14 months.



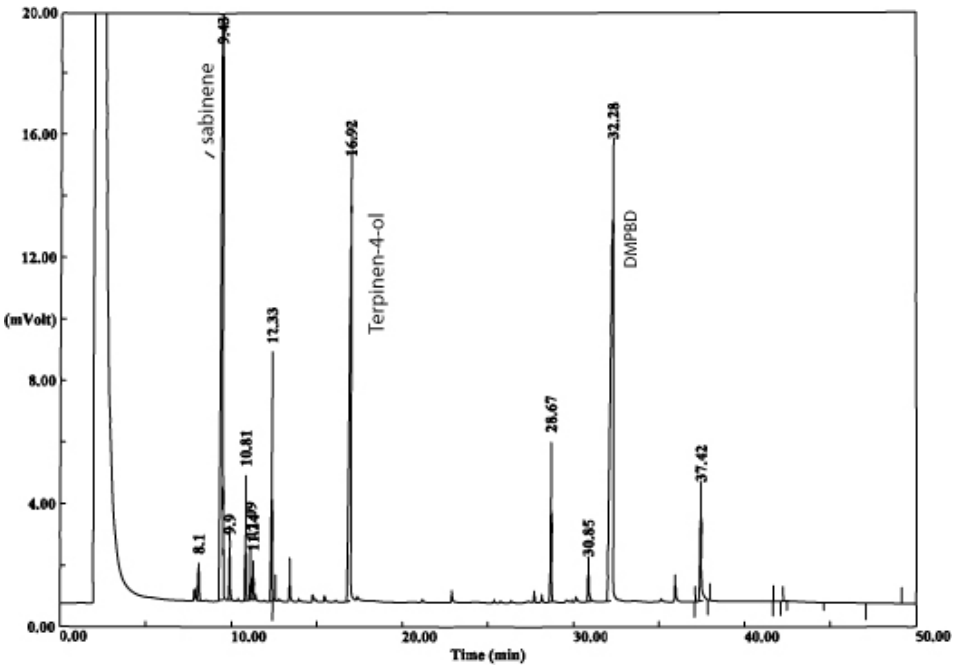
Appendix Figure C6 GC chromatogram of cassumunar ginger (*Zingiber montanum*) at 16 months.



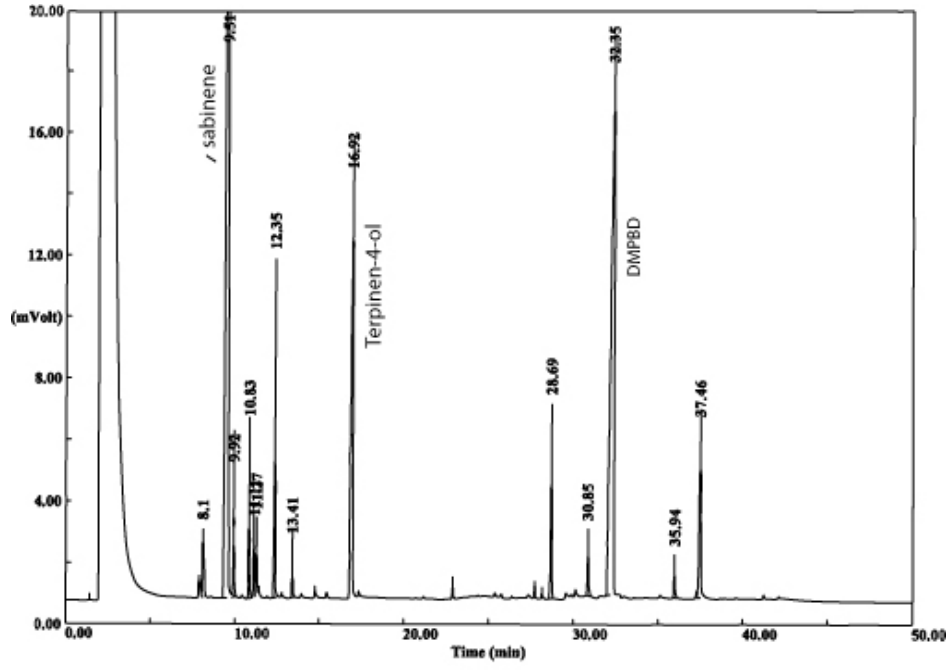
Appendix Figure C7 GC chromatogram of cassumunar ginger (*Zingiber montanum*) at 18 months.



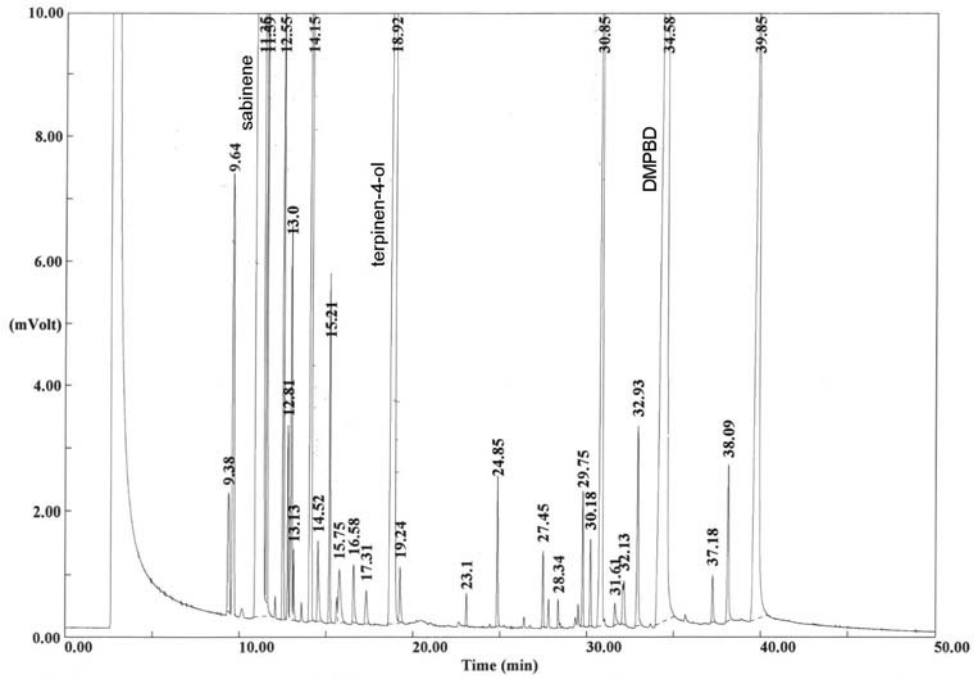
Appendix Figure C8 GC chromatogram of cassumunar ginger at (*Zingiber montanum*) 20 months.



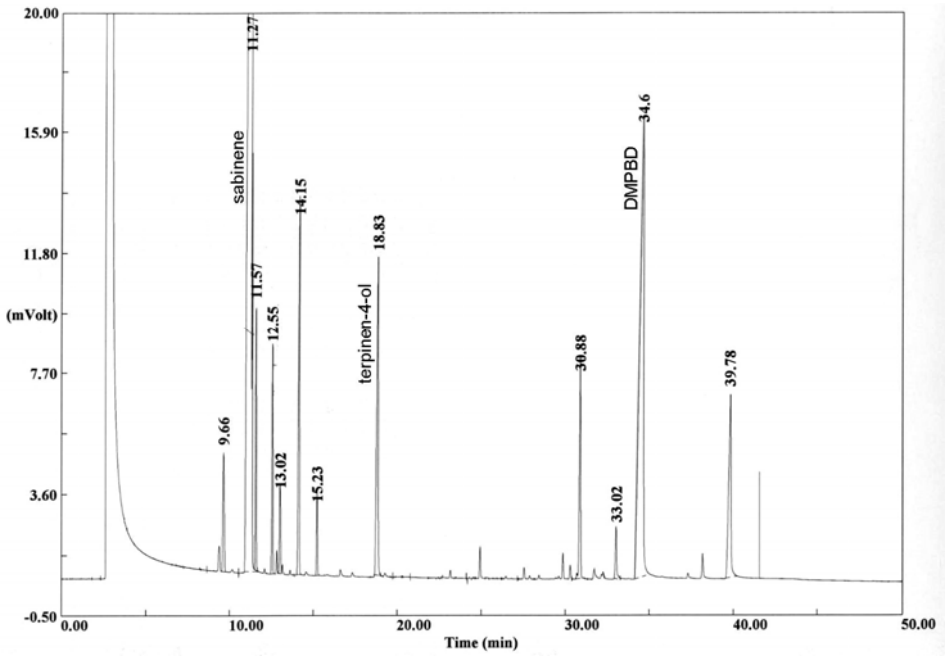
Appendix Figure C9 GC chromatogram of cassumunar ginger (*Zingiber montanum*) at 22 months.



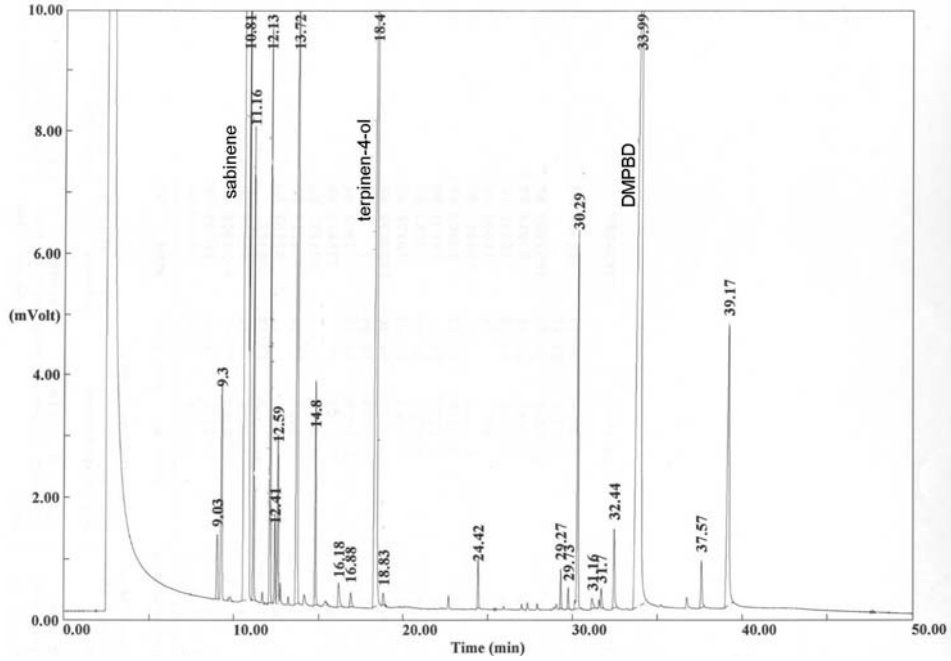
Appendix Figure C10 GC chromatogram of cassumunar ginger (*Zingiber montanum*) at 24 months.



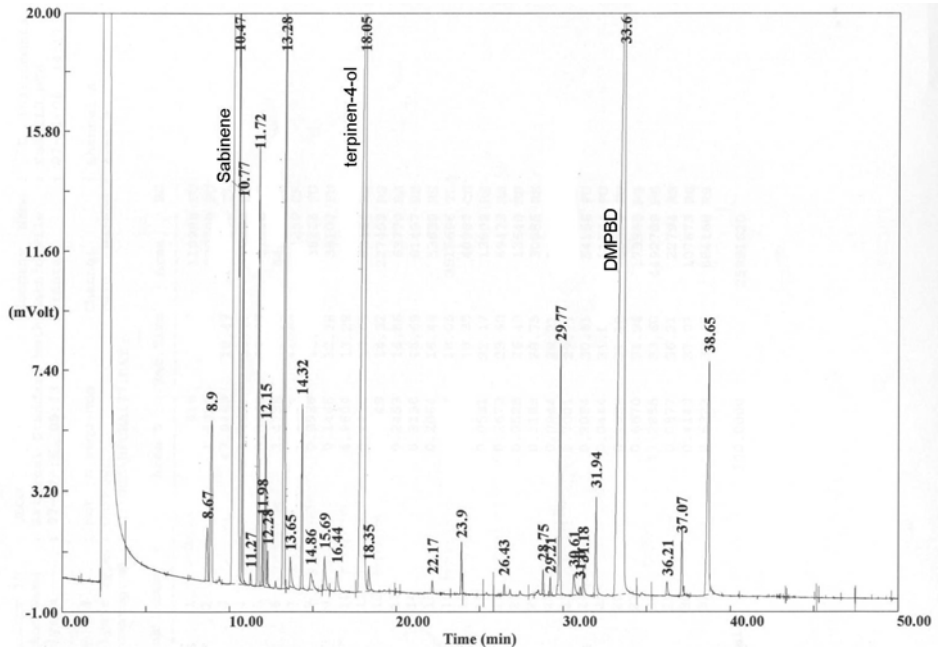
Appendix Figure C11 GC chromatogram of cassumunar ginger (*Zingiber montanum*) under sunlight..



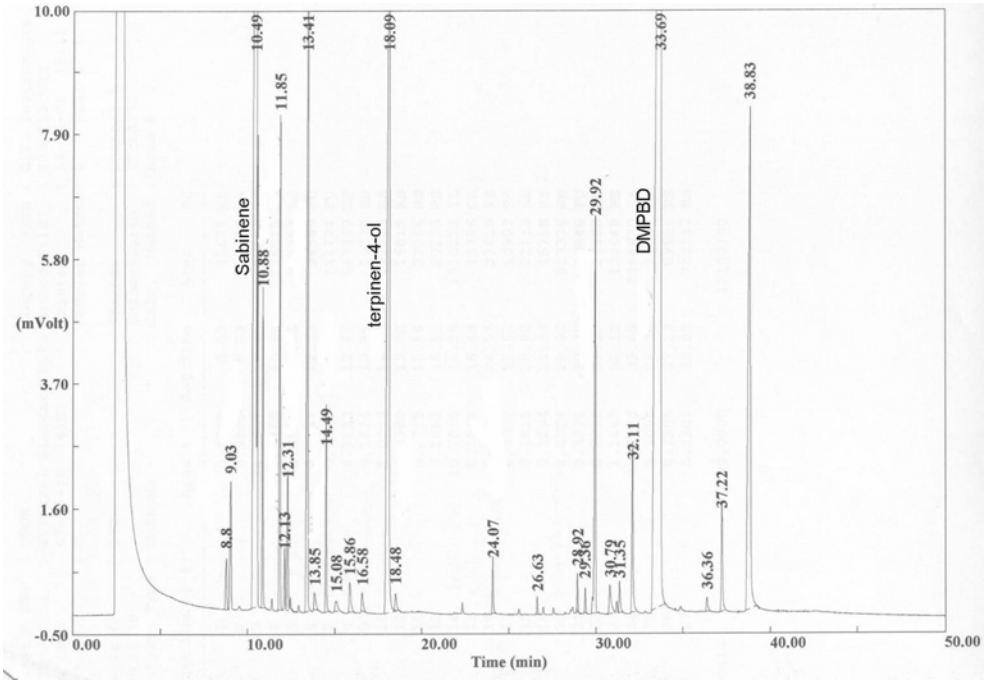
Appendix Figure C12 GC chromatogram of cassumunar ginger (*Zingiber montanum*) under 50% of light intensity.



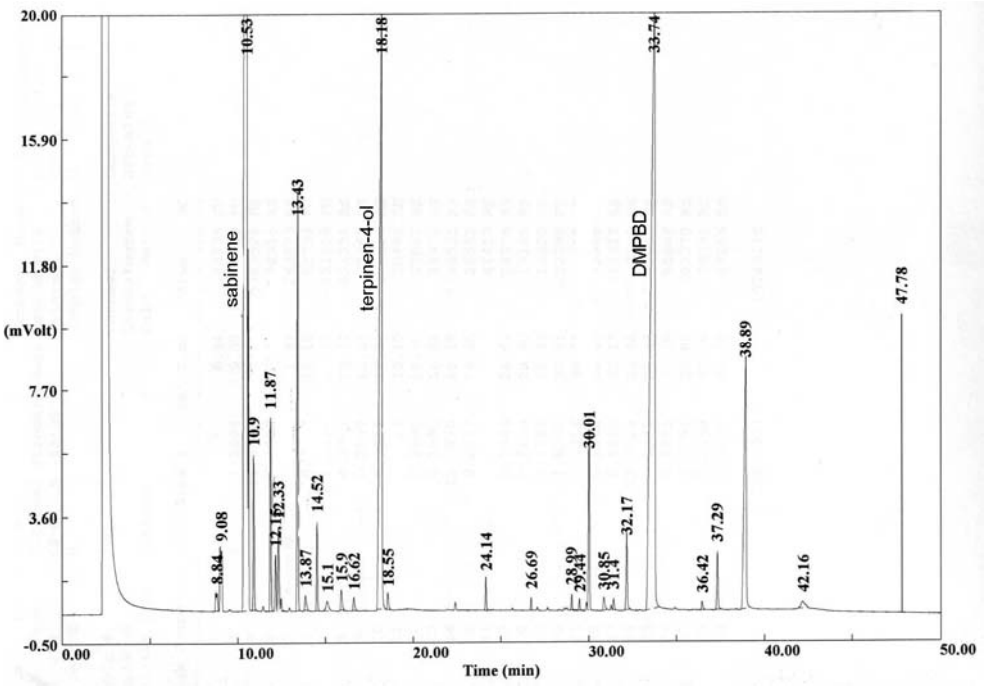
Appendix Figure C13 GC chromatogram of cassumunar ginger (*Zingiber montanum*) under 25% of light intensity.



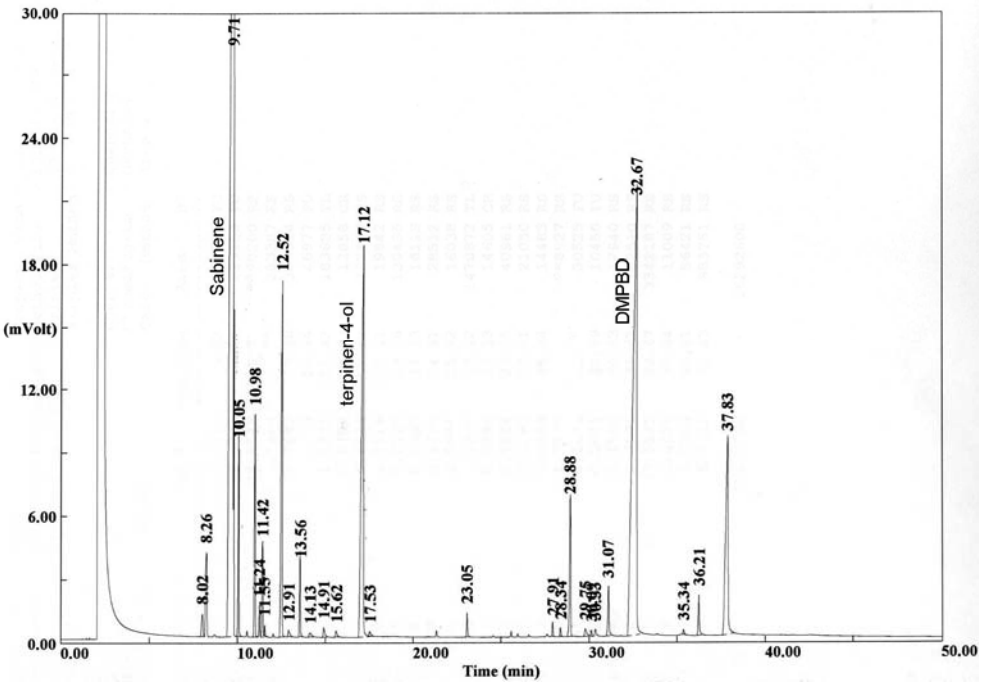
Appendix Figure C14 GC chromatogram of cassumunar ginger (*Zingiber montanum*) treated with 0 days water deficit (control).



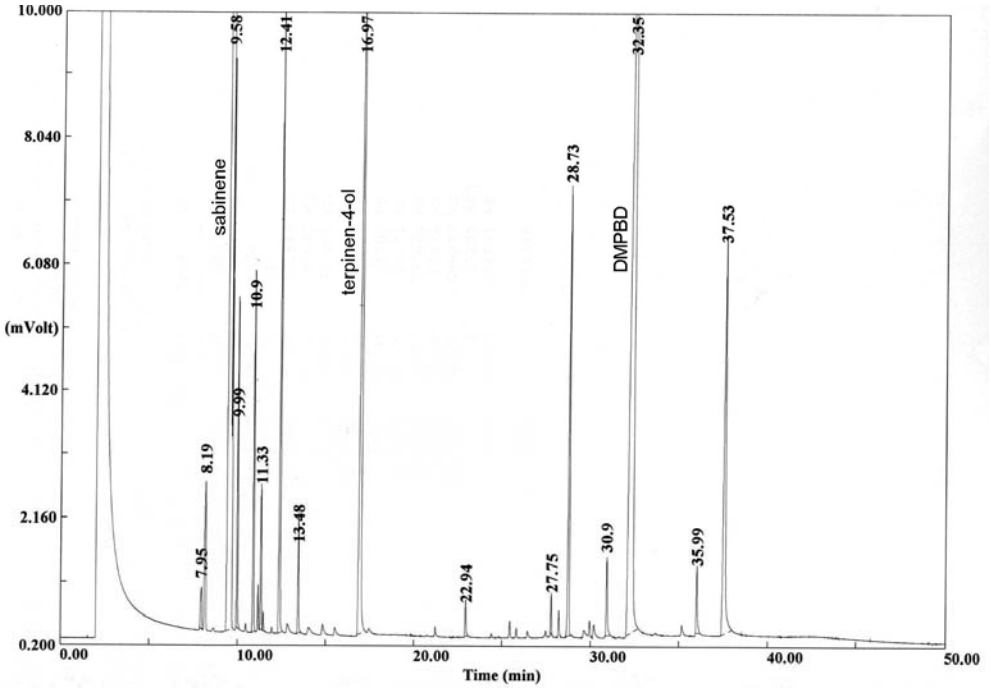
Appendix Figure C15 GC chromatogram of cassumunar ginger (*Zingiber montanum*) treated with 30 days water deficit.



Appendix Figure C16 GC chromatogram of cassumunar ginger (*Zingiber montanum*) treated with 60 days water deficit.



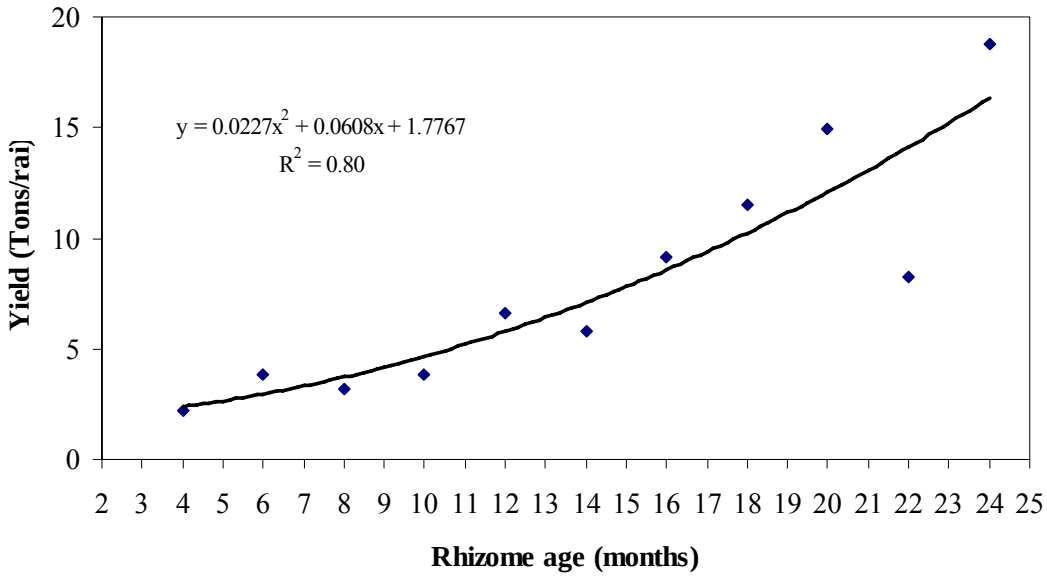
Appendix Figure C17 GC chromatogram of cassumunar ginger (*Zingiber montanum*) treated with 90 days water deficit.



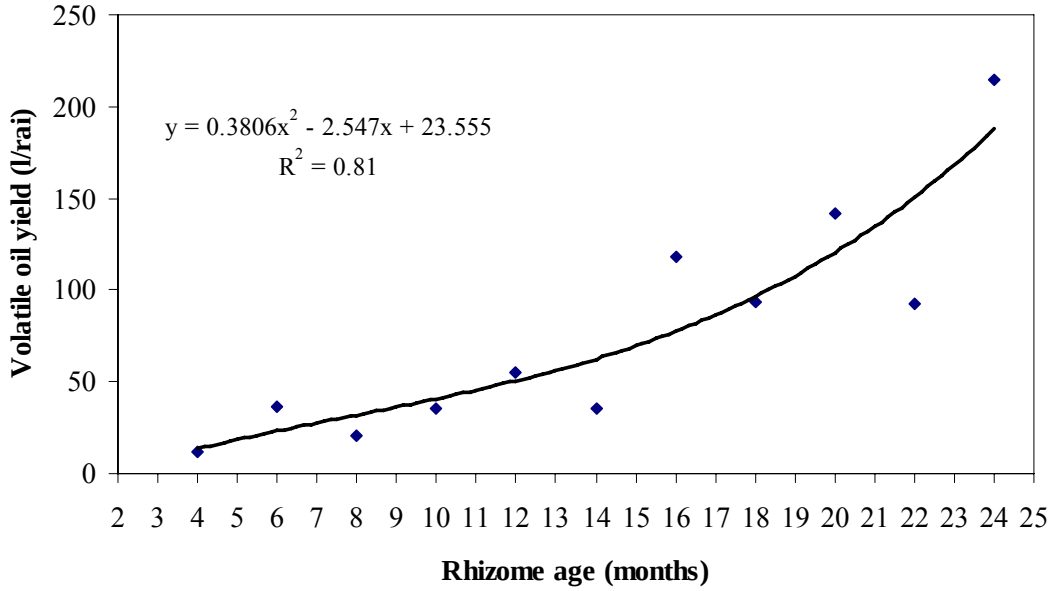
Appendix Figure C18 GC chromatogram of cassumunar ginger (*Zingiber montanum*) treated with 120 days water deficit.

Appendix D

The productivity



Appendix Figure D1 The rhizome productivity of cassumunar ginger (*Zingiber montanum*) based on fresh weight at various rhizome age.



Appendix Figure D2 Volatile oil productivity of cassumunar ginger (*Zingiber montanum*) based on fresh weight at various rhizome age.

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