)F NITROGEN AND PHOSPHORUS ON GROWTH,)WERS AND ESSENTIAL OIL OF CHAMOMILE ı chamomilla L.) GROWN UNDER KENYA CONDITIONS !!

BY

Emongor Vallantino Erone.

(B.Sc.Agric. Univ. Nbi)

submitted in partial fulfilment of the of Master of Science in Agriculture in the University of Nairobi.

June, 1988.

THE EFFECT OF NITROGEN AND PHOSPHORUS ON GROWTH, YIELD OF FLOWERS AND ESSENTIAL OIL OF CHAMOMILE (*Matricaria chamomilla* L.) GROWN UNDER KENYA CONDITIONS

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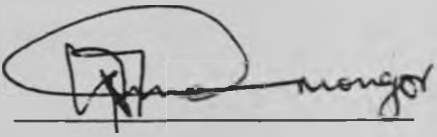
DECLARATION

This thesis is my original work and has not been presented for a degree in any University.

Signed  Date 15·6·88

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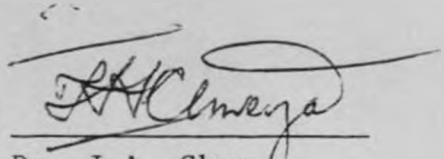
Signed  Date 15·6·88

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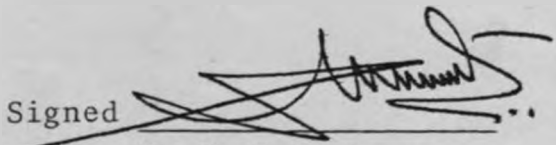
Signed  Date 17·6·88

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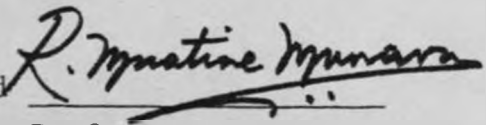
Signed  Date 22·6·88

Prof. R.M. Munavu

DEDICATED TO MY PARENTS

DISMAS ENYIKOT IDUKITA

and

ESTHER NASULA ERONI

and

MY SON STEPHEN ENYIKOT .

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ABSTRACT

An experiment was carried out at the Field Station, University of Nairobi, Kabete Campus to find out firstly, the effect of nitrogen, (0, 50, 100 and 150 kg N/ha) and phosphorus (0, 17.47, 34.93 and 52.41 kg P/ha) rates, and their interaction on growth, flower and essential oil yields and quality of chamomile plants; and secondly whether chamomile plants can grow under Kenyan conditions and at an altitude above 1800 m above sea level. The treatments were laid down in a split-plot design with three replicates. The variety Max et Oljea was used. The essential oil was extracted by steam distillation using Clevinger apparatus and its composition was determined by gas liquid chromatography.

The results showed that vegetative growth and dry matter production of chamomile plants were significantly increased by nitrogen application. Nitrogen at 50 kg N/ha increased significantly both fresh and dry flower yields of the plants.

Increasing nitrogen application from 0 to 100 kg N/ha, increased essential oil yield per unit dry flower weight and per plant from 0.627 to 1.036% and

from 5.85 to 16.64 kg/ha., respectively. However, application of more than 50 kg N/ha did not significantly increase essential oil yield from the plants. Essential oil yield per unit dry flower weight increased with increasing phosphorus application. Increasing application of phosphorus from 0 to 17.47 kg P/ha. increased essential oil yield from 0.728 to 0.914%. Applying more than 17.47 kg P/ha. decreased the essential oil yield.

Increasing application of nitrogen and phosphorus, from 0 to 50 kg N/ha, and 0 to 17.47 kg P/ha. increased chamazulene concentration in the essential oil from 6.89 to 8.60%, and from 7.07 to 8.25%, respectively. However, application of more than 50 kg N/ha and 17.47 kg P/ha. led to a decrease of chamazulene. Nitrogen fertilization significantly decreased bisabololoxides "A" and "B" concentration in the essential oil of chamomile flowers. Increasing application of nitrogen from 0 to 150 kg N/ha. decreased bisabololoxides "A" and "B" concentration in the essential oil from 41.56 to 31.58% and from 21.69 to 13.19%, respectively. Nitrogen fertilization increased farnesene content in the essential oil of

chamomile flowers. However, applying more than 50 kg N/ha. increased farnesene concentration.

The interactions between nitrogen nad phosphorus, had no significant influence on chamomile plant growth, development, yield and quality of the essential oil.

In general, the results showed that nitrogen played a vital role in the growth and development of chamomile plants and in the biosynthesis of the essential oil and its components from the plants. Phosphorus fertilization did not significantly influence the growth and development of the plants. However, it is recommended that further research should be done in the areas of plant nutrition, ecological zones, plant breeding and varietal evaluation, plant biochemistry and the economic evaluation of chamomile growing in Kenya.

CHAPTER ONE

1. INTRODUCTION

The discovery of the healing and curative properties of plants is as old as the human race. Egyptian papyri, dating back as far as 2000 B.C., record the common use in Egypt of plants like mustard, linseed, squill and myrrh (Balbaa, 1983; and Trease and Evans, 1972). Early in the Christian era, gardens of food, culinary and medicinal plants were established in connection with monasteries in various parts of Europe, and the knowledge of many medicinally important plants were kept alive during the dark ages (Balbaa, 1983; and Trease and Evans, 1972). Certain drugs are now obtained almost exclusively from cultivated plants. Among these plants are cardamon, ginger, cinnamon, fennel, opium, linseed and chamomile (Trease and Evans, 1972; and Balbaa, 1983). In some cases both wild and cultivated plants are used.

In many cases it is advisable to cultivate medicinal plants because of the improved quality of the required drugs (Trease and Evans, 1972; Balbaa, 1983; and Franz, 1983a). The improvement may be as a result of confining collection of species, varie-

ties or hybrids which have the desired characters. The improvement of drug quality may also be due to better development of the plants owing to better agricultural and improved post-handling practices.

Chamomile is an annual from the family of compositae. The genus Matricaria has about 50 species native to Europe, Mediterranean region, Asia Minor, Egypt, Congo, and Eastern and Southern African countries (Bailey, 1949; Watt and Breyerbrandwijk, 1962; Masefield et al., 1971; Trease and Evans, 1972). Some of the chamomile varieties are grown for ornamentals, but most of the varieties are grown for medicinal purposes. The medical product is contained in the flowers of chamomile.

Chamomile flowers yield an essential oil which is light blue in colour when fresh. The essential oil is used for the manufacture of drugs and in the treatment of diseases such as convulsions in children, diarrhoea, colic and acidity, hysteria, allergy, sleeplessness (Kling, 1923; Jelicic et al., 1972; Isaac and Schimpke, 1975; Martindale, 1977; Trease and Evans, 1978; and Sticher, 1977) to name a few. Furthermore, the

essential oil is used for making drugs for the treatment of stomach ulcers induced by chemical stress or heat coagulation (Szelenyi et al., 1979; and Isaac, 1980). The essential oil also promotes epithelization, granulation, and shows antibacterial and antimycotic effects (Isaac, 1979) through the activity of (-)-α-bisabolol. The essential oil is further used for flavouring liqueurs and for making cosmetics (Bailey, 1949; Kirk and Othmer, 1952; Masefield et al., 1971; and Trease and Evans, 1972). The essential oil may also be used for the treatment of allergies and inflammation of body tissues (Jelicic et al., 1972).

The flowers of Chamomile are also used for making chamomile tea. The Chamomile tea helps in digestion and stops pain in the bladder region (Isaac, 1979). The steam from water mixed with Chamomile flowers is used to cure soar throat and hot compresses for croup, diptheria, rheumatism, sciatica, gout and lumbago (Kling, 1923, Isaac, 1979). Chamomile flowers have an antispasmodic and tonic effect for fever, particularly typus, for skin conditions, when used as an emollient in bath and as a perfume (Dragendorff, 1895; Trease and Evans, 1972; and Kirk and

Othmer, 1952). Chamomile flowers flour is used to rub on gums of young children to reduce pains when they are teething (Isaac, 1979). Lastly, chamomile flowers can also be used for making herb beers (Masefield et al., 1971).

Since chamomile essential oil and flowers are very useful in pharmacy and beer industry, it would be worthwhile if our Kenyan farmers adopted the plant as a cash crop. Chamomile dried flowers are very expensive and the present Kenyan market price is in the range of Kshs. 600 to Kshs. 1000 per kilogramme of dried flowers (provided the essential oil percentage is 0.5 percent and above). At present, the world demand of chamomile is very high, while the production is low (Kubeczka, 1978; and Franz, 1983a). If Kenyan farmers adopt chamomile, it would earn them income which would raise their living standards and general welfare. If large scale production of chamomile flowers is done in Kenya, it would earn the country foreign exchange from exporting the flowers and the essential oil (especially to the European countries where there is ready market for chamomile). The large scale production would also lead to construction of agro and agri-industries,

which in turn will create employment in the country. It is therefore, important that agronomic and quality studies on the plant are initiated in the country.

The objectives of this study were therefore:-

(1) To find the effect of nitrogen and phosphorus, and their interaction on the

   (a) Growth of chamomile

   (b) Flower yield of chamomile

   (c) Essential oil yield of chamomile

   (d) Quality of the essential oil of chamomile.

(2) To find whether chamomile can grow under Kenyan conditions and at an altitude above 1800 M. above sea level.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Botany of Chamomile

Chamomile is an annual plant from the family of compositae. The flowers are often strongly scented. The leaves of chamomile are 1-3 times finely dissected. Each dried flower-head is hemispheral and about 12-20 mm in diameter. The florets are of a white to pale bluff colour, while the outer florets hiding the involucre of bracts. A few hermaphrodite tubular florets are usually found near the apex of the solid receptacle. A transition between typical tubular florets and typical ligulates is often seen. The ligulate florets show three teeth (or occassionally two), the centre one being the most developed. There are four principle veins. The corolla is contracted near its base into a tube from which a bifid style projects. The ovary is inferior and devoid of purpose. Each floret arises in the axil of a thin membranous bract or palea, which has a blunt apex. The receptacle is conical, ovoid or naked. At the base of the receptacle is an involucre consisting of two or three rows of oblong bracts which have membranous margins (Bailey, 1949; Kirk and Othmer, 1952; Masefield et al., 1971; Trease

and Evans, 1972, 1978; and Martindale, 1977).

2.2 Essential oil

The essential oil content of Chamomile flowers is largely highest in developed flower heads. The maximum oil content is generally reached approximately one week after the beginning of flowering (Franz, 1980). The oil content of chamomile flowers is in the range of about 0.2-1.5% (Trease and Evans, 1972; Martindale, 1977; Schroder, 1978; Waly, 1980; and Franz, 1980, 1983a). The essential oil of Chamomile flowers is composed of Chamazulene, (-)-α-bisabolol, bisaboloxides "A", "B" and "C", cis-spiroether (cis-en-in-dicycloether), trans-spiroether, matricine, flavanoids (there are 17 flavanoids in chamomile (Horhammer et al., 1968), bisabolonoxide, and farnesene (Isaac and Schimpke, 1965; Jelicic-Hadlovic, 1972; Stern, 1972; Trease and Evans, 1972; Martindale, 1977; Jakovlev et al., 1979; Szelenyi et al., 1979; Isaac, 1979, 1980; and Breinlich et al., 1968). The composition of the essential oil depends on the stage of development of the flowers (Franz et al., 1978b; Franz, 1982; and Balbaa, 1983). Farnesene and (-)-α-bisabolol are

predominant in the flower buds. While in fully developed flowers, a higher relative content of chamazulene and (-)-α-bisabolol are predominant (Franz, 1980). Gasic et al. (1983), reported that Chamomile essential oil contained 3.00-13.26% farnesene, 1.26-14.47% bisabololoxide B and 1.54-18.55% cis-en-indicyloether (cis-spiroether) per unit weight extracted dry flowers. Mrlianova and Felklova (1983) reported that most samples of essential oil from cultivated and wild Chamomile flower heads contained more than 50% bisabololoxides A + B. Konoralova et al. (1981) reported that Chamomile essential oil contained chamazulene in the range of 1.03-12.9%. However, in the essential oil of chamomile chamazulene and (-)-α-bisabolol concentrations,are highest in the uppper parts of blossoming flowers, although they are also present at low concentrations in the stems and roots (Franz, 1983a). The formation of bisabololoxide A and B are controlled by a dominant gene, while the formation of (-)-α-bisabolol is controlled by a recessive gene (Franz, 1982).

2.30 Harvesting of chamomile

The flowers are collected in dry weather and carefully dried. The crop is often damaged by wet weather and the discoloured flowers then obtained fetch a much lower price than those having a good colour (Trease and Evans, 1972). The first harvesting of the flower heads can be done when the oldest flower heads of 50% of plants have started to wilt. A second harvesting can follow after 14 days. Only flower heads with more than 40 percent tubular florets are picked. The picked flower heads are dried at 35°C for 5 days, after which the flower yield may be determined (Schröder, 1978; and Trease and Evans, 1972). However, the number of flower heads which a plant is able to produce and the total flower yield is correlated positively to the number of foliage leaves formed before flower formation starts (Hummel and Stürner, 1955; and Schröder, 1978).

2.40 Propagation of chamomile

Chamomile can be propagated sexually or asexually (Trease and Evans, 1972; and Schröder, 1978). In sexual propagation, the seed is either directly

sown in the field or the seed is raised in nursery first, and then later transplanted to the field. However, transplanting is necessary due to the small size of the seeds and it helps in selecting vigorous and uniformly growing seedlings. The seedlings are transplanted when they have reached 6-7 leaf stage (Verzár-petri et al., 1978). For commercial production of chamomile, direct seeding is done using precise machines. Thinning is done when the seedlings are about four weeks old (6-7 leaf stage) in order to get the desired plant population.

In asexual propagation, offsets or stem cuttings are used. When cuttings are used, stock plants of different stages of development may be used provided that the plants have not flowered (Trease and Evans, 1972; and Schröder, 1978). Rooting of chamomile takes place at a relative humidity of more than 90% and temperature of about 20°C (Schröder,1978). Treating the cut surface of the stem cuttings with auxins neither influences the period of time to root nor the plant development (Schröder, 1978). Leaf cuttings may also be used in asexual propagation. However, the leaf cuttings develop roots without shoots and flower formation (Schröder, 1978). Shoot cuttings

of unlignified parts possessing at least one leaf, root faster and develop strong roots compared to leaf cuttings (Schröder, 1978).

2.5 Day length

Medicinal plants may be short day, long day or day neutral. Chamomile has varicties (cultivars) falling in all the three categories of photoperiodic classes. However Matricaria chamomilla L. (used in this study) is a quantitative long-day plant (Saleh, 1978; and Franz et al., 1975). Short day length and low temperatures promote the development of short and leafy lateral shoots and retard flower formation (Schröder, 1978).

2.6 Soils

Chamomile, grows in well drained and fertile soils. The soil pH suitable for chamomile growing is pH 5.0, though it can be grown in a soil with a pH range of 4-7 (Eggens and Hilton, 1971). It can also grow well in partially salty soil, for example Singh (1970), found that chamomile had an exceptionally high sodium (Na^+) uptake (66 Meq/100 g dry

matter) and tolerance of high levels of sodium ions (Na^+). Chamomile does not grow well in acid, marshy or sandy soils.

2.7 Processing

Dried chamomile flowers are used in the extraction of the essential oil. The essential oil is extracted by steam distillation using Clevinger apparatus (Trease and Evans, 1972; Martindale, 1977; Kirk and Othmer, 1952; Hölzl and Demuth, 1975; Haggag et al., 1975; and Franz, 1983). The extracted essential oil can then be used either in the pharmaceutical or beer industries.

2.8 Effect of nitrogen and phosphorus on chamomile growth

2.8.1 Nitrogen

Nitrogen is an important plant nutrient. The forms most commonly assimilated by plants are the nitrate and ammonium ions. In most well aerated soils, nitrate is the principle form of available nitrogen, and plants adapted to such soils grow well with nitrate as the sole source of nitrogen. Many

such plants can also utilize ammonium, but suffer various impairments when only ammonium ions furnish nitrogen (Barker et al., 1966; Street and Sheat, 1958; and Black, 1968). For example, the structure of chloroplasts are affected under conditions of ammonium toxicity (Puritch and Barker, 1967; and Mengel and Kirkby, 1979). However, some plants such as rice utilize ammonium nitrogen more effectively than nitrate at all their stages of growth (Kelley, 1911; Theli- and Beaumont, 1934; Bonner, 1946; and Mengel and Kirkby, 1979). Ammonia can also be absorbed by plant roots particularly under conditions of high pH where the presence of NH_3 is favoured. Urea ($CO(NH_2)_2$) may also be absorbed by plants (Tisdale and Nelson, 1966). The urea is rapidly and directly absorbed through the leaf epidermis. But it is unlikely that large quantities of urea nitrogen as such are absorbed by plant roots, for urea hydrolyses to ammonium nitrogen in most soils. There are also complex materials, such as water-soluble amino acids and nucleic acids, which may be absorbed and utilized by higher plants (Tisdale and Nelson, 1966).

An adequate supply of nitrogen is associated

with vigorous vegetative growth (Beevers and Hageman, 1969; Beevers, 1976; and Mengel and Kirkby, 1979). Excessive quantities of nitrogen can, under some conditions, prolong the growing period and delay crop maturity. This is most likely to occur when adequate supplies of other plant nutrients are not present (Beevers, 1976).

Since nitrogen is a constituent of proteins, purines, pyrimidines, and many coenzymes, an interference with protein synthesis and hence, growth, is the major biochemical effect of nitrogen deficiency (Epstein, 1972; Hewitt and Smith, 1974; and Mengel and Kirkby, 1979). Lack of nitrogen leads to reduced photosynthesis which in turn causes a nitrogen deficient plant to lack not only essential amino acids, but also the machinery for synthesis of necessary carbohydrates and carbon skeletons for all manner or organic syntheses. Plants deprived of nitrogen show decreased cell division, expansion and elongation, prolonged dormancy and therefore, delayed swelling of buds in some plants (Frank, 1965; and Batholomew and Clark, 1965). All morphological parts are reduced in size, and leaves and fruits are also reduced (Frank, 1965; Batholomew and Clark, 1965;

Epstein, 1972; and Mengel and Kirkby, 1979).

Krejcik (1973) reported that a solitary chamomile plant under optimum conditions can grown to 88 cm tall and 60 cm wide and will bear most of its flowers 50-80 cm above the ground. He further reported that when chamomile was cultivated, the height at which flowers were borne was lowered by the soil type and row width. Majority of the flowers were borne between 35-65 cm above the ground which was suitable for machine harvesting compared to hand harvesting.

Franz and Kirsch (1974) working with chamomile plants grown in pots and supplied with nitrogen at 0-4.5 g N/pot and 0-4 g K_2O/pot, reported that nitrogen nutrition increased vegetative growth of chamomile. However, nitrogen nutrition delayed the transition of chamomile plants from the vegetative phase to reproductive phase. The workers further reported that potassium nutrition advanced flowering time. However, El-Hamidi *et al.* (1965), reported that addition of nitrogen and potassium increased the height, spreading line, fresh and dry weights of chamomile plants.

Ruminska (1978) in Poland reported that using 0.75-4.50 g N/pot increased the fresh matter of chamomile plant. Gindich and Sheberstov (1971) reported that in the early stages of growth, wild chamomile plants responded the best to phosphorus nutrition. However, in the later stages, the plants required all the three major elements, particularly nitrogen and potassium. Gindich and Sheberstov (1971) also reported that potassium and nitrogen increased the vegetative growth of chamomile, but that, high nitrogen retarded flowering.

Meawad et al. (1984) reported that a combination of nitrogen fertilization at 60 kg N/ha (ammonium sulphate - 21%) and the lower concentrations of each of gibberellin (10-100 ppm), ethephon (1-10 ppm), cycocel (50-500 ppm) and B-9 (50-500 ppm), were very effective in increasing chamomile plant height, total dry matter, and flower yield.

2.8.2 Phosphorus

Plants absorb most of their phosphorus as the primary orthophosphate ($H_2PO\bar{4}$) and secondary orthophosphate ($HPO_4 2-$) ions. The relative amount of these two ions, to be absorbed by plants, are affected

by the soil reaction (pH) surrounding the roots. Lower pH (<5) values will increase the absorption of the $H_2PO_4^-$ ion, whereas higher pH (about 7.7) values will increase absorption of the HPO_4^{2-} form. Plants may also absorb certain soluble organic phosphates. Nucleic acid and phytin are taken in by plants from sterile sand or solution cultures. Both compounds may occur as degradation products of the decomposition of soil organic matter and as such could be utilized directly by growing plants. Because of their instability in the presence of active microbial population, however, nucleic acid and phytin importance as sources of phosphorus for higher plants under field conditions is limited (Tisdale and Nelson, 1966; Black, 1968; and Mengel and Kirkby, 1979).

Phosphorus stimulates root growth and promotes rapid development of the plants (Houghland, 1947; Glover, 1953; Yamashita and Goto, 1963; Black, 1968; and Mengel and Kirkby, 1979). If soil phosphorus availability is high, young plants absorb phosphorus rapidly. By the time they have accumulated 25% of the seasonal total dry matter, they may have absorbed as much as 50% of the seasonal total

phosphorus. The relative response of crops to phosphorus fertilization is greatest early in the season and decrease, as maturity is approached (Black, 1968). In plants that have an adequate supply of phosphorus, the proportion of the seasonal total phosphorus taken up during early growth characteristically exceeds the proportion of the seasonal total dry matter produced (Tisdale and Nelson, 1966; Black, 1968; and Mengel and Kirkby, 1979). Phosphorus also enhances the maturity of some crops (Haddock, 1947; Glover, 1953; Van Goor, 1954; Black, 1968; Arnon, 1972; and Mengel and Kirkby, 1979). The availability of fertilizer phosphorus tends to decrease relative to availability of soil phosphorus as the season progresses. This effect is probably due to several factors, including reaction of the fertilizer with the soil, preferential removal of water from the upper part of the soil where the fertilizer is located, and extension of roots into soil that does not contain fertilizer phosphorus (Black, 1968).

Since phosphorus is required for the synthesis of adenosine triphosphate (ATP) and numerous other phosphorylated compounds, its deficiency therefore, causes immediate and severe disruptions of metabolism

and development. Phosphorus deficiency causes dormancy of buds and suppresses lateral bud development or tillering, and growth habits often thin and erect. Purple taints are particularly characteristic but are not always produced. Apart from the work of Gindich and Sheberstov (1971) described above, there is hardly any literature dealing with the effect of phosphorus on chamomile growth.

2.8.3 Other factors affecting growth of chamomile

Other factors affecting chamomile growth and development apart from nutrition include growing site (climate), photoperiodism, water availability and variety differences. Franz et al. (1978) reported that fourteen chamomile varieties grown in two locations, Rauischholzhausen and Weihenstephan (Federal Republic of Germany), differed morphologically and in their chemical properties. Wild populations (e.g. Menemen) and local cultivar (bohemia) grew to a short height and developed meagre flowers. Shalaby and Verzár-petri (1978) working on Roman chamomile (Achillea millefollium) variety "Collina becker" reported that chamomile grown in various sites in Hungary (Kerpes, Sagvariliget and Daranypuszta) showed differences in the growing behaviour. The

plants grown under the phytotron conditions were shorter but heavier than those grown in the three different sites. The plants grown under the phytotron completed their life cycle in three months, while those grown in the three sites completed theirs in about five months. Franz (1983a) reported that the more southernly (West Germany) chamomile was grown the less the flower and oil yields. Investigations with chamomile cultivated in Munich and Izmir showed differences in growth (Franz, 1983a).

The growth of officinal plants e.g. chamomile was reported to be stimulated by irrigation, whereas development remained practically unaffected in conditions of no rainfall (Penka, 1978). He further reported that the growth characteristics (fresh weight, water content, dry matter and height) of chamomile plants were on average, from 15-50 percent higher compared to non-irrigated chamomile plants in conditions of no rainfall. The inter-relationship of water and nutrient availability to plants was also evident (Penka, 1978). High water supply produced an increase in flower yield with increasing nitrogen fertilization in chamomile plants (Penka, 1978). Low

water supply along with additional nitrogen fertilization resulted in yield depression of chamomile and other plants in general (Taysi et al., 1977; Vömel et al., 1977; and Penka, 1978).

The genetical differences of chamomile cultivars and varieties affect growth, flower and oil yield, and quality of the essential oil (oil composition). Franz and Hölzl (1978) reported that wild chamomile (Menemen and Bohemia varieties) grew to a small plant height. The improved varieties E29, Pohorelicky and BK2 (Federal Republic of Germany) which are tetraploids grew vigorously and homogeneously.

2.9 Effect of nitrogen and Phosphorus on the flower yield of Chamomile

Golcz et al. (1971), working with tetraploid chamomile varieties, reported that applying NPK fertilizer increased the flower yield of chamomile. They further reported that nitrogen had the greatest influence on the growth and development of chamomile plants and flowers. The best results were obtained using NPK or NP fertilizer (Golcz et al., 1971).

Sheberstov et al. (1972) got similar results. Franz and Kirsch (1974) working in pot experiments, reported that chamomile plants grown in pots and supplied with nitrogen at 0-4.5 g. N per pot and K_2O at 0-4 g per pot, increased the flower size, number and total flower yield. Potassium nutrition advanced flowering time. An N:K ratio of about 1:2 ensured an adequate numbers of shoots with flower buds.

Experiments in India by Singh (1977) showed that increasing nitrogen from 0 to 40 kg N/ha increased the fresh and dry flower yield of chamomile from 2281 to 4041 kg/ha and 456 to 806 kg/ha, respectively. He further reported that increasing phosphorus application from 0 to 80 kg P_2O_5/ha increased the fresh flower yield from 3092 to 3230 kg/ha. However, he also reported that increasing potassium from 0 to 120 kg K_2O/ha decreased the fresh flower yield of chamomile from 3236 to 3086 kg/ha. Franz (1981) reported that under middle European conditions, nitrogen application not only increased the flower yield, but also improved the essential oil quality.

El-Hamidi et al. (1965) reported that nitrogen and phosphorus increased flower head size and total flower yielf of chamomile, but a decrease was observed when unbalanced levels of nitrogen and phosphorus were applied. An N:P ratio of 2:2, gave the highest flower yield of chamomile. Meawad et al. (1984) reported that a combination between nitrogen fertilizer at 60 kg N/ha and lower concentration of each of gibberellin (10-100 ppm), ethephon (1-10 ppm), cycocel (50-500 ppm) and succinic acid-2,2, dimethyl hydrazide (50-500 ppm) were more effective in increasing the flower head size and total flower yield of chamomile than nitrogen fertilization on growth regulators alone.

2.10 Other factors affecting flower production of chamomile

Other factors affecting flower production of chamomile apart from nutrition are growing site (climatic factors) and genetical differences among the varieties, clones and cultivars. Franz et al. (1978) reported that chamomile plants grown in two locations (Rauischholzhausen and Weihenstephan - Federal Republic of Germany) differed in their flower yields. The varieties 'Menemen' and 'Bohemia'

produced few flowers, which had a diameter not exceeding 20 mm. Varieties, BK2, H'74, Pohorelicky, PL 1071 and PL 1073 (tetraploids - Federal Republic of Germany) produced flowers with a diameter of about 30 mm and the flower yield was higher than that of Menemen and Bohemia. The tetraploid varieties produced heavier flowers (each flower weighing averagely 40-45 mg) than Bohemia (30 mg) and Menemen (25 mg). Therefore, tetraploid varieties yielded more than the local vareities (Bohemia and Menemen), (Franz et al., 1978). Chamomile grown in the Southern hemisphere yields less flowers compared to the plants grown in other hemispheres (Franz, 1983a). Investigations with chamomile cultivated in Munich and Izmir showed flower yield differences of about 100 percent between the two sites.

2.11 The effect of nitrogen and phosphorus on the essential oil yield and content (or composition)

El-Hamidi et al. (1965) reported that nitrogen, phosphorus and potassium application increased oil percentage and total oil yield per plant of chamomile. However, nitrogen and phosphorus increased the

chamazulene percentage in the essential oil and a N: P ratio of 2:2 gave the highest oil yield of chamomile. Increased nitrogen application caused a noticeable decrease in the chamazulene percentage in the essential oil (El-Hamidi et al., 1965). The highest oil percentage per unit weight of dried flowers and oil yield per plant was obtained on 79 and 143 days, respectively, after transplanting, then a decrease was observed (El-Hamidi, et al., 1965). However, NPK levels had no effect on the physical properties of the essential oil. Jolivet (1977) reported that NPK fertilizer application gave the highest essential oil yield of chamomile.

Franz (1981) reported that under middle European conditions nitrogen and phosphorus fertilization increased the essential oil yield of chamomile. Increasing nitrogen from 0 to 40 kg N/ha increased the oil yield while a further nitrogen increase decreased it. Nitrogen application increased the percentage of (-)-α-bisabolol in the essential oil, but decreased the percentage of bisabololoxide B(and other bisabololoxides) in the essential oil. Increasing phosphorus application from 0 to 80 kg P_2O_5/ha increased the essential oil of chamomile and further

phosphorus application decreased it. Phosphorus application generally decreased the percentage of (-)-α-bisabolol in the essential oil of chamomile, but increased the percentage of bisabololoxide B. Franz (1983b) using the same rates of fertilizer application he used in 1981 reported that essential oil content of chamomile was increased to a certain extent when nitrogen and phosphorus was applied. He further reported that potassium application decreased the percentage of the essential oil of chamomile flowers. Franz (1983b) did an additional experiment, using radioactive labelled ^{14}C-Na-acetate (application of the acetate solution into the cavity of the receptacle) and confirmed and explained the results he got in 1981 and 1983b. He reported that plants well supplied with nitrogen showed lower incorporation rates of ^{14}C-Na-acetate in compounds of the essential oil of chamomile, and contained less bisabololoxides, but high (-)-α-bisabolol. Nitrogen deficient plants showed high incorporation rates of ^{14}C-Na-acetate in compounds of the essential oil, and increased bisabololoxides, but decreased (-)-α-bisabolol.

Singh (1977) showed that nitrogen and phos-

phorus application increased the flower and essential oil yield of chamomile per hectare. Increasing nitrogen from 0 to 40 kg N/ha increased the total essential oil yield from 7.2 to 7.63 kg/ha. However, he reported that increasing potassium application (from 0 to 120 kg K_2O/ha) reduced the total essential oil yield from 7.7 to 7.0 kg/ha. Furthermore, increasing nitrogen and potassium above 40 kg N/ha and 120 kg K_2O per hectare, respectively, decreased the oil percentage per unit weight of dry chamomile flowers.

Meawad et al. (1984) reported that essential oil yield per unit weight of dry flowers was increased as a result of nitrogen fertilization (60 kg N/ha) combined with gibberellin (10-100 ppm), ethephon (1-10 ppm), cycocel (50-500 ppm) and B-9 (50-500 ppm), as compared to nitrogen fertilization or growth regulators alone. The combination of nitrogen and growth regulators under study increased the percentage of chamazulene in the essential oil.

2.12 Other factors affecting essential oil yield and quality of Chamomile

Other factors apart from nitrogen and phos-

phorus nutrition that influence essential oil yield and quality includes environmental, genetical and plant factors. Blazek and Stary (1961) reported different essential oil contents in flowers of chamomile from different origins. The choice of localities with a particular type of climate influences yield and quality of the essential oil of chamomile (Franz, 1983).

Schröder (1978) reported that chamomile varieties "M", "E" and "CH" (Federal Republic of Germany) showed variability in their oil contents per unit weight of dry chamomile flowers. He reported that the mean essential oil content per unit weight of dry chamomile flowers was 1.0 percent. He also reported that wide variation in oil contents were in the "M" variety with average values of 0.57% and 1.5%, essential oil per unit weight of dry flowers. Franz et al. (1978) reported that polyploid cultivars (BK-2, H'2, H'74, Bodegold, Pohorelicky, PL-107, PL-1073 - Federal Republic of Germany) and selections E29 and CH 29 produced the highest essential oil yield and content than Menemen (wild variety). They further reported that tetraploid varieties and selections "E29" and "CH29" produced

essential oil with high chamazulene percentage (more than 20%) than Menemen (which had no chamazulene).

Shalaby and Verzár-petri (1978a) reported that there were fluctuations in the oil composition during different stages of growth in wild Chamomile plants. They reported that chamazulene and α-pinene increased at the beginning of growth, reached a maximum at budding stage and then decreased. Franz et al. (1978) reported that the content of essential oil of chamomile differed clearly not only in the sowing and harvest dates but also in the flowering stages. In stage I (ligulate flowers beginning until full developed, tubular florets closed) the oil content varied much more than in stage II (tubular flowers partially open, until fully developed). However, generally the flowers in stage II contained more essential oil than those of stage I. The least oil content was evident in stage III (flower heads start to decay). Franz et al. (1978a) reported that the composition of the essential oil was not only dependent on the flower development stage, but also on the date of harvesting. Bisaboloids generally were higher in stage II than in stages I and III.

Chamazulene increased from stage I to stage II too, whereas the hydrocarbons decreased (Franz et al., 1978). Franz (1980) reported that the relative amount of farnesene and (-)-α-bisabolol was highest in stage I and lowest in stage IV (flower heads decayed). He further reported that the essential oil content of chamomile flowers was highest in largely developed flower heads, and the maximum oil content was reached approximately one week after the start of flowering. Marczal and Verzár-petri (1980) got similar results as Franz et al. (1978); Franz (1980); and Shalaby and Verzár-petri (1978). Similar results had also been obtained by Ruminska (1965), Schantz and Salonen (1956) and Poethke and Bulin (1969).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental site

The experiment was carried out during the short rains starting from August 1986 to the end of February 1987. The experiment was conducted at Kabete Campus, Field station farm of the University of Nairobi. The area lies at an altitude of 1,940 m. above sea level, and between latitudes 1^{o} 14' 20" South to 1^{o}15'15" South, and longitudes 36^{o}44' East to 36^{o}45' East. The rainfall is bimodal and evenly distributed with peaks in April and November. The seasonal data, annual averages for the last 5 years are given in appendix 1. The details of radiation, sunshine hours per day, maximum and minimum temperatures and total rainfall during the experimental period are presented in appendix 2.

The soils are dark reddish brown clays overlying on dark and red clays (Nyandat and Michieka, 1970). The soils are deep, well drained and have fairly high water holding capacity. The soils have a blocky structure which allows good root penetration and development. They are depleted of soluble

bases and silica but are rich in iron oxide, haematite, goethite and aluminium in the form of clay minerals, metahalloysite and hydrated halloysite, as a consequence of excessive leaching (Nyandat and Michieka, 1970). Although the clay minerals are predominantly kaolin (metahalloysite), they contain about 15 percent illite. The soil pH ranges from 5.2 to 7.2 and 5.2 to 7.7, respectively (Nyandat and Michieka, 1970). The soils have been classified as humic nitosols according to FAO/UNESCO.

3.2 Soil analysis

Before the seedlings of chamomile were transplanted from the nursery to the seedbed, five random soil samples were collected from the entire experimental plots at a depth of 0-20 cm from the surface of the soil on 19th August, 1986. This was repeated on 28th February, 1987 after the last chamomile flower harvest.

The soil samples were air dried in the laboratory and they were passed through a 2 mm sieve. What passed through the sieve was used for all soil analysis.

The soil pH (reaction) was determined with a pH meter (PYE UNICAM, U.K.) on one part of soil to two and half parts water (1:2.5) or 0.01M.KCl.

Soil available nitrogen was determined by the Kjeldahl method followed by distillation and titration as described by Bremner (1960). The method is used in the Soil Science Department of the University of Nairobi.

Soil available phosphorus was determined by buffered 0.5M. $NaHCO_3$ method of Olsen *et al.* (1954). The method is used in the Department of Soil Science of the University of Nairobi. The soil analysis results before transplanting the seedlings of chamomile to the seedbed and after the last flower harvest are given in Appendix 3 and 4, respectively.

3.3 Experimental design

The treatments consisted of 4 levels of phosphorus fertilizer and 4 levels of nitrogen fertilizer. The 4 levels of phosphorus fertilizer were:- 0(control), 17.47, 34.93 and 52.41 kg P per hectare, applied as double superphosphate (45% P_2O_5) at transplanting time. The 4 levels of

nitrogen fertilizer were: 0 (control), 50, 100 and 150 kg N per hectare, applied as calcium ammonium nitrate (26% N) two weeks after transplanting. This gave 16 treatment combinations. The spacing used was 40 cm between the rows and 30 cm between the plants.

The 16 treatment combinations were laid down in a split-plot experimental design with three replications (blocks). The main plots were the four levels of phosphorus fertilizer and the sub plots the four levels of nitrogen fertilizer. Each main plot was 6 m x 6 m (36 m^2), while each sub plot was 3 m x 3 m (9 m^2). Each sub plot had 7 rows of plants with 2 guard rows on each side. During the determination of leaf area and dry matter accumulation in the plant, destructive harvesting was done, and an area of 2 m^2 (1 m x 2 m) was set aside within the subplots, in order not to affect the required plant population per unit area required for the estimation of flower yield. An area of 2m x 2m ($4m^2$) was used for the estimation of flower yield of chamomile in each sub plot. The remaining $3m^2$ was used to determine plant height, number of tillers

per plant and spreading line of the plants.

3.4 Cultural practices

The seeds of chamomile (variety-max et oljea) were sown in the nursery on 22nd July, 1986. The seedlings were transplanted when they had 6-7 true leaves (4 weeks after emergence). The experimental plots were tractor ploughed and disc harrowed two weeks in advance before transplanting.

During transplanting (19th August, 1986) each main plot received an appropriate level of phosphorus fertilizer. Two weeks later each sub plot received an appropriate level of nitrogen fertilizer.

Weed control was done by hand cultivation (hoeing between rows and within plants). The weeding was done at an interval of two weeks. The predominant weeds in the experimental area were:- Pennisetum clandestinum, Oxalis latifolia H.B.K., Oxalis corniculata and Cyperus rotundus. Irrigation was done whenever necessary to avoid water stress in plants.

During the growing season there were signs

of aphids attack on the plants. The aphids were successfully controlled by using Metasystox (at a rate of 1 ml of Metasystox in 2 litres of water). During the dry spell in September, 1986, there were signs of powdery mildew attack. This was successfully controlled by spraying the plants with Benlate (at a rate of 10 g of Benlate in 20 litres of water).

3.5 Growth studies

Changes in leaf area, plant height, dry matter accumulation in plants, fresh weights of plants, spreading line of plants and number of tillers (offsets) per plant were determined after two weeks of top dressing with nitrogen. The data on growth was collected at a two week interval until the last flower harvest was done. All the plants used for data collection were randomly selected. Three plants were used for dry matter and leaf area determination and the three plants were just cut below the ground level using a pair of scissors. The leaf area was determined by using a portable leaf area meter Model LI-3000. The dry matter weights of the three plants were determined on materials dried on a ventilated

oven at 105°C for 48 hours. Plant height, number of tillers per plant, and spreading line of the plants were determined on intact plants. Plant height and spreading line of plants were determined using a metric meter ruler. The number of tillers per plant were counted at an interval of two weeks.

3.6 Determination of flower Yield

The first harvesting of flower heads was undertaken when 50 percent of the plants had flowered. Subsequent harvests were done after every 14 days. There were 8 harvests in total. At any harvesting date, only flower heads with more than 40 percent open tubular florets were picked. After every harvest the fresh flower weights were determined. The fresh flowers were then dried in an air ventilated oven at 35°C for 5 days (to constant weight). After drying at 35°C, the flower dry weights were determined. The fresh and dry weights were used to estimate the weight of fresh flowers that could give 1 kg of dry flowers.

3.7. Determination of the essential oil yield and quality

Determination of the quantity of the essential oil in the dried flower samples was based on steam distillation of the essential oil. Clevinger apparatus were used for the essential oil extraction using the method described by Hölzl and Demuth (1975); Trease and Evans (1972 and 1978), Tyler et al. (1976), and Leon (1975). The qualitative analysis of the essential oil was done using gas liquid chromatographtechniques (GLC) as outlined by Haggag et al. (1975), Trease and Evan (1972 and 1978), Kirk and Othmer (1952) and Grant (1971), with slight modification on the conditions of the GLC. The conditions of the GLC used were as follows:

APPARATUS	:	GOW-Mac series 69-750
COLUMN	:	2.5 M long, 0.25 cm in diameter
PACKING	:	5% OV-1 on CHROMOSORB W/HP (100-120)
TEMPERATURE	:	Linear temperature programming, 85-175^{o}, 2.5^{o}C per minute
DETECTOR	:	Flame ionization detector
INJECTOR	:	Temperature 220^{o}C

DETECTOR	:	Temperature 220^{o}C
COLUMN	:	Temperature 170^{o}C
CARRIER GAS	:	Nitrogen (flow 25 cm^{3}/ minute)
ATTENUATION	:	16
CHART SPEED	:	1 cm per minute
RANGE	:	10^{-11}

3.8 Statistical analysis

The data collected were subjected to analysis of variance (Little and Hills, 1978; Snedecor and Cochran, 1967; Steel and Torrie, 1981; and Cochran and Cox, 1957). The means were separated using the Duncan's Multiple range test (Little and Hill, 1978 and Steel and Torrie, 1981).

CHAPTER 4

4.0 RESULTS

4.1 Effect of nitrogen and phosphorus on vegetative growth.

Nitrogen fertilization significantly increased the vegetative growth (plant height, leaf area, spreading ability of plants, number of tillers and dry matter production) of chamomile plants. Tables 1, 2, 3, 4 and 5 show that vegetative growth of top-dressed plants was significantly higher than those that were not and that vegetative growth increased with increasing nitrogen application rates. However, vegetative growth did not significantly increase with application of above 50 kg N/ha. Top-dressing the plants with more than 100 kg N/ha led to insignificant decrease in vegetative growth.

Phosphorus fertilization did not significantly influence vegetative growth of chamomile plants. Similarly, the interaction between nitrogen and phosphorus fertilizer rates for vegetative growth was not significant.

Table 1. Effect of nitrogen top-dressing on the height of chamomile plants

N Rates kg N/ha	Plant height (cm)				
	Days after transplanting				
	38	45	59	80	112
0	9.74[a]	14.65[a]	20.35[a]	31.09[a]	51.31[a]
50	10.63[ab]	17.50[b]	24.90[ab]	49.05[b]	82.58[b]
100	11.97[b]	18.19[b]	29.47[b]	55.24[c]	84.74[b]
150	11.09[b]	17.67[b]	27.44[b]	51.73[c]	82.07[b]

Figures followed by same letter(s) down the column are not significantly different according to Duncan's multiple range test (5% probability level).

Table 2. Effect of nitrogen top-dressing on leaf area of chamomile plants.

N rates kg N/ha	Leaf area per plant (cm^2)		
	Days after transplanting		
	38	45	59
0	102.81[a]	154.61[a]	160.48[a]
50	142.39[b]	198.17[a]	201.73[a]
100	182.95[c]	284.67[b]	273.51[b]
150	150.75[b]	255.87[b]	254.97[b]

Figures followed by same letter(s) down the column are not significantly different according to Duncan's multiple range test (5% probability level).

Table 3. Effect of nitrogen top-dressing on chamomile plant spread.

N rates kg N/ha	Plant spread (cm) Days after transplanting 36	45	59	84
0	19.39[a]	24.64[a]	25.90[a]	25.90[a]
50	23.65[b]	28.40[b]	29.77[b]	30.36[b]
100	25.71[b]	32.37[b]	33.78[b]	30.94[b]
150	24.76[b]	31.46[b]	32.96[b]	31.03[b]

Figures followed by same letter(s) down the column are not significantly different according to Duncan's multiple range test (5% probability level).

Table 4. Effect of nitrogen top-dressing on tillering ability of chamomile plants

N rates kg N/ha	Number of tillers per plant Days after transplanting 45	59	74	93	107
0	1.00[a]	1.27[a]	3.33[a]	5.86[a]	5.87[a]
50	2.07[b]	2.46[b]	5.67[b]	11.24[b]	11.27[b]
100	2.59[c]	3.11[c]	7.13[c]	13.69[c]	13.67[c]
150	2.42[bc]	2.97[c]	6.73[c]	12.36[b]	12.35[b]

Figures followed by same letter(s) down the column are not significantly different according to Duncan's multiple range test (5% probability level).

Table 5. Effect of nitrogen top-dressing on dry matter production of chamomile plants.

N rates kg N/ha	Dry matter production (g/3 plants) Days after transplanting 38	45	92	106	123	153	175
0	2.02[a]	4.77[a]	83.51[a]	222.37[a]	264.64[a]	313.08[a]	294.16[a]
50	3.64[a]	8.40[b]	113.19[b]	342.53[b]	409.72[b]	495.38[b]	434.66[b]
100	6.25[b]	12.81[c]	122.30[b]	437.87[c]	502.10[b]	607.13[c]	522.47[b]
150	5.08[cb]	11.14[bc]	114.35[b]	473.39[c]	525.44[b]	645.12[c]	549.82[b]

Figures followed by same letter(s) down the column are not significantly different according to Duncan's multiple range test (5% probability level).

4.2 Effect of nitrogen and phoshorus on dry flower yield.

Nitrogen fertilization significantly increased dry flower yield of chamomile plants. Table 6 shows that dry flower yield increased with increasing nitrogen application rates and that plants top-dressed with nitrogen significantly produced more flowers than those that were not. However, the table also shows that there was no significant increase in flower yield with the application of more than 50 kg N/ha. Figure 1 shows that dry flower yield generally increased slowly 78 days after transplanting, but it increased rapidly from 120 days after transplanting (4th flower harvest) and reached highest level 134 days after transplanting, and there after the dry flower yield decreased.

Phosphorus fertilization, and nitrogen and phosphorus interaction had no significant influence on dry flower yield of chamomile plants.

4.2.1 Effect of nitrogen and phosphorus on total cumulative dry flower yield.

There was a significant effect of nitrogen fertilization on cumulative dry flower yield. Table 7 shows that cumulative dry flower yield increased

Table 6. Effect of nitrogen top-dressing on dry flower yield of chamomile plants

N rate kg N/ha	Dry flower yield ($g/4m^2$) Days after transplanting							
	78	92	106	120	134	148	162	176
0	1.01[a]	3.80[a]	17.05[a]	56.68[a]	121.01[a]	78.67[a]	69.32[a]	25.30[a]
50	2.62[b]	13.19[b]	32.22[b]	87.99[b]	192.79[b]	117.42[b]	108.98[b]	43.55[b]
100	4.73[c]	19.89[b]	35.65[b]	92.33[b]	206.49[b]	135.18[b]	111.01[b]	37.10[b]
150	3.40[bc]	14.16[b]	33.88[b]	87.93[b]	217.49[b]	133.90[b]	115.95[b]	42.51[b]

Figures followed by same letter(s) down the column are not significantly different according to Duncan's multiple range test (5% probability level).

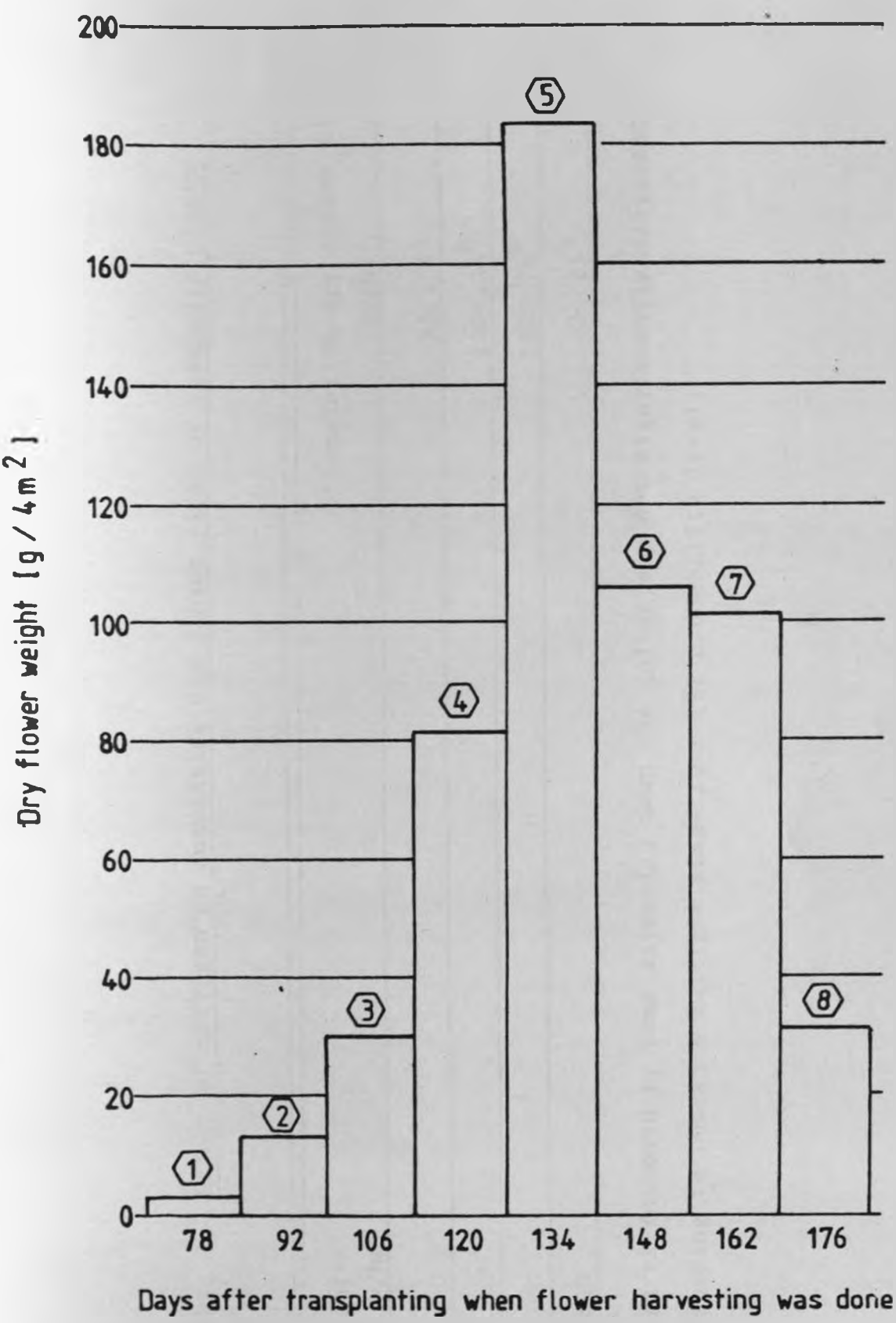

Fig. 1. Effect of time of flower harvest on dry flower yield

Table 7. Effect of nitrogen on cumulative dry flower yield of chamomile plants

N rates kg N/ha	Cumulative dry flower field (kg/ha)
0	932.13[a]
50	1504.38[b]
100	1625.78[b]
150	1623.35[b]

Figures followed by same letter(s) down the column are not significantly different according to Duncan's multiple range test (5% probability level).

with increasing nitrogen application rates and that top-dressed chamomile plants significantly produced more flowers than those that were not. However, there was no significant difference on cumulative dry flower yield among chamomile plants that were top-dressed with nitrogen above 50 kg N/ha.

Phosphorus fertilization, and the interaction between nitrogen and phosphorus fertilizer rates, had no significant effect on total cumulative dry flower yield of chamomile plants.

4.3 Effect of nitrogen and phosphorus on essential oil yield.

Nitrogen fertilization significantly increased essential oil yield per unit dry flower weight and per plant. Tables 8 and 9, show that essential oil yield per unit dry flower weight and per plant, respectively, increased with increasing nitrogen application rates. The tables also show that top-dressing chamomile plants with more than 100 kg N/ha did not significantly increase the essential oil yield per both unit dry flower weight and plant. It can be seen that application of more

Table 8. Effect of nitrogen on essential oil yield per unit dry flower weight of chamomile plants

N rates kg N/ha	Essential oil yield per unit dry flower weight (%)				
	Days after transplanting				
	106	120	134	148	176
0	1.013[a]	0.546[a]	0.481[a]	0.545[a]	0.551[a]
50	1.365[a]	0.953[bc]	0.729[b]	0.658[b]	0.642[b]
100	1.929[b]	1.047[c]	0.873[c]	0.676[b]	0.655[b]
150	1.127[a]	0.880[b]	0.819[bc]	0.624[b]	0.602[b]

Figures followed by same letter(s) down the column are not significantly different according to Duncan's multiple range test (5% probability level).

Table 9. Effect of nitrogen on essential oil yield per chamomile plant

N rates (kg N/ha)	Essential oil yield (kg/ha)
0	5.85[a]
50	13.08[b]
100	16.64[b]
150	13.16[b]

Figures followed by same letter(s) down the column are not significantly different according to Duncan's multiple range test (5% probability level).

both unit dry flower weight and plant. However, the decrease was not significant. Increasing application of nitrogen from 0 to 100 kg N/ha increased essential oil yield per unit dry flower weight and per plant from 0.627 to 1.036% and 5.85 to 16.64 kg/ha, respectively.

The effect of phosphorus fertilization on essential oil was significant during the last two flower harvests (Table 10). Table 10 shows that low phosphorus fertilizer rates tended to increase essential oil yield per unit dry flower weight. Application of more than 34.93 kg P/ha significantly decreased essential oil yield per unit dry flower weight. Figures 2 and 3, respectively, show that essential oil yield per unit dry flower weights decreased with advancing plant age irrespective of nitrogen application.

The interaction between nitrogen and phosphorus fertilizer rates on essential oil yield per unit dry flower weight and per plant was not significant.

Table 10. Effect of phosphorus on essential oil yield per unit dry flower weight of chamomile plant

P rates kg P/ha	Essential oil yield per unit dry flower weight (%)				
	Days after transplanting				
	106	120	134	148	176
0	1.026[a]	0.735[a]	0.698[a]	0.586[a]	0.594[a]
17.47	1.673[a]	0.768[a]	0.871[a]	0.638[a]	0.594[a]
34.93	1.423[a]	1.066[a]	0.583[a]	0.666[b]	0.644[b]
52.41	1.312[a]	0.856[a]	0.750[a]	0.613[a]	0.589[a]

Figures followed by same letter(s) down the column are not significantly different according to Duncan's multiple range test (5% probability level).

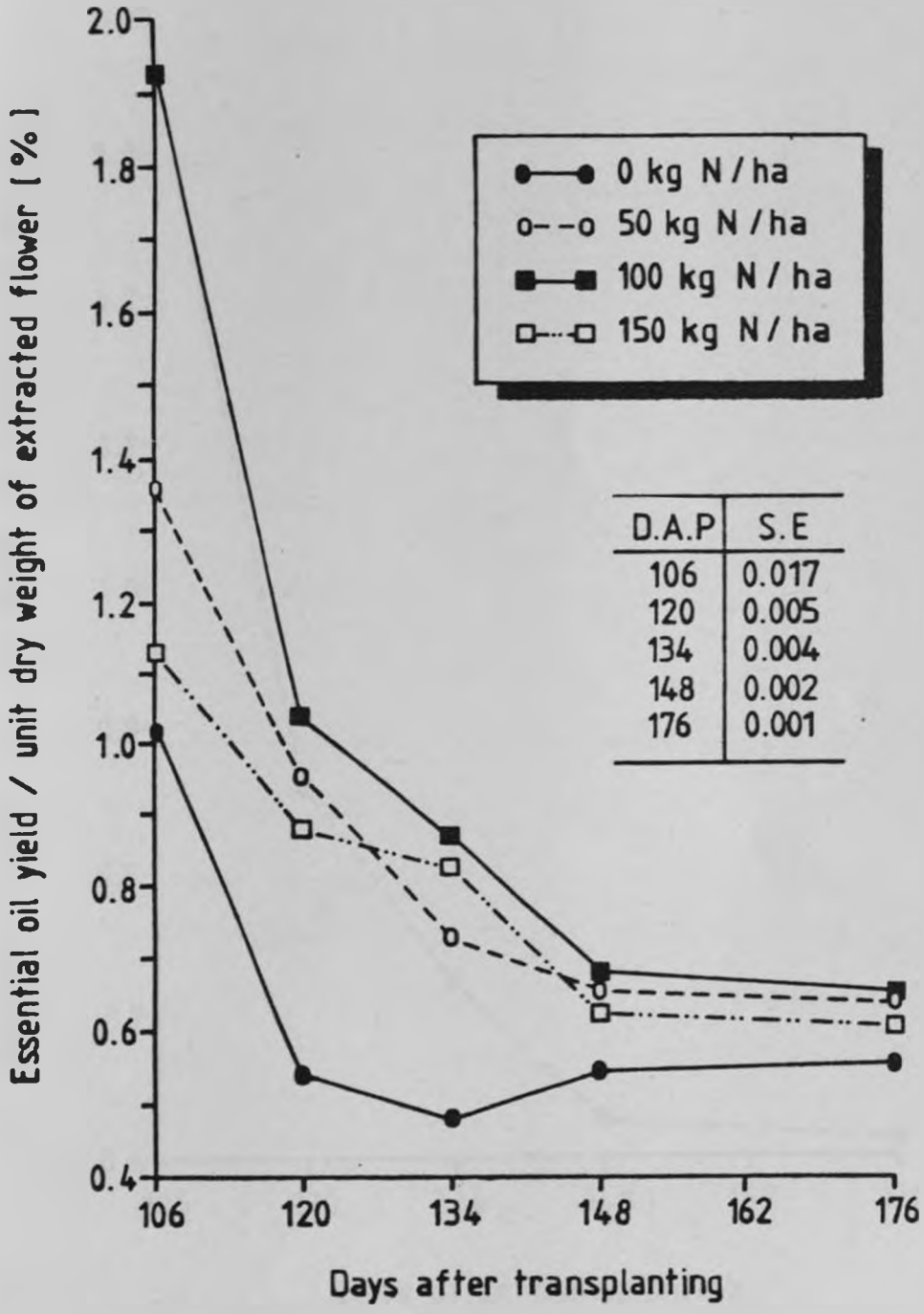

Fig. 2. Effect of nitrogen on essential oil yield per unit dry flower weight as plant age increases

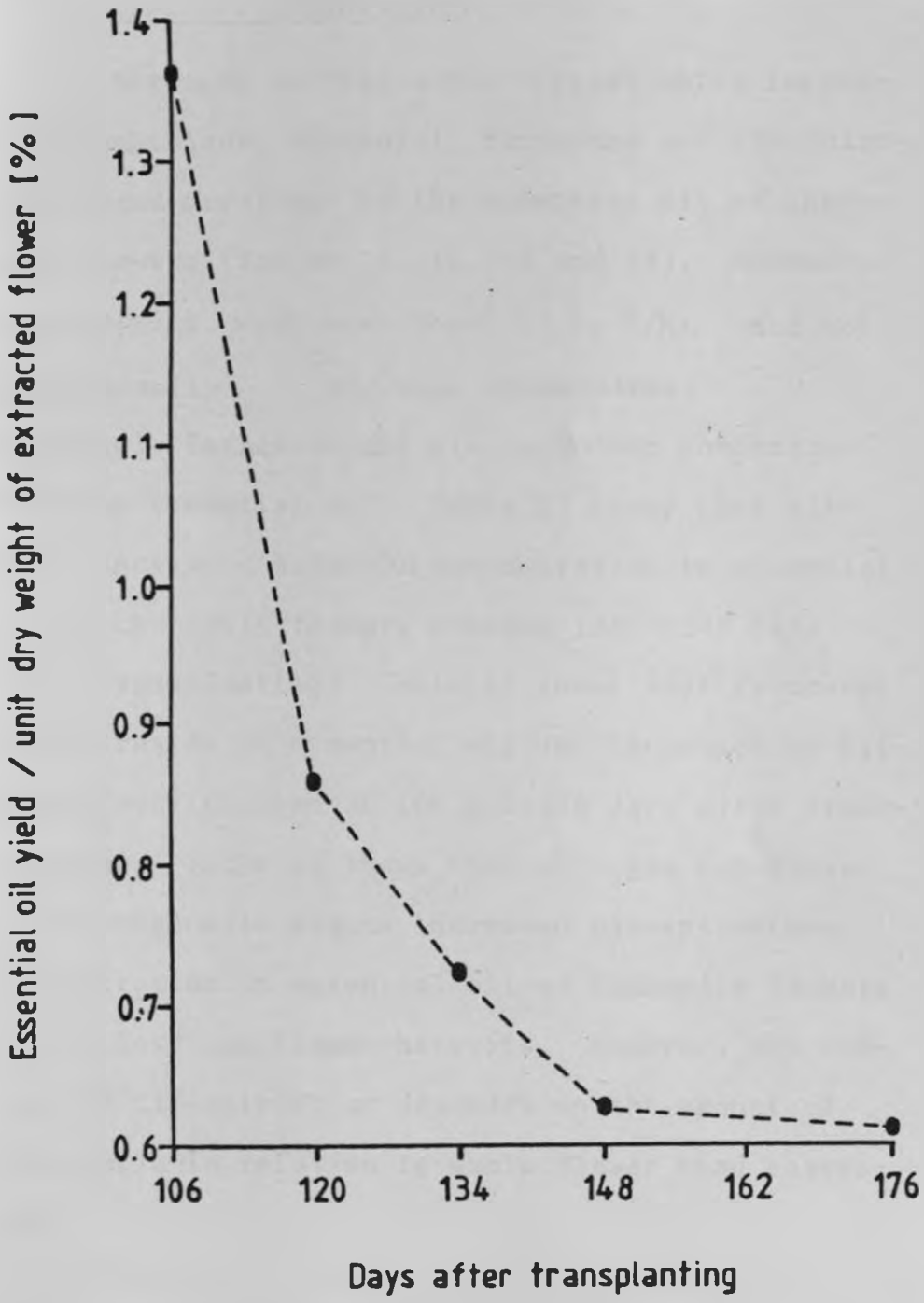

Fig. 3. Effect of time of harvest on essential oil of chamomile flowers.

4.4 Effect of nitrogen and phosphorus on essential oil composition.

Nitrogen fertilization significantly increased chamazulene, bisabolol, farnesene and cis-spiroether concentrations in the essential oil of chamomile flowers (Tables 11, 12, 13 and 14). However, top-dressing with more than 50 kg N/ha did not significantly increase chamazulene, bisabolol, farnesene and cis-spiroether concentrations in essential oil. Table 12 shows that nitrogen increased bisabolol concentration in essential oil of chamomile flowers between 134 - 148 days after transplanting. Table 13 shows that farnesene concentration in essential oil was increased by nitrogen fertilization on 106 and 176 days after transplanting. Table 14 shows that nitrogen top-dressing to chamomile plants increased cis-spiroether concentration in essential oil of chamomile flowers in the last two flower harvests. However, the content of cis-spiroether depended on the amount of receptacle in relation to whole flower head harvested.

Nitrogen fertilization significantly decreased bisabololoxides "A", "B" and (A + B) in the

Table 11. Effect of nitrogen top-dressing on chamazulene percentage in the essential oil of chamomile flowers

N rates kg N/ha	Chamazulene content in essential oil (%)				
	Days to transplanting				
	106	120	134	148	176
0	14.86[a]	5.27[a]	6.76[a]	4.70[a]	2.87[a]
50	15.88[a]	7.84[b]	7.92[a]	7.21[c]	4.15[b]
100	13.79[a]	7.89[b]	7.90[a]	6.69[bc]	3.84[b]
150	13.71[a]	6.96[ab]	7.54[a]	5.63[ab]	3.43[ab]

Figures followed by same letter(s) down the column are not significantly different according to Duncan's multiple range test (5% probability level).

Table 12. Effect of nitrogen on levarotatory alpha bisabolol percentage in the essential oil of chamomile flowers

N rates kg N/ha	Levarotatory alpha bisabolol in the essential oil (%) Days after transplanting				
	106	120	134	148	176
0	5.18[a]	5.85[a]	5.47[a]	5.14[a]	4.21[a]
50	5.86[a]	6.13[a]	6.63[b]	5.56[ab]	5.04[a]
100	6.60[a]	6.44[a]	7.43[bc]	6.47[b]	5.59[a]
150	6.70[a]	6.80[a]	7.86[c]	5.88[ab]	5.31[a]

Figures followed by same letter(s) down the column are not significantly different according to Duncan's multiple range test (5% probability level).

Table 13. Effect of nitrogen on farnesene percentage in the essential oil of chamomile flowers.

N rates kg N/ha	Farnesene content in essential oil (%) Days after transplanting				
	106	120	134	148	176
0	10.12[a]	16.57[a]	15.17[a]	12.20[a]	8.58[a]
50	12.31[b]	17.13[a]	15.89[a]	15.93[a]	10.31[b]
100	13.67[b]	15.28[a]	14.93[a]	14.96[a]	10.34[b]
150	15.21[b]	16.38[a]	15.11[a]	14.37[a]	8.44[a]

Figures followed by same letter(s) down the column are not significantly different according to Duncan's multiple range test (5% probability level).

Table 14. Effect of nitrogen on cis-spiroether concentration in the essential oil of chamomile pflowers.

N rates kg N/ha	Cis-spiroether content in essential oil (%)				
	Days after transplanting				
	106	120	134	148	176
0	7.40[a]	3.23[a]	16.02[a]	5.14[a]	5.13[a]
50	7.67[a]	3.01[a]	15.40[a]	8.61[b]	7.64[b]
100	9.21[a]	3.55[a]	15.00[a]	6.29[a]	6.28[a]
150	10.28[a]	3.78[a]	14.68[a]	4.36[a]	5.80[a]

Figures followed by same letter(s) down the column are not significantly different according to Duncan's multiple range test (5% probability level).

Table 15. Effect of nitrogen on bisabololoxide A percentage in the essential oil of chamomile flowers.

N rates kg N/ha	Levorotatory alpha bisabololoxide "A" in essential oil (%)				
	Days after transplanting				
	106	120	134	148	176
0	46.98[a]	46.11[a]	36.14[a]	28.23[a]	51.32[a]
50	40.15[b]	38.80[b]	31.32[b]	33.34[b]	45.80[b]
100	38.49[b]	38.36[bc]	29.16[c]	29.69[c]	40.39[c]
150	32.68[b]	33.04[c]	26.46[d]	27.44[c]	38.29[c]

Figures followed by same letter(s) down the column are not significantly different according to Duncan's multiple range test (5% probability level).

Table 16. Effect of nitrogen on bisabololoxide B percentage in the essential oil of chamomile flowers.

N rates kg N/ha	Levorotatory bisabololoxide "B" content in essential oil (%) Days after transplanting				
	106	120	134	148	176
0	19.33[a]	17.71[a]	21.64[a]	22.83[a]	26.93[a]
50	14.04[b]	16.05[b]	17.94[b]	20.17[b]	22.38[b]
100	11.16[b]	15.14[b]	14.36[c]	16.74[b]	18.08[b]
150	10.44[b]	11.78[c]	12.93[c]	14.49[b]	16.33[b]

Figures followed by same letter(s) down the column are not significantly different according to Duncan's multiple range test (5% probability level).

and 17). Tables 15, 16 and 17, show that in all flower harvest dates top-dressed plants produced flowers that contained significantly lower bisabololoxides concentrations in the essential oil than those that were not. Top-dressing of 150 kg N/ha to chamomile plants resulted with the production of flowers with lowest bisabololoxides contents in the essential oil in all flower harvests. However, in the case of bisabololoxide B, top-dressing plants with 150 kg N/ha did not significantly reduce its concentration whereas in the case of bisabololoxides "A" and (A + B) whose concentrations significantly reduce.

Phosphorus fertilization significantly increased chamazulene percentage in the essential oil. Table 18 shows that between 120 - 134 days after transplanting, application of 17.47 kg P/ha significantly increased chamazulene content in the essential oil. However, application of more than 17.47 kg P/ha to chamomile plants decreased chamazulene in the essential oil.

However, phosphorus had no significant effect in the contents of bisabololol,farnesene, cis-

Table 17. Effect of nitrogen on bisabololoxides (A + B) percentage in the essential oil of chamomile flowers.

N rates kg N/ha	Bisabololoxide (A+B) content in essential oil (%)				
	Days after transplanting				
	106	120	134	148	176
0	67.14[a]	66.75[a]	53.85[a]	61.09[a]	78.26[a]
50	58.20[b]	56.74[b]	47.37[b]	53.50[b]	68.19[b]
100	47.65[c]	52.72[b]	44.33[c]	46.43[c]	58.46[c]
150	42.62[c]	46.01[c]	38.24[d]	41.93[c]	54.62[c]

Figures followed by same letter(s) down the column are not significantly different according to Duncan's multiple range test (5% probability level).

Table 18. Effect of phosphorus on chamazulene percentage in the essential oil of chamomile flowers.

P rates kg P/ha	Chamazulene content in the essential oil (%)				
	Days after transplanting				
	106	120	134	148	176
0	12.98[a]	6.53[a]	6.66[a]	5.89a	3.30[a]
17.47	14.04[a]	8.52[b]	8.88[b]	5.72[a]	4.08[a]
34.93	16.04[a]	6.69[a]	8.10[a]	5.96[a]	3.15[a]
52.41	15.66[a]	6.99[a]	6.47[a]	6.66[a]	3.81[a]

Figures followed by same letter(s) down the column are not significantly different according to Duncan's range test (5% probability level).

the essential oil. Similarly, the interaction between nitrogen and phosphorus treatments on chamazulene, bisabolol, farnesene, cis-spiroether and bisabololoxides contents in the essential oil was not significant.

The effect of nitrogen, phosphorus and their interactions on trans-spiroether concentration in the essential oil of chamomile flowers could not be determined because trans-spiroether was present in trace amounts.

Bisabololoxides generally increased in the essential oil of chamomile flowers with increasing plant age. Figure 4 shows that bisabololoxides (A + B) concentration in the essential oil decreased with increasing plant age and reached the lowest level 134 days after transplanting, after which they increased rapidly irrespective of nitrogen fertilizer application. However, despite the increase of bisabololoxides (A + B) concentration in the essential oil after 134 days after transplanting, nitrogen fertilization significantly decreased them. Chamazulene and farnesene contents in essential oil decreased with increasing plant age. Farnesene

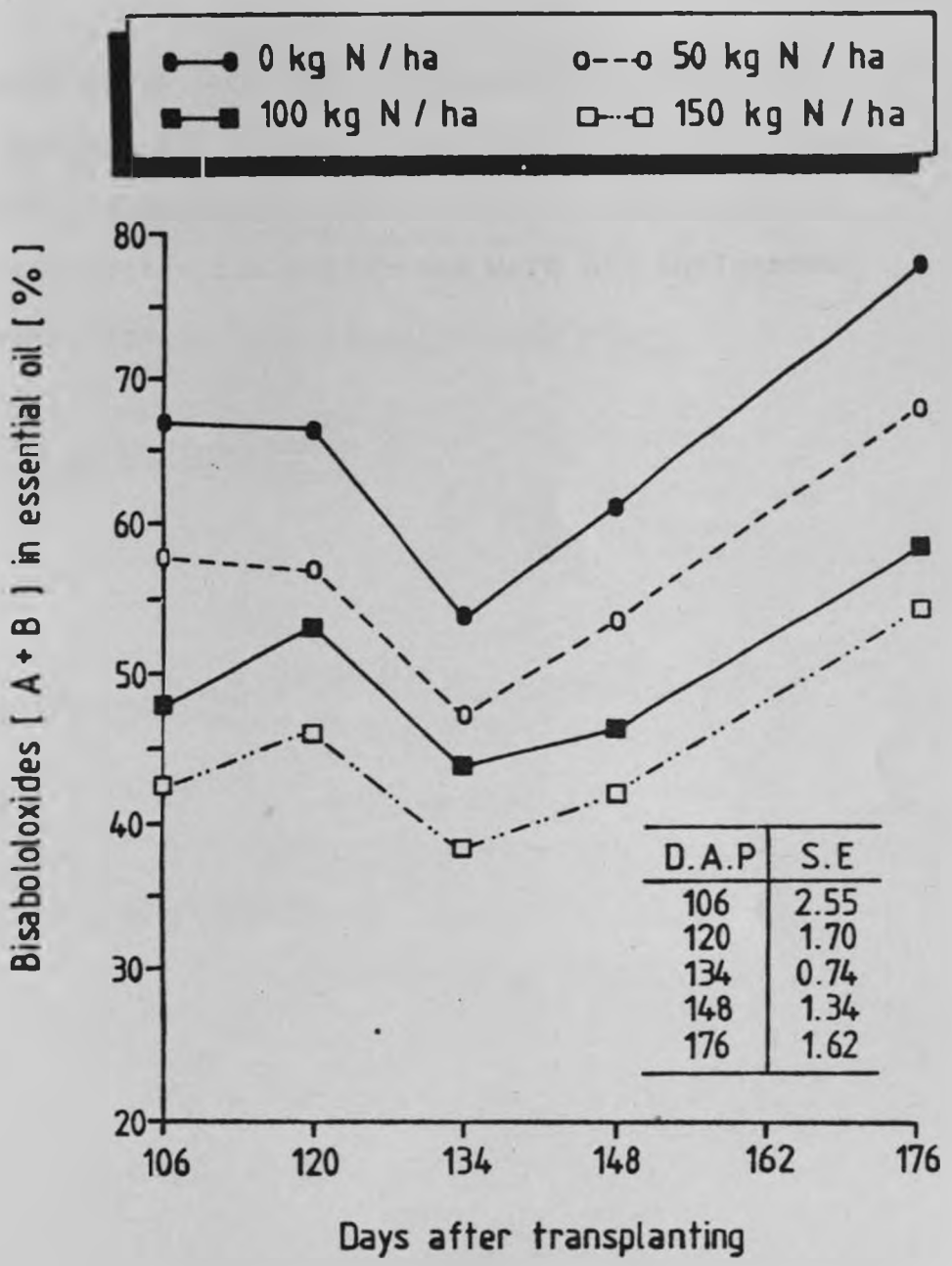

Fig. 4. The effect of nitrogen on (-)-α-bisabololo-(A + B) in the essential oil of chamomile flowers.

D.A.P. - Days after transplanting

S.E. - Standard error.

concentration increased with advancing plant age and reached the highest level 120 days after transplanting after which it decreased. Bisabolol and cis-spiroether concentrations were not influenced by plant age.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Vegetative growth

Results of this study showed that nitrogen significantly increased the vegetative growth of chamomile plants. Plants that were top-dressed with 100 kg N/ha had most vegetative growth but this was not significantly different from 50 and 150 kg N/ha rates. Top-dressing chamomile plants with more than 100 kg N/ha decreased vegetative growth. The increase in vegetative growth of chamomile plants due to nitrogen fertilization has been reported by Franz and Kirsch (1974) in the Federal Republic of Germany, El-Hamidi et al. (1965) in Egypt, Ruminska and El-Gamal (1978) in Poland and Egypt, respectively, Gindich and Sheberstov (1971) in Poland, Meawad et al. (1984) in Egypt, Franz (1974) in Federal Republic of Germany, Agena (1974) in Egypt, and Ruminska (1978) in Poland. However, Golcz et al. (1965) in Poland,, and Ruminska and El-Gamal (1978) in Poland and Egypt, respectively, reported a decrease in vegetative growth with high doses of nitrogen.

The increase in vegetative growth with nitrogen fertilizer application may be attributed to the

role of nitrogen in plant growth and development. Nitrogen plays an active role in the development of new cells, resulting in their growth, enlargement and elongation (Letham, 1961a; Bartholomew and Clark, 1965; Frank, 1965; Black, 1968; Agena, 1974; Brady, 1984; and Meawad et al., 1984). In the present study the increasing of dry matter by nitrogen application could be due to the fact that nitrogen increases carbohydrate concentration in plants, which is then utilized to form more protoplasm and cells rather than for deposition of carbohydrates to thicken cell walls (Baumeister, 1939; Letham, 1961; Hasegawa et al., 1962; and Black, 1968). The increase in dry matter production could also be because nitrogen plays an active role in the development and division of new cells, resulting in their growth and enlargement (Agena, 1974; Abou-Zeid and El-Sherbeeny, 1978; Sacks and Kofranek, 1963; Moore, 1979; and Meawad et al., 1984). While the decrease in vegetative growth at higher rates of nitrogen fertilization above 100 kg N/ha could be due to ammonium ion toxicity (Street and Sheat, 1958; Barker et al., 1966; Puritich and Barker, 1968; Black, 1968; and Mengel and Kirkby, 1979).

Dry matter production increased with time of

vegetative growth as expected (Laughlin, 1978 and 1983; Badaway, 1979; Hornok, 1980; Vomel, 1984; and Meawad et al., 1984). The general decrease of dry matter production that occurred after 153 days after transplanting may be attributed to the onset of senescence (Thomas et al., 1973; Blackman, 1961; and Russell, 1961).

5.2 Flower yield

Nitrogen significantly increased both fresh and dry flower yields (five kilogrammes fresh flower weight approximately yielding one (1) kg. dry flower weight). Chamomile plants top-dressed with 50, 100 and 150 kg N/ha respectively, did not significantly differ in their flower yields. These results are in agreement with results reported by Franz (1974), Franz and Kirsch (1974), Golcz et al. (1971), Sheberstov et al. (1972), Franz (1981, 1983b), Singh (1977), and Meawad et al. (1984). These workers reported that nitrogen fertilization increased flower yield of chamomile plants. The increase in flower yield due to nitrogen fertilization could be because of the fact that nitrogen increases the development of new cells, resulting in their growth, enlargement and differentiation, hence leading to

large flower size and many number of flowers per plant (Agena, 1974; Abou-Zeid and El-Sherbeeny, 1974; and Meawad et al., 1984).

Top-dressing chamomile plants with 100 kg N/ha resulted in plants producing the highest dry flower yield after which a decrease was observed. Franz (1974) reported similar results, when he found that application of more than 1.5 g N/pot. Decrease in flower yield with nitrogen high doses could be due to ammonium ion toxicity as discussed elsewhere. Maximum flower yield was obtained 134 days after transplanting (5th flower harvest), and thereafter a decrease was observed. This could have occurred because either the available nitrogen around the root zone had got depleted or there was an onset of senescence (Blackman, 1961; Russell, 1961; and Thomas et al., 1973).

5.3 Essential oil yield

Nitrogen fertilization significantly increased essential oil yield both per unit dry flower weight and plant. Essential oil yield increased with increasing nitrogen application. Increasing application of nitrogen from 0 to 100 kg N/ha

increased essential oil yield per unit dry flower weight and per plant from 0.627 to 1.036%, and from 5.85 to 16.64 kg/ha, respectively. Application of more than 100 kg N/ha decreased essential oil yield. El-Hamidi et al. (1965), Franz (1974), Franz and Kirsch (1974), Singh (1977), Jolivet (1977), Franz (1981, 1983b), Agena (1974), Sacks and Kofranek (1963), Abou-Zeid and El-Sherbeeny (1974), Moore (1979), Meawad (1981), and Meawad et al. (1984) obtained similar results. The workers reported that nitrogen fertilization increased essential oil yield of chamomile flowers.

The increasing of essential oil yield by nitrogen application could firstly be because nitrogen increased carbohydrate accumulation in chamomile plants which was then utilized in the formation of more essential oil cells in the secretory ducts or cavities in glandular hairs (Baumeister, 1939; Letham, 1962; and Black, 1968). Secondly, nitrogen may have played an active role in the development of new essential oil cells, cavities and ducts (Agena, 1974; Verzalova and Nespor, 1971; Meawad, 1981; and Meawad et al., 1984). Thirdly, nitrogen may have increased essential oil yield by increasing the concentration of auxins and gibberellins in the whole

plant and flower heads (Sacks and Kofranek, 1963; Abou-Zeid and El-Sherbeeny, 1974; Agena, 1974; Moore, 1979; and Meawad, et al., 1984). Fourthly, nitrogen may have increased the concentration of ethaphon, which increases carbohydrate synthesis as well as nitrogen content, all involved in the synthesis of essential oil cells, cavities and ducts of chamomile flowers (Bosila and Udalova, 1977; and Meawad et al., 1984).

The highest essential oil yield per unit dry flower weight and per plant was obtained 78 and 134 days after transplanting (1st and 5th flower harvests), respectively, after which a decrease was observed. The results of this study are in agreement with the results reported by El-Hamidi et al. (1965) who reported that the highest essential oil yield per unit dry flower weight and per plant was obtained 79 and 143 days after transplanting, respectively.

Essential oil yield per unit dry flower weight decreased with increasing plant age, though nitrogen fertilization significantly increased it. Shalaby and Verzar-petri (1978), Franz et al. (1978a), Franz (1980), Marczal and Verzar-petri (1980), Ruminska (1965), Schantz and Salonen (1966), and

Poethke and Bulin (1969) reported similar results. The researchers reported that essential oil yield per unit dry flower weight decreases with advancing plant age. The decrease in essential oil may have occurred because of high nutrient competition within the plant for the synthesis of other plant materials like dry matter and flowers, in expense of essential oil. From 153 - 176 days after transplanting, the decrease in essential oil may be accounted for by the onset of senescence.

Phosphorus fertilization increased essential oil yield per unit dry flower weight. Increasing application of phosphorus from 0 to 17.47 kg P/ha increased essential oil yield per unit dry flower weight from 0.728 to 0.914%, after which a decrease was observed. These results are inconformity with the results reported by El-Hamidi et al. (1965), and Franz (1981). El-Hamidi et al. (1965) reported that an N:P ratio of 2:2 gave the highest essential oil yield. However, in this study the highest essential oil yield was obtained using an N:P ratio of 2.5:1. Franz (1981) reported that phosphorus fertilization increased essential oil yield. Phosphorus increased essential oil yield probably because it increased

the biosynthesis of adenosine triphosphate (ATP) and pyrophosphates, which are needed in the synthesis of farnesyl pyrophosphate. Farnesyl pyrophosphate is a precursor for the synthesis of sesquiterpenes which constitute the essential oil (Martin, 1972; Wagner and Wolff, 1977; Harborne, 1973; and Thomas et al., 1973). The sesquiterpenes that constitute essential oil include chamazulene, bisabolol, bisabololoxides "A", "B" and "C", bisabololoxides, farnesene (Bonner, 1965; Herout, 1970; Goodwin, 1971; Martin, 1972; Harborne, 1973; Thomas et al., 1973; and Wagner and Wolff, 1977). Therefore, if phosphorus increased synthesis of farnesyl pyrophosphate, then this explains why phosphorus fertilization increased essential oil yield.

The variety Max et oljea which was used in the experiment gave a mean essential oil content of 0.84% per unit dry flower weight extracted under Kenyan conditions. The same variety grown under Yugoslavian conditions yielded a mean essential oil content of 0.68% per unit dry flower weight (Kornhauser, 1986). The variation in essential oil in the same variety could be due to environmental differences between Kenya and Yugoslavia. Similar observations were

reported by Franz (1983a), Blazek and Stary (1961), Schroder (1978), Schilcher (1973) and Schantz and Salonen (1966). The workers reported that essential oil yield and its composition differed according to the locality where the plant is grown.

5.4 Essential Composition

5.4.1 Chamazulene

Nitrogen fertilization increased chamazulene percentage in the essential of chamomile flowers. Increasing application of nitrogen from 0 to 50 kg N/ha increased chamazulene in essential oil from 6.89 to 8.60%, thereafter a decrease was observed. Similar results have been reported by Agena (1974), Meawad et al. (1984), Franz (1981, 1983b). Agena (1974) and Meawad et al. (1984) reported that nitrogen application to chamomile plants increased chamazulene in the essential oil of chamomile flowers. Franz (1981, 1983b) reported that chamomile plants rich in nitrogen, amino acid metabolism leads to the biosynthesis of chamazulene and bisabolol. The increasing chamazulene by nitrogen fertilization could firstly be because nitrogen increased the development of new cells that synthesize chamazulene, resulting in their growth and enlargement (Agena, 1974; and Meawad et

al., 1984). Secondly, may be nitrogen increased the metabolism of basic hydrocarbons (farnesene) to oxygenated compounds (Franz et al., 1978b). Thirdly, may be nitrogen increased amino acid metabolism, therefore leading to the biosynthesis of chamazulene (Franz, 1981, 1983b).

Phosphorus fertilization increased Chamazulene in essential oil of chamomile flowers. Phosphorus probably either increased the metabolism of basic hydrocarbons to oxygenenated compounds (Franz et al., 1978b) or phosphorus increased the synthesis of matricine which is a precursor in the synthesis of chamazulene (Isaac, 1974).

Chamazulene decreased with advancing plant age. Chamazulene decreased from 14.66% at 106 days after transplanting to 3.59% at 176 days after transplanting. The decrease of chamazulene with increasing plant age could be either due to the increase of bisabololoxides "A" and "B" in expense of chamazulene or the metabolism of basic hydrocarbons (matricine and farnesene) to bisabololoxides "A" and "B" (Franz et al., 1978b). However, the decrease of chamazulene, 153 days after transplanting could be accounted for by the onset of senescence.

5.4.2 Bisabololoxides "A" and "B" and bisabolol

Nitrogen significantly decreased bisabololoxides "A" and "B" contents in the essential oil of chamomile flowers. Bisabololoxides decreased significantly with increasing nitrogen application rates. Increasing application of nitrogen from 0 to 150 kg N/ha decreased bisabololoxides "A" and "B" in essential oil from 41.56 to 31.58%, and from 21.69 to 13.19%, respectively. However, top-dressing chamomile plants with more than 50 kg N/ha did not significantly decrease bisabololoxides. On the contrary, nitrogen significantly increased bisabolol content in essential oil, and yet bisabololoxides are derived from bisabolol (Isaac, 1974). However, application of more than 50 kg N/ha to chamomile plants did not significantly increase bisabolol content. Franz (1981, 1983b) reported that in nitrogen rich plants amino acid metabolism leads to the biosynthesis of bisabolol, matricine, and farnesene, whereas amino acid metabolism in nitrogen deficient plants leads to the biosynthesis of bisabololoxides "A" and "B". He also reported that nitrogen fertilization increased bisabolol and decreased bisabololoxides contents

in the essential oil of chamomile flowers. He further reported that edaphic factors (e.g. high nitrogen application) which led to a faster flower development, resulted in essential oil with high bisabololoxides contents. On the contrary, high nitrogen application kept the plants and flowers in a physiologically younger stage, the essential oil content per unit dry flower weight increased, and a higher amount of bisabolol was obtained. In that case, it shows that the influence of plant nutrition on secondary metabolism is not a direct but an indirect relationship. The modified nutrition led to a different ontogenesis.

Nitrogen significantly decreased bisabololoxides contents and on the contrary, increased bisabolol content in the essential oil. This could be due to increased amino acid metabolism and therefore, leading to the biosynthesis of more bisabolol at the expense of bisabololoxides (Franz, 1981, 1983b). Nitrogen could have either increased the concentration of the enzyme that converts bisabololoxides to bisabolol or nitrogen decreased the concentration of the enzyme that converts bisabolol to bisabololoxides, hence leading to more bisabolol. However, these enzymes have not been known at present (Bonner, 1965;

and Wagner and Wolff, 1977). From the above explanation and results of Franz (1981, 1983b) discussed elsewhere, it shows that the influence of plant nutrition on secondary metabolism is not a direct but an indirect one. The modified nutriton led to a different ontogenesis.

Bisabololoxides (A + B) were predominant in the essential oil of chamomile flowers because they constituted averagely 54.21% (bisabololoxide A, 37.21%, bisabololoxide B 17%). Franz (1982), Mrlianova and Felklova (1983), Schilcher (1973, 1977), Gasic et al. (1983) and Franz et al. (1978a) reported similar results. These workers reported that the concentration of bisabololoxides (A + B) was over 50% in the essential oil of chamomile flowers. Bisabololoxides (A + B) were predominant in the essential oil because of the fact that the biosynthesis of bisabololoxides "A" and "B", and bisabolol are controlled by dominant and recessive genes, respectively (Franz, 1982; Mrlianova and Felklova, 1983; and Gasic et al., 1983).

Bisabololoxides (A + B) concentration in the essential oil increased with advancing plant age. In fact bisabololoxide A content shot up to 65% at

176 days after transplanting (8th flower harvest). On the contrary, bisabolol content decreased with increasing plant age (decreased from 6.08 to 5.04%. Similar results have been reported by Franz et al. (1978b) and Franz (1980). The researchers found that bisabololoxides and bisabolol contents in the essential oil of chamomile flower increased and decreased, respectively, with advancing plant age. The increase in bisabololoxides and the decrease of bisabolol contents with increasing plant age could either be due to the decrease of available nitrogen for plant metabolism, hence amino acid metabolism being directed to the synthesis of bisabololoxides as discussed elsewhere, or the decrease/increase of bisabolol/bisabololoxides, or the decrease of en-in-dicycloethers (cis- and trans-spiroether) in essential oil (Franz et al., 1978b)

5.4.3 Farnesene

Nitrogen fertilization increased farnesene content in the essential oil of chamomile flowers. However, top-dressing chamomile plants with more than 50 kg N/ha did not significantly increase the farnesene content. No work has been reported on the

effect of nitrogen on farnesene content in the essential oil. However, nitrogen could have increased farnesene either by increasing amino acid metabolism and therefore, leading to the biosynthesis of more farnesene (Franz, 1981, 1983b), or it reduced the metabolism of basic hydrocarbons to oxygenated compounds, or it increased the biosynthesis of farnesyl pyrophosphate which is a precursor in the synthesis of farnesene (Bonner, 1965; Goodwin, 1971; Harbone, 1973; Martin, 1972; Thomas et al., 1973; Leon, 1975; Tyler et al., 1976; Wagner and Wolff, 1977; and Sticher, 1977). Farnesene is directly formed from farnesyl pyrophosphate via an intermediate farnesol alcohol (Martin, 1972; Robinson, 1975; and Sticher, 1977).

5.4.4 Cis-spiroether

Nitrogen fertilization did not significantly increase cis-spiroether content in the essential oil, except in the last two flower harvests. In these two flower harvests (148 and 176 days after transplanting) application of more than 50 kg N/ha to chamomile plants did not significantly increase cis-spiroether concentration.

There is no work reporting the effect of nitrogen on the concentration of cis-spiroether. The

increase of cis-spiroether concentration from nitrogen application could be due to increased amino acid metabolism and therefore, leading to the biosynthesis of cis-spiroether, at the expense of bisabololoxides. However, initially there was no significant effect of nitrogen on cis-spiroether concentration. This may be because of the fact that there was less amount of receptacle in relation to the whole flower head harvested. This is because the amount of cis-spiroether in the essential oil of chamomile flower directly depends on the relative amount of receptacle in relation to whole flower head that is harvested (Franz et al., 1978b., and Franz, 1980).

Cis-spiroether concentration decreased with increasing plant age. This was also reported by Franz et al. (1978b) and Franz (1980). This could be due to the increase of bisabololoxides with increasing plant age at the expense of cis-spiroether (Franz, 1980).

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Under Kenyan conditions, application of 17.47 kg P/ha (40 kg P_2O_5/ha) during transplanting of chamomile seedlings and two weeks later top-dressing them with 50 kg N/ha would ensure their good growth and development with high flower and essential oil yields. The essential oil will also be of high quality.

The flower and essential oil yields, and essential oil composition were greatly influenced by nitrogen fertilization. The study showed that nitrogen played a vital role in growth and development of chamomile plants and in the biosythesis of essential oil and its composition. However, phosphorus fertilization did not significantly influence the growth and development of chamomile plants.

The flower yield increased with plant age up to 134 days after transplanting, thereafter a decrease occurred. The essential oil yield per unit dry flower weight decreased with increasing plant age. However, the composition of essential oil also varied with plant

age. The highest essential oil yield per unit dry flower weight and per plant occurred 78 and 134 days after transplanting, respectively. Therefore, this implies that the farmer should continue harvesting chamomile flowers up to 176 days after transplanting because essential oil per unit dry flower weight under Kenyan conditions at this particular time is above 0.5% which is acceptable in the world market. However, if the market demands flowers with essential oil content of above 0.8% per unit dry flower weight, with high chamazulene and bisabolol, and low bisabololoxide content, then farmers should stop flower harvesting 134 days after transplanting.

The amount of en-in-dicycloethers (cis-spiroether and trans-spiroether) in essential oil depends on the proportion of receptale harvested in relation to whole flower head.

The study also showed that chamomile can grow in Kenya and at an altitude of 1800 M. above sea level.

6.2 Recommendations

(1) It is recommended that further research should be done in the field of plant nutrition (including trace elements) as it affects secondary metabolism, since

there is scanty/very little literature dealing with plant nutrition of chamomile.

(2) It is recommended that research should be done on the effect of time of harvest of chamomile flowers in different ecological zones of Kenya on the essential oil yield and its composition.

(3) It is recommended that research should be done to find varieties which produce high essential oil yield per unit dry flower weight and per plant and essential oil with high quality through both varietal evaluation and plant breeding.

(4) Economic analysis of growing the crop (chamomile) should also be done to find the cost: benefit ratio.

(5) Biochemical studies should be done to see how to increase quality and quantity of the essential oil.

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APPENDIX 1: MEAN CLIMATIC DATA FOR KABETE FOR THE LAST 5 YEARS (1982 - 1986)

MONTH	RADIATION MJM^{-2}	SUNSHINE HOURS/DAY	MAXIMUM TEMPERATURE ^{o}C	MAXIMUM TEMPERATURE ^{o}C	TOTAL RAINFALL (MM)
JANUARY	25.06	9.74	24.76	12.62	2.80
FEBRUARY	26.38	9.36	26.08	13.02	55.92
MARCH	24.93	9.00	25.78	13.74	71.06
APRIL	19.52	6.80	23.84	14.48	193.38
MAY	18.11	5.82	22.08	13.62	138.70
JUNE	19.69	5.26	21.78	11.78	23.84
JULY	16.03	4.18	20.92	10.64	21.42
AUGUST	15.91	4.64	21.60	10.62	15.64
SEPTEMBER	20.79	6.08	23.66	11.30	23.66
OCTOBER	21.78	6.68	23.58	12.88	82.62
NOVEMBER	19.95	6.42	22.56	13.52	147.96
DECEMBER	22.92	7.68	22.56	12.96	132.46

APPENDIX 2: RADIATION, SUNSHINE HOURS PER DAY, MAXIMUM AND MINIMUM TEMPERATURES, AND TOTAL RAINFALL DURING THE EXPERIMENTAL PERIOD.

MONTH	RADIATION MJM^{-2}	SUNSHINE HOURS/DAY	MAXIMUM TEMPERATURE ^{o}C	MAXIMUM TEMPERATURE ^{o}C	TOTAL RAINFALL (MM)
1986 SEPTEMBER	20.02	5.60	23.20	11.10	4.30
OCTOBER	21.99	7.70	25.10	13.10	40.40
NOVEMBER	18.62	5.00	21.80	13.50	202.00
DECEMBER	30.34	7.50	22.80	12.50	91.50
JANUARY 1987	24.39	8.90	23.80	12.70	79.50
FEBRUARY	25.22	8.20	25.40	12.50	95.50

APPENDIX 3: SOIL ANALYSIS PRIOR TRANSPLANTING (ONE WEEK BEFORE TRANSPLANTING).

SAMPLE	KG OF AVAILABLE N/HA	AVAILABLE KG P_2O_5/HA	pH WATER	^{p}H POTASSIUM CHLORIDE
1	49.73	39.85	6.00	5.45
2	43.74	40.08	6.00	5.60
3	21.57	50.38	6.40	5.60
4	48.54	57.25	6.00	5.60
5	4.97	41.22	6.00	5.60
MEAN ($\bar{X}$)	33.71	45.75	6.08	5.57

APPENDIX 4: SOIL ANALYSIS AT THE END OF THE GROWING SEASON.

SAMPLE	AVAILABLE N KG N/HA.	AVAILABLE P KG P_2O_5/HA.	P^H WATER	P^H POTASSIUM CHLORIDE
NITROGEN CONTROL PLOTS	27.41	-	6.40	5.60
PLOTS SUPPLIED WITH 50 KG N/HA	50.03	-	5.90	5.60
PLOTS TREATED 100 KG N/HA	61.87	-	6.10	5.90
PLOTS TREATED 150 KG N/HA	64.86	-	6.50	5.80
PHOSPHORUS CONTROL PLOTS	-	41.73	6.00	5.45
PLOTS TREATED 40 KG P_2O_5/HA	-	50.44	6.50	6.00
PLOTS TREATED 80 KG P_2O_5/HA	-	73.38	6.10	5.90
PLOTS TREATED 120 KG P_2O_5/HA	-	118.24	6.10	5.80

APPENDIX 5: SUMMARIZED ANOVA TABLE FOR PLANT HEIGHT.

SOURCE OF VARIATION	df	DAYS AFTER TRANSPLANTING WHEN SAMPLING WAS DONE				
		38	45	59	80	112
		MEAN SUM OF SQUARES				
SUB PLOTS	47					
MAIN PLOTS	11					
BLOCKS	2	NS 5.1420185	NS 12.45929	NS 8.291505	NS 15.9388	NS 27.2768
PHOSPHORUS	3	NS 0.8523276	NS 6.2122433	NS 4.1091567	NS 48.344433	NS 29.2464
MAIN PLOT ERROR	6	1.3552717	4.7627317	3.569065	145.87773	65.153483
NITROGEN	3	** 10.407917	** 30.529877	** 185.41559	** 1390.043	** 3052.5267
NITROGEN AND PHOSPHORUS INTERACTION	9	NS 1.0350744	NS 2.6390578	NS 15.7729	NS 15.7729	NS 42.266011
SUB PLOT ERROR	24	0.8611388	1.2737792	31.052304	19.586504	34.650754

NOTE: df - degrees of freedom
NS - Not significant
* - Significant at P = 0.05
** - Significant at P = 0.01.

APPENDIX 6: SUMMARIZED ANOVA TABLE FOR LEAF AREA.

SOURCE OF VARIATION	df	DAYS AFTER TRANSPLANTING WHEN SAMPLING WAS DONE		
		38	45	59
		MEAN SUM OF SQUARES		
Sub plots	47			
Main plots	11			
Blocks	2	NS 2663.321	NS 4272.0915	NS 5981.119
Phosphorus	3	NS 2534.222	NS 10496.885	NS 10252.489
Main plot error	6	2407.3802	21910.622	21301.498
Nitrogen	3	** 13040.527	** 40707.813	** 31733.490
Nitrogen x Phosphorus (interaction)	9	NS 1052.8781	NS 9035.891	NS 10955.426
Sub plot error	24	1196.099	6315.3304	6418.7092

NOTE: df - degrees of freedom
NS - Not significant
* - Significant at P = 0.05
** - Significant at P = 0.01

APPENDIX 7: SUMMARIZED ANOVA TABLE FOR PLANT SPREAD.

		DAYS AFTER TRANSPLANTING WHEN SAMPLING WAS DONE			
		36	45	59	84
SOURCE OF VARIATION	df	MEAN SUM OF SQUARES			
Sub plots	47				
Main plots	11				
Blocks	2	NS 23.90909065	NS 2.63005	NS 0.03299	NS 0.009435
Phosphorus	2	NS 37.4133271	NS 8.43997	NS 3.20749	NS 3.3396233
Main plot error	6	6.02454	9.1856883	12.658845	7.7267
Nitrogen	3	** 93.51326	** 146.29315	** 153.62872	** 151.13542
Nitrogen and phosphorus interaction	9	NS 4.3675889	NS 6.1764911	NS 5.9328756	NS 5.8028467
Sub plot error	24	1.501475	6.1076583	3.2065146	4.4569767

NOTE: NS - Not significant
* - Significant at P = 0.05
** - Significant at P = 0.01
df - Degrees of freedom.

APPENDIX 8: SUMMARIZED ANOVA FOR NO. OF TILLERS/PLANT.

		DAYS AFTER TRANSPLANTING WHEN SAMPLING WAS DONE				
		45	59	74	93	107
SOURCE OF VARIATION	df	MEAN OF SQUARES				
Sub plots						
Main plots						
Blocks		NS 0.3000287	NS 0.5238537	NS 1.126178	NS 5.6650405	NS 5.677734
Phosphorus		NS 0.1746844	NS 0.3596677	NS 3.5284027	NS 2.5634303	NS 2.6243917
Main plot error		0.6956515	1.0096765	3.6039768	11.012388	10.936272
Nitrogen		** 6.1206733	** 8.4252067	** 34.842893	** 141.57081	** 140.4985
Nitrogen and phosphorus interaction		NS 0.22107	NS 0.2677292	NS 1.5923309	NS 2.2949594	NS 2.2016706
Sub plot error		0.1879708	0.1904569	1.2403622	2.2302308	2.2341373

NOTE: df - degrees of freedom
NS - Not significant
* - Significant at P = 0.05
** - Significant at P = 0.01

APPENDIX 9: SUMMARISED ANOVA TABLE FOR DRY MATTER PER 3 PLANTS.

SOURCE OF VARIATION	df	DAYS AFTER TRANSPLANTING WHEN SAMPLING WAS DONE						
		38	45	92	106	123	153	175
		MEAN SUM OF SQUARES						
Sub plots	47							
Main plots	11							
Blocks	2	NS 21.4656	NS 65.59938	NS 2498.262	** 144779.37	* 84496.42	* 112256.06	* 64104.165
Phosphorus	3	NS 3.1145361	NS 4.0346277	NS 2315.6817	NS 13746.351	NS 11032.692	NS 11518.08	NS 10333.864
Main plot error	6	4.3819413	13.725293	1155.4077	4042.1328	10289.651	14634.537	11890.343
Nitrogen	3	** 40.05441	** 147.95379	** 3484.996	** 151362.05	** 167922.93	** 264685.66	** 158938.65
Nitrogen and phosphorus interaction	9	NS 2.4322222	NS 6.8050078	NS 26.294922	NS 10132.534	NS 11699.29	NS 15371.298	NS 11914.663
Sub plot error	24	4.2665771	15.908598	143.82885	10169.777	13050.652	18827.131	13126.833

NOTE: df - degrees of freedom
NS - Not significant
* - Significant at P = 0.05
** - Significant at P = 0.01

APPENDIX 10: EFFECT OF NITROGEN AND PHOSPHORUS ON THE DRY FLOWER YIELD OF CHAMOMILE - SUMMARY OF ANOVA TABLE.

		DAYS AFTER TRANSPLANTING WHEN FLOWER HARVEST WAS DONE							
		78	92	106	120	134	148	162	176
SOURCE OF VARIATION	df	MEAN SUM OF SQUARES							
Sub plots	47								
Main plots	11								
Blocks	2	NS 4.1865156	NS 412.61502	NS 422.78833	NS 1320.2673	NS 24241.993	NS 3011.0772	NS 18776.416	NS 1169.8301
Phosphorus	3	NS 5.7404917	NS 433.71113	NS 699.94817	NS 1743.6588	NS 864.625	NS 1932.8353	NS 1762.1827	NS 120.50119
Main plot error	6	8.0241532	284.43503	752.7159	2484.1358	18542.568	3378.4378	10486.257	1395.7808
Nitrogen	3	** 28.984558	** 532.33317	** 964.41983	** 3254.8555	** 22733.727	** 8333.958	** 5563.00	** 841.16993
Nitrogen and phosphorus interaction		NS 4.4357814	NS 101.91557	NS 82.64817	NS 331.449	NS 861.32956	NS 500.24768	NS 652.08214	NS 84.500609
Sub plot	24	4.131655	66.807708	90.050317	550.50704	1658.5218	428.19013	612.74925	146.13999

NOTE: df - degrees of freedom
NS - Not significant
* - Significant at P = 0.05
** - Significant at P = 0.01

APPENDIX 11: EFFECT OF NITROGEN AND PHOSPHORUS ON THE TOTAL FLOWER YIELD - SUMMARY ANOVA TABLE.

SOURCE OF VARIATION	df	SUM OF SQUARES	MEAN SUM OF SQUARES	F OBSERVED	F EXPECTED	F EXPECTED 5%
Sub plots	47	2125817.20				
Main plots	11	1298616.70				
Blocks	2	347619.84	173809.92	NS 1.196		5.14
Phosphorus	3	79134.05	26378.017	NS 0.182		4.76
Main plot error	6	871862.81	145310.47			
Nitrogen	3	616224.44	205408.15	** 30.670	4.72	3.01
Phosphorus x Nitrogen	9	50236.91	5581.8789	NS 0.833		2.30
Sub plot error	24	160739.15	6697.4646			

NOTE: NS - Not significant
* - Significant at P = 0.05
** - Significant at P = 0.01
df - degrees of freedom.

APPENDIX 12: SUMMARIZED ANOVA FOR ESSENTIAL OIL YIELD PER UNIT WEIGHT OF EXTRACTED DRY FLOWERS.

		DAYS AFTER TRANSPLANTING WHEN FLOWER HARVESTING WAS DONE				
		106	120	134	148	176
SOURCE OF VARIATION	df	MEAN SUM OF SQUARES				
Sub plots	47					
Main plots	11					
Blocks	2	NS 0.00105415	NS 1.2725×10^{-4}	NS 2.7221×10^{-4}	NS 5.27655×10^{-5}	NS 3.29378×10^{-5}
Phosphorus	3	NS 0.0085246	NS 2.65476×10^{-3}	NS 1.70869×10^{-3}	** 1.42822×10^{-4}	* 8.029×10^{-5}
Main plot error	6	0.0161546	7.9258×10^{-4}	1.03893×10^{-3}	1.36151×10^{-5}	1.51169×10^{-5}
Nitrogen	3	** 0.0199245	** 5.7067×10^{-3}	** 3.62816×10^{-3}	** 4.08316×10^{-4}	** 2.59024×10^{-4}
Nitrogen and Phosphorus interaction	9	* 0.00857184	* 8.3551×10^{-4}	NS 1.82384×10^{-4}	NS 4.76075×10^{-5}	NS 2.75253×10^{-5}
Sub plot error	24	0.00366976	3.32179×10^{-4}	1.98636×10^{-4}	3.55082×10^{-5}	1.40659×10^{-5}

NOTE: df - degrees of freedom
NS - Not significant
* - Significant at P = 0.05
** - Significant at P = 0.01

APPENDIX 13: SUMMARIZED ANOVA FOR PERCENTAGE CHAMAZULENE IN THE ESSENTIAL OIL

		DAYS AFTER TRANSPLANTING WHEN FLOWER HARVESTING WAS DONE				
		106	120	134	148	176
SOURCE OF VARIATION	df	MEAN OF SQUARES				
Sub plots	47					
Main plots	11					
Blocks	2	NS 3.188556	NS 5.8217521	* 27.357256	NS 13.765228	NS 6.345029
Phosphorus	3	NS 21.393283	** 12.94341877	* 16.194105	NS 2.0554917	NS 2.1235622
Main plot error	6	22.20949	1.08772708	2.763337	3.1372907	1.4326042
Nitrogen	3	NS 12.523233	* 18.00312433	NS 3.5237553	** 14.953236	* 3.6961623
Nitrogen and Phosphorus	9	NS 19.785228	NS 3.210348367	NS 1.588723	NS 1.8580352	NS 1.16577
Sub plot error	24	16.315065	4.7722608	1.7969222	1.9625667	0.7175826

NOTE - df - degrees of freedom
NS - Not significant
* - Significant at P = 0.05
** - Significant at P = 0.01

APPENDIX 14: SUMMARIZED ANOVA FOR PERCENTAGE BISABOLOL.

		DAYS AFTER TRANSPLANTING WHEN FLOWER HARVESTING WAS DONE				
		106	120	134	148	176
SOURCE OF VARIATION	df					
Sub plots	47					
Main plots	11					
Blocks	2	NS 3.0406405	* 70.03308	NS 21.382981	* 7.9914435	NS 29.492569
Phosphorus	3	NS 3.1001137	NS 0.472975	NS 0.639711	NS 4.337461	NS 6.6454667
Main plot error	6	9.4065172	9.7635117	7.6946508	1.489305	11.573877
Nitrogen	3	NS 5.9455125	NS 1.9750527	** 14.847661	* 3.7935443	NS 4.2995053
Nitrogen and Phosphorus interaction	9	NS 10.07912	NS 0.942287	NS 0.9056871	NS 0.5163315	NS 1.5781649
Sub plot error	24	2.9092923	1.1692491	1.7945222	1.2378785	2.399125

NOTE: df - degrees of freedom
NS - Not significant
* - Significant at P = 0.05
** - Significant at P = 0.01

APPENDIX 15: SUMMARIZED ANOVA FOR PERCENTAGE BISABOLOLOXIDE B

		DAYS AFTER TRANSPLANTING WHEN FLOWER HARVESTING WAS DONE				
		106	120	134	148	176
SOURCE OF VARIATION	df	MEAN SUM OF SQUARES				
Sub plots	47					
Main plots	11					
Blocks	2	NS 36.286928	NS 96.0734	NS 11.473775	NS 20.56328	** 220.92909
Phosphorus	3	NS 208.51433	NS 67.455533	NS 41.944383	NS 27.822117	** 22.848916
Main plot error	6	46.09487	108.72267	67.908187	56.429432	15.910098
Nitrogen	3	** 195.58957	** 181.1039	** 74.832897	** 162.85638	** 269.73184
Nitrogen and phosphorus interaction		NS 45.227073	NS 15.215126	NS 8.0191911	NS 4.7509278	NS 6.8475344
Sub plot error	24	26.579094	9.3796779	2.2002946	7.0858508	11.084593

NOTE: df - degrees of freedom
NS - Not significant
* - Significant at P = 0.05
** - Significant at P = 0.01

APPENDIX 16: SUMMARIZED ANOVA FOR PERCENTAGE BISABOLOLOXIDE A IN THE ESSENTIAL OIL

		DAYS AFTER TRANSPLANTING WHEN FLOWER HARVESTING WAS DONE				
		106	120	134	148	176
SOURCE OF VARIATION	df	MEAN SUM OF SQUARES				
Sub plots	47					
Main plots	11					
Blocks	2	NS 48.345155	NS 17.131318	NS 95.62845	NS 105.13489	NS 49.54325
Phosphorus	3	NS 59.467137	* 163.85421	NS 79.94623	NS 46.92386	NS 31.575133
Main plot error	6	83.685488	25.044193	77.877347	46.53329	34.005948
Nitrogen	3	** 527.42553	** 292.9113	** 201.21625	** 266.2271	** 410.08392
Nitrogen and phosphorus interaction	9	NS 25.474491	NS 64.337693	NS 4.2805911	NS 12.663161	NS 17.836583
Sub plot error	24	30.174305	32.815861	4.6415233	17.274181	13.812981

NOTE: df - Degrees of freedom
NS - Not significant
* - Significant at P = 0.05
** - Significant at P = 0.01

APPENDIX 17: SUMMARIZED ANOVA FOR PERCENTAGE BISABOLOLOXIDES (A + B) IN THE ESSENTIAL OIL

		DAYS AFTER TRANSPLANTING WHEN FLOWER HARVEST WAS DONE				
		106	120	134	148	176
SOURCE OF VARIATION	df	MEAN SUM OF SQUARES				
Sub plots	47					
Main plots	11					
Blocks	2	NS 40.96298	NS 35.34765	NS 46.3669	NS 108.75586	* 407.1327
Phosphorus	3	NS 470.91927	NS 118.33345	NS 102.03213	NS 97.367027	NS 98.004667
Main plot error	6	142.68113	110.23228	197.85767	85.100718	76.71475
Nitrogen	3	** 1439.7494	** 903.22297	** 505.82363	** 844.0068	** 1344.9599
Nitrogen and phosphorus interaction	9	NS 72.857014	NS 93.045909	NS 5.5903778	NS 11.64702	NS 21.7017
Sub plot error	24	78.332558	34.597738	6.532975	21.405688	31.441483

NOTE: df - Degrees of freedom
NS - Not significant
* - Significant at P = 0.05
** - Significant at P = 0.01

APPENDIX 18: SUMMARIZED ANOVA FOR PERCENTAGE FARNESENE IN THE ESSENTIAL OIL

		DAYS AFTER TRANSPLANTING WHEN FLOWER HARVESTING WAS DONE				
		106	120	134	148	176
SOURCE OF VARIATION	df	MEAN SUM OF SQUARES				
Sub plots	47					
Main plots	11					
Blocks	2	NS 47.951944	NS 9.865815	NS 5.52768	NS 15.786894	* 84.825705
Phosphorus	3	NS 58.53045	NS 129.71549	NS 39.850183	NS 42.686077	NS 4.871386
Main plot error	6	21.617543	37.089465	22.83674	18.901005	15.474468
Nitrogen	3	* 82.321517	NS 10.852853	NS 2.1391933	NS 29.916028	** 13.155958
Nitrogen and phosphorus interaction	9	NS 27.212456	NS 22.828343	NS 3.9056144	NS 6.7484986	NS 3.2096037
Sub plot error	24	22.637494	21.1928180	10.566969	10.716316	2.061613

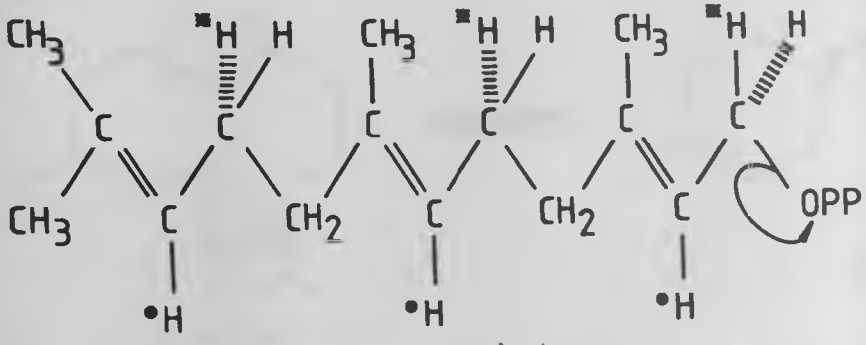
NOTE: df - Degrees of freedom
NS - Not significant
* - Significant at P = 0.05
** - Significant at P = 0.01

APPENDIX 19: SUMMARIZED ANOVA FOR PERCENTAGE CIS-SPIROETHER IN THE ESSENTIAL OIL

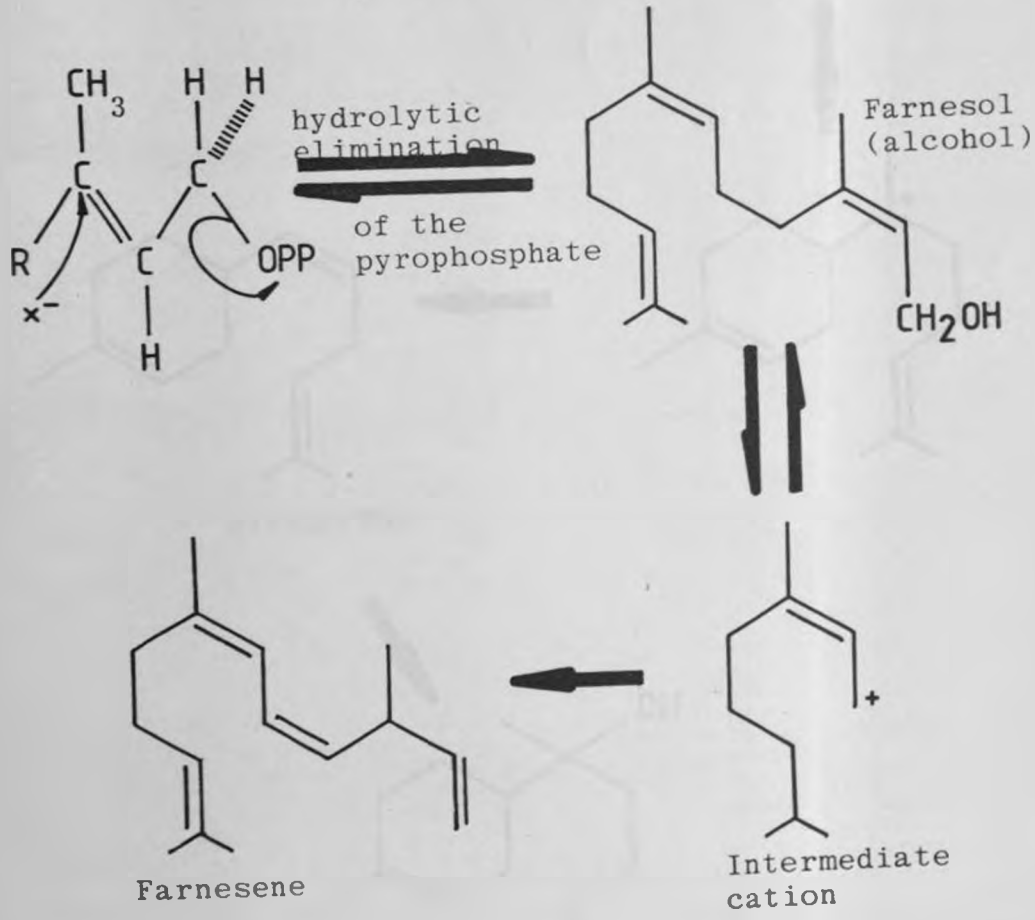
		DAYS AFTER TRANSPLANTING WHEN FLOWER HARVESTING WAS DONE				
		106	120	134	148	176
SOURCE OF VARIATION	df	MEAN SUM OF SQUARES				
Sub plots	47					
Main plots	11					
Blocks	2	NS 13.571653	NS 32.114728	NS 5.53823	NS 25.517006	NS 18.341331
Phosphorus	3	NS 32.801614	NS 14.927158	NS 39.876667	NS 4.2120053	NS 11.334261
Main plot error	6	20.897722	6.2373823	22.842948	5.4340537	6.3407008
Nitrogen	3	NS 8.872775	NS 1.426175	NS 4.0287367	* 41.229193	** 13.472261
Nitrogen and phosphorus interaction	9	NS 16.930463	NS 1.5179481	NS 4.3217833	NS 2.60645221	NS 1.4390222
Sub plot error	24	20.949476	1.3476515	10.96221	9.7439167	1.5848278

NOTE: df - Degrees of freedom
NS - Not significant
* - Significant at P = 0.05
** - Significant at P = 0.01

APPENDIX 20. FORMATION OF FARNESENE FROM FARNESYL PYROPHOSPHATE (Martin, 1972)



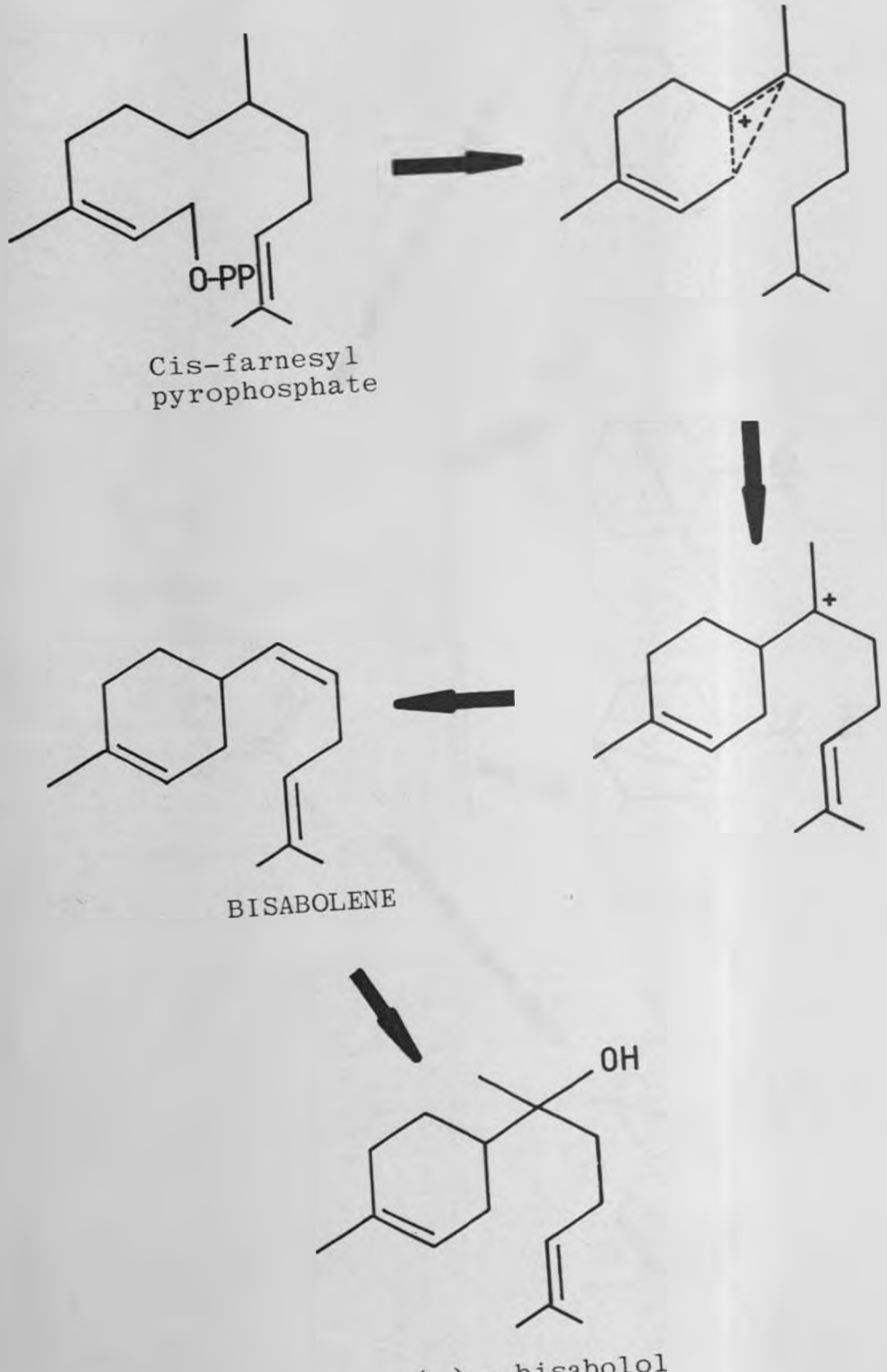
Farnesyl pyrophosphate



Farnesene

Intermediate cation

APPENDIX 21. BIOGENETIC FORMATION OF(-)-α-BISABOLOL (Martin, 1972)

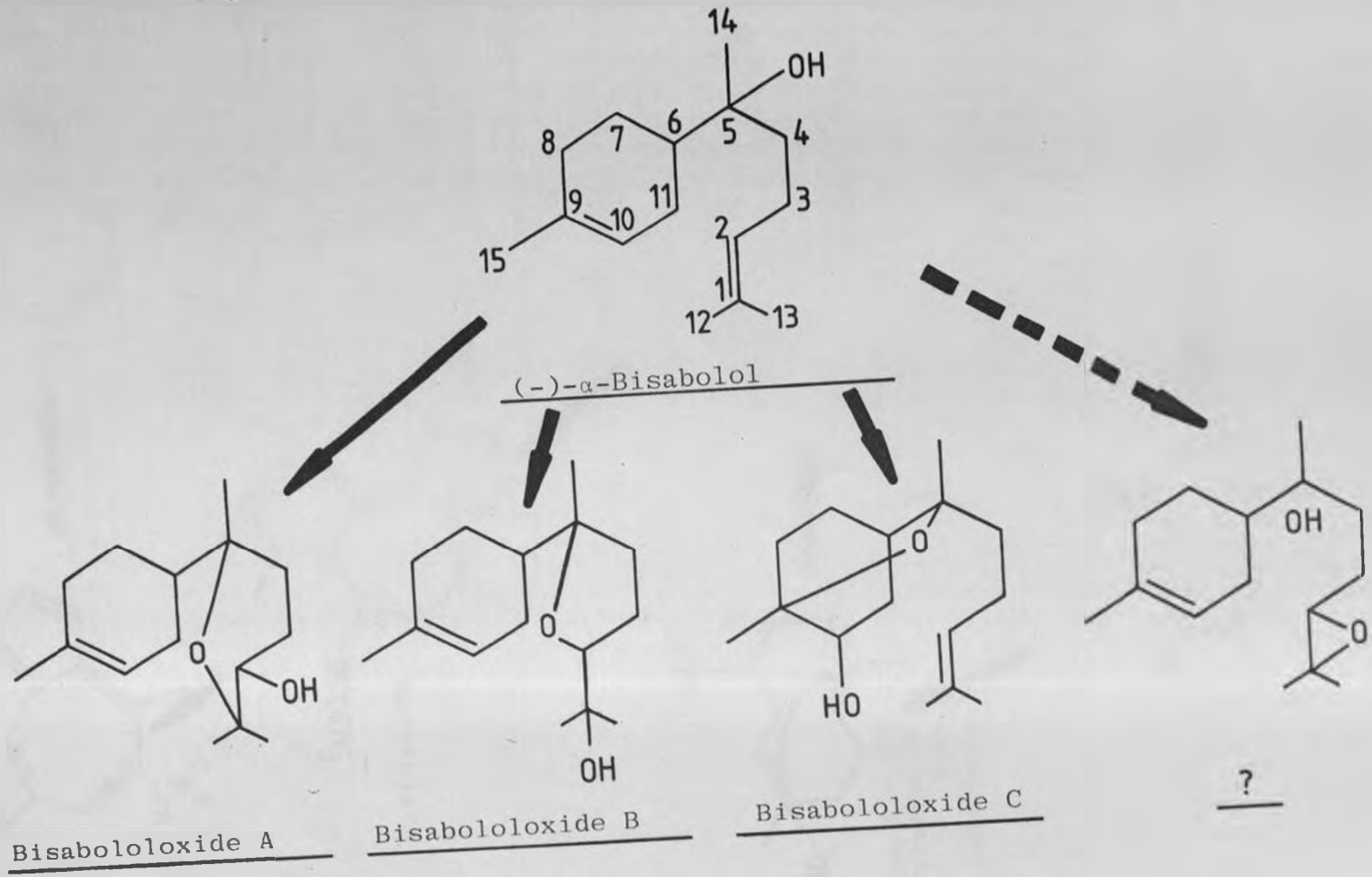


Cis-farnesyl pyrophosphate

BISABOLENE

(-)-α-bisabolol

APPENDIX 22. BIOGENETIC FORMATION OF BISABOLOLOXIDES FROM (-)-ALPHA-BISABOLOL (O. ISAAC, 1974).



APPENDIX 23. BIOGENETIC FORMATION OF MATRICINE (Martin, 1972).