MINERAL NUTRIENT STUDIES OF SOME ECONOMICALLY IMPORTANT PLANTS

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Chapter 1

INTRODUCTION
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INTRODUCTION

It is common knowledge that the source of direct energy on this globe is the sun. The secret of capturing the solar energy is known to the plants. The plants are, in fact, miniature factories of nature for the production of food materials, essential oil, gums and resins, alkaloids, steroidal substances, glycosides and other compounds of medicinal value, fibres, woods, and many other raw materials of daily use. Hunger and disease are the two extremely vital problems, which are connected with the survival of man. In addition to food bearing plants, medicinal and aromatic plants play an important role in curing of diseases.

The earliest mention of the use of medicinal plants in India is found in the Rigveda, having been written between 1500 and 1600 B.C. Sushruta and Charaka (c. 1000 B.C.) recorded the properties and uses of about 700 medicinal plants in a classified manner. Probably, the world's first symposium on medicinal plants was held in relation to diseases on the slopes of the Himalayas, presided by Bharadwaj Kishi, the father of Indian Medicine (Atal and Kapoor, 1977).

The use and cultivation of aromatic plants was also undertaken in India like the medicinal ones. Some plants like ocimum, marigold, jasmine and sweet smelling roses are aromatic. The
aromatic plants were attracted in the east due to the hot climate. The aroma of such plants has a soothing effect on the nerves.

It has been recorded that the process of distillation of essential oils, the chief content of aromatic plants, was also conceived in India, turpentine being the earliest distillate, and the essential oil producing centre of Kannauj is world famous (Atal and Kapoor, 1977).

During the past three decades an attempt has been made to lay more stress on the medicinal and aromatic plants, due to their obvious significance. The research and development units of these plants have been established in different parts of India for their particular importance. Some of these are well known, e.g., Richardson Hindustan (Mentha), CIPLA (Dioscorea), Glaxo (Solanum khasianum), SAC Laboratories (Ammi majus), Sandoz (Digitalis), CIBA-Geigy (Rauwolfia), and Meghalaya Phytochemicals (Cymbopogon) (Atal and Kapoor, 1977).

It was estimated in the First International Congress on Medicinal Plants Research held at the University of Munich, Germany that 60,000 plants species were existed on the earth. Among them, only five percent had been specifically investigated chemically and pharmacologically. This shows the scope of research work in the field of medicinal plants.
To strengthen national economy, it is necessary to make intensive research in identifying and characterizing new medicinal and aromatic plants. It seems that emphasis has been given on the chemical constituents and their economical uses of these plants. In order to obtain such economically important plants, one should be careful about ideal growth of these plants. Keeping this view in mind, agricultural requirements for these plants become one of the important aspects. Among these, nutritional studies are of paramount importance. There has not been much work done on the nutritional requirements of such plants.

Recent work at Aligarh (Afzal, 1976) has revealed that there exists considerable relationship in the basal uptake of the nitrogen and phosphorus requirements for Datura innoxia Mill. Wasiudin (1975) worked out the effect of nitrogen and phosphorus on the growth and yield characteristics of kasondi (Cassia occidentalis Linn.), Kasni (Cichorium intybus Linn.) and fennel (Roeniculum vulgare Mill.).

It is, therefore, proposed to study the nutritional requirements of three important species of Cymbopogon, namely, citronella grass (C. winterianus), palmarosa grass (C. martini) and lemongrass (C. flexuosa) under field conditions. Citronella oil is widely used as a raw material in perfumery, soaps, cosmetics and pharmaceutical industries. Oil of palmarosa and its separated
Fraction geraniol is widely used in perfumery. And lemongrass oil is used in flavours, cosmetics and perfumes. The oil has also been found important in other uses such as bactericidal, as insect repellent and in medicines. As much work has not been done anywhere, including India, on the nutritional physiology of *Cymbopogon*, it is proposed to apply the knowledge gained at Allisrh, and elsewhere, to this "essential oil bearing" grass. It is also proposed to assess the effectiveness of foliar fertilization with this important grass with an aim of fertilizer economy, high potential and better quality of essential oil. It is, therefore, planned to extend the work on the following aspects:

1. To establish the optimum basal dose of nitrogen for three species of *Cymbopogon*.
2. To establish the optimum basal dose of phosphorus for three species of *Cymbopogon*.
3. To establish the optimum basal dose of potassium for three species of *Cymbopogon*.
4. To investigate fertilizer economy with sub-optimal combined basal dose of nitrogen, phosphorus and potassium followed by a supplemental foliar spray of nitrogen and/or phosphorus to obtain ideal yield of herb and essential oil of selected best species of *Cymbopogon* from above trials.
Chapter 2

REVIEW OF LITERATURE
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Historical account:

There had been a substantial built up in the agricultural "know-how" since antiquity. The experimental plant sciences developed simultaneously with the advancement of civilization. It was once believed that the entire nutritional requirements of crop plants are provided by applying adequate quantities of organic fertilizers to the soil before sowing. Would (1903) credits Cato as one of the pioneer writers on the nutrition of plants.

Van Helmont conducted the first quantitative experiment in plant nutrition. In a famous experiment he investigated the source of the materials that plants are composed of. John Woodward recognized the importance of mineral matter for the growth of plants. He conducted that the plants grew better in water containing dissolved solids than in distilled water. For the first time de Saussure in 1741 chemically analysed soils and concluded that the composition of the plants varied with the soil, the plant part analysed and the age of the plant. Sprengel gave the importance of nutrient elements absorbed from the soil. Boussingault is credited for laying the foundation of the modern agricultural science. Liebig's main contribution to plant nutrition was with the humus theory according to which organic matter of the soil
is the source of the carbon that plants absorb. We considered that soil contributes soluble inorganic constituents. Jacxs in 1860 prepared solutions of salts supplying the major essential mineral nutrients and he grew plants to maturity in these nutrient solutions. Ever since, the growing of the plants in nutrient solutions has been a favorite research technique in plant nutrition.

2.2 The role of nitrogen, phosphorus, and potassium (NPK) in plant nutrition

The various essential elements perform many important functions in the plant: some have roles in osmotic phenomena, some are also fundamental constituents of metabolic products, and other may act catalytically in the regulation of certain chemical processes within the cell. Out of the list of macronutrients, intensive work has been done on nitrogen, phosphorus, and potassium. It is due to the reason that these macronutrients are required by plants in large quantities for their growth and development. Their roles in plants are summarized below:

2.2.1 Role of Nitrogen

Nitrogen constitutes about 80% by volume in the atmosphere. For higher plants, "Nitrogen cycle" provides a source of nitrogen. The nitrate or ammonium salts, formed in the soil by various agents, are absorbed readily by roots and converted
to organic forms of nitrogen (Webster, 1959). Nitrogen encourages the vegetative development of plants, imparting a healthy green colour to the leaves. Its deficiency results in various disorders, e.g., retardation of growth and root development, turns foliage yellowish or pale green, hastens maturity, causes shrivelling of grain and lowers crop yield. The elder leaves are affected first. An excess of nitrogen produces leathery (and sometimes crinkled), dark green leaves and succulent growth. It also delays maturation of plants; increases susceptibility to diseases and causes 'lodging' of cereal crops by inducing an undue lengthening of stem internodes.

Nitrogen occupies a distinctive place as its compounds include some of the most important plant constituents. The nitrogenous compounds found in plants include structural proteins, catalytic proteins i.e., enzymes, chlorophyll, nucleic acids, amino acids, peptides, purines, pyrimidines, nucleosides, nucleotides, alkaloids and vitamins.

2.2.2 Role of Phosphorus

Phosphorus is present in the soils in two forms, inorganic and organic. It is essential to supply fresh quantities of this nutrient to the soil every season for healthy growth of crop plants. Some of the important cell constituents that have phosphorus as their integral part include sugar esters and
their derivatives; phospholipids; nucleotides; nucleic acids; nucleoproteins; as well as co-enzymes including nicotinamide adenine dinucleotide, nicotinamide adenine dinucleotide phosphate, pyridoxal phosphate and thiamine pyrophosphate. Phosphorus plays a key role in the energy transfer in respiration, photosynthesis and other processes through the energy rich bonds of adenine triphosphate and similar compounds (Devlin, 1978).

Phosphorus influences the vigour of plants and improves quality in crops. It encourages formation of new cells, promotes root growth and hastens leaf development, emergence of ears, formation of seed and grain and maturation of crops. It also increases resistance to disease and strengthens stem of cereal plants. If phosphorus is deficient in the soil, plants fail to make quick start, do not develop a satisfactory root system, remain stunted in growth and sometimes develop a tendency to show a reddish or purplish discolouration of stem and foliage due to an abnormal increase in sugar content and formation of anthocyanin pigments.

It is obvious that phosphorus influences both the vegetative as well as the reproductive phase of plant life and "involved in practically every synthetic reaction of the cell" (newitt, 1967).

2.2.3 Role of Potassium

Potassium is present in the soil, in a non-exchangeable or
fixed form, and exchangeable form, and a soluble form.

Although there is a relatively high content of this element in the soil, most of it is non-exchangeable and therefore, unavailable to the plant. Unlike nitrogen and phosphorus, potassium does not form an integral part of any plant constituent. However, it is abundantly present in soluble form in the cytoplasm and in the vacuolar sap. Meristematic tissues and metabolically active regions like buds, young leaves, root tips, developing fruits, etc. accumulate high concentration of the element (Meyer and Anderson, 1952).

Potassium is the only monovalent cation essential for plants. Potassium enhances the plant's ability to resist disease, insect attack, cold and other adverse conditions. It is known to be a cofactor for a number of enzyme systems (Evans and Sorger, 1961). As such, its deficiency causes some disorders in most of the physiological processes, including photosynthesis, carbohydrate and nitrogen metabolism and respiration. It also influences the hydration of the protoplasm (Mason and Mc Elroy, 1963).

It plays an essential part in the formation of starch and the production and translocation of sugars, and is thus of special value to carbohydrate-rich crops like sugarcane, potato and sugar beet.
2.2. Effect of Nitrogen and Phosphorus alone and in combination on growth, yield and oil quality of medicinal and aromatic plants

Medicinal and aromatic plants show a direct response to nitrogen application as regards to their growth characteristics. Several investigators have studied the beneficial effects of nitrogen application in enhancing the yield characteristics and oil quality of these plants under different conditions. Phosphorus influences the vigour of the plants and improves quality in some medicinal and aromatic plants. However, it may be admitted that not much attention has been paid to these plants inspite of their recognised importance.

The combined effect of nitrogen and phosphorus on Cinchona ledgeriana has been reported by Loustalot and Winters (1918). They noted positive results by the application of nitrogen and phosphorus with regard to growth of this plant. Nitrogen was beneficial to increase the total alkaloid content but did not affect quinine percentage. Similarly phosphorus also increased the total alkaloid content but did not affect quinine percentage.

Greezlov (1952) studied the fertilizer requirements of Digitalis species recommended the dose of 30-40 kg. nitrogen and 8-10 kg.
superphosphate per hectare during spring season.

Putta and Singh (1961) studied the effect of nitrogen on the fresh flower and oil yield of *Matricaria chamomilla*. It was observed that 10 kg. nitrogen per hectare, as ammonium sulphate, proved optimum for fresh flower and oil yield, while the oil percentage was decreased significantly. N1 combination increased the oil percentage of the flower over nitrogen alone significantly, while FA and Na decreased oil content.

Sinha and Saxena (1965) studied the reproductive characters in linseed (*Linum usitatissimum* L.) as affected by different levels of nitrogen, and to a lesser extent phosphorus enhanced the flower buds formation. The uptake of nitrogen was related to the influence of pH. They also found that 33 ppm of nitrogen and 12.4 ppm of phosphorus was the optimum dose for best growth and yield of linseed in one year, and 99 ppm of nitrogen and 6.4 ppm of phosphorus during the following year.

Kinra et al. (1970) conducted an experiment to study the effect of various levels and time of nitrogen application on the yield of linseed (*Linum usitatissimum* L.) 'type 12b'. They observed that 0.75 kg. nitrogen per hectare gave higher yield than control. The
effect of different times of nitrogen application did not differ significantly. The treatment 67.2 kg. nitrogen per hectare also resulted in the highest return per unit of investment. The yield was increased by 13, 17 and 19% over the control when 33.6, 67.2 and 100.8 kg. nitrogen per hectare were applied respectively.

Atal and Bradu (1973) studied the effect of nitrogen on Jammu lemongrass (Cymbopogon pendulus Wats.). They noted that 325 kg. nitrogen per hectare gave a fresh herbage yield of 20.5 tonnes per hectare corresponding to 157.7 kg. per hectare of oil.

The application of different levels of nitrogen and phosphorus has been studied by Mahajan et al. (1973). They found that seed yield of celery (Apium graveolens L.) increased consistently with higher uptake of nitrogen. The increase was significant only at 100 kg. nitrogen per hectare and higher doses over the control. The yield was increased by 27.3, 32.4 and 36.2% when phosphorus was applied at the rate of 33.22 and 44 kg. per hectare respectively over the control.

Luhan et al. (1974) studied the effect of nitrogen on the seed yield and oil quality of Anethum graveolens. They suggested that
20 kg. nitrogen per hectare or at least 66 kg. nitrogen per hectare were favourable for better seed yield and oil quality. Afridi et al. (1977) studied the effect of different levels of nitrogen on the growth and alkaloid content of _Lutra innoxia_ L. at Aligarh. The length of plant, number of branches, fresh and dry weight, and alkaloid percentage of leaves was found to be significantly higher in the treated plants, over control. They observed that 6 kg. nitrogen per hectare, as urea, proved optimum for all the selected parameters under the local conditions.

Bains et al. (1977) studied the effect of four row spacing (30, 45, 60, and 75 cm.) and three levels of nitrogen (100, 120, and 150 kg. per hectare) on celery _Aegium graveolens_ L., on a well drained loam soil of Amritsar during 1969-70 and 1970-71. They found that the celery crop gave the highest yield at a row spacing of 30 cm., applied with 150 kg. nitrogen per hectare. In another experiment conducted on the response of celery to the application of five levels (0, 50, 100, 150 and 200 kg. per hectare) of nitrogen and four levels of phosphorus (0, 11, 22 and 33 kg. phosphorus per hectare), they observed that an increase in the level of nitrogen increased the yield consistently. 100 kg. per hectare and higher dose of nitrogen was significant over the control. However, phosphorus levels gave nonsignificant increase in yield. The combined dose of...
200 kg. and 33 kg. P₂O₅ per hectare was noted most remunerative. Bradu (1977) studied the fertilizer requirement of *Ammi majus* L. They reported that a dose of 25 kg. per hectare of superphosphate was applied in the furrows before sowing. The effect of phosphorus on *Ipecac* (*Cephalis ipecacuanha*) was studied by Chatterjee (1977). He reported that application of 100 g. of superphosphate per unit size (22.5 x 10 cm.) gave maximum yield of total alkaloid.

Ganguly and Thvgarjan (1977) studied the effect of nitrogen on the growth of *citronella* (*Cymborogon winterianus*). They observed that a dose of 20 kg. nitrogen per hectare proved to be optimum, depending upon the soil conditions. Phosphorus appeared to influence tillering but the effect of phosphorus and potassium was not appreciable on yield.

The fertilizer requirements of *Pyrethrum* (*Chrysanthemum cinerariaefolium*) was studied by Gulati *et al.* (1977). They found that 60 kg. N and 40 kg. P₂O₅ per hectare was optimum fertilizer requirements under normal soils for ideal growth and yield of *Pyrethrum*.

Haul and Choudhary (1977) studied the nutritional requirements of "Mysore hybrid" (*Syn. Eucalyptus tereticornis* and *E. francis*). They reported that application of nitrogen and phosphorus at the rate of 375 kg. and 250 kg. per hectare respectively was proved
to be optimum for good seedling growth. Addition of potassium did not give any significant result.

Raman et al. (1977) studied the fertilizer requirements of *Pyrethrum* (*Chrysanthemum cinerariaefolium*). They reported that phosphorus was necessary specially on the more leached and exhausted soil of the high altitude. The application of 12 kg. P₂O₅ per hectare gave higher flower yield, 9% by number and 11.1% by weight than those of 80 and 40 kg. P₂O₅ per hectare.

Singh et al. (1977) studied the effect of nitrogen on the fresh herb yield of Jammu lemongrass (*Cymbopogon pendulus* Hata.). Eight levels of nitrogen were compared in a randomised block design with five replications (N=0, 100, 200, 300, 400, 500, 600 and 700). It was observed that treatments of N=100 and N=400 kg. per hectare gave significant value than those for other treatments. However, 520 kg. nitrogen per hectare showed the maximum profit of Rs. 2864.00 per hectare but maximum return per rupee invested was under the 250 kg. nitrogen per hectare level to limit fertilizer application.

Husain (1977) studied the mineral nutrient requirements of belladonna (*Atropa belladonna L.*). It was revealed that 60 kg. per hectare phosphorus and 40 kg. per hectare of nitrogen should be supplied as a basal dose of nitrogen and phosphorus should be repeated every year in the beginning of spring season, followed by top dressing of nitrogen.
Afsar et al. (1978) studied the effect of different levels of phosphorus on the growth and alkaloid content of Lactuca innoxia Mill. They concluded that the length of the plants, number of branches, fresh weight and dry weight of shoot and alkaloid percentage of leaves were significantly increased as the result of all the applied doses (0, 30, 60 and 90 kg. P₂O₅ per hectare, as monocalcium superphosphate) in comparison with control. The application of 90 kg. P₂O₅ per hectare proved best for all the parameters to ensure continuous supply of large quantity of the alkaloid for the pharmaceutical industry.

Afridi et al. (1978) studied the effect of nitrogen and phosphorus on the fruit yield, percentage of oil and its carvone content of oil of dill (Anethum sowa L.) . They found that total fruit yield and percentage of oil and its carvone content were being 62.1, 98.9 and 3.7 % higher respectively than in the control, in the plants supplied with 60 kg N per hectare. A dose of 35 kg. P₂O₅ per hectare proved optimum for total fruit yield among phosphorus levels which was 8.5 % higher than the control. Regarding the interaction of nitrogen and phosphorus, the combination N₀P₀ proved best for both total yield and percentage of oil as well.

Wisra and Kapoor (1976) studied the nitrogen and phosphorus requirements of Matricaria chamomilla L. . They noted that crop responded well to fertilization and 50-60 kg. P₂O₅ per
hectare was the optimum dose for the yield of fresh flower. The effect of nitrogen and phosphorus on growth, development and diosgenin content in different species of Dioscorea was studied by Nandi and Chatterjee (1978). They found that the treatments $N_0P_{150}$ and $N_{150}P_{150}$ inhibited panicle formation, whereas $N_{150}P_{300}$ increased it in D. bulbifera var. pulchella. On the other hand D. rentaphylla and D. composita showed the opposite response when supplied with $N_{300}P_{150}$ and $N_{150}P_{300}$ and $N_0P_{150}, N_{150}P_{150}$ and $N_{150}P_{300}$ respectively. They also noted that yam yield was increased by $N_{300}P_{150}, N_0P_{150}, N_{050}P_0$ and $N_{150}P_{300}$. The diosgenin content increased in $N_0P_{150}$ and was least affected in $N_{300}P_{150}$.

The effect of sowing date and levels of nitrogen on the yield of fennel (Foeniculum vulgare) was studied by Randhawa et al. (1978, a). They concluded that the crop sown on 21 October gave the highest seed yield and this yield of seed increased with each successive increase in the level of nitrogen up to 75 kg. per hectare.

In another study, Randhawa et al. (1978, b) further observed the effect of sowing date, seed rate and nitrogen fertilizer on the growth and yield of isabgol (Plantago ovata). They noted that crop sown on 21 October, using 7.5 or 10 kg. seed per hectare receiving 20 or 40 kg. nitrogen per hectare gave better yield than later sowings.
Rao et al. (1976) studied the effect of graded levels of nitrogen on the tuber yield and diosgenin content of one year old crops of *Dioscorea floribunda*. They noted that the diosgenin content of the tuber was not influenced by varying nitrogen levels but 300 kg. nitrogen per hectare gave the maximum tuber yield.

Shelke and Morey (1976) observed the growth, yield and quality of Japanese mint (*Mentha arvensis*) as influenced by various levels of nitrogen and topping. They conducted a field experiment with four nitrogen levels (0, 40, 80 and 120 kg. per hectare) and three topping treatments (no topping, topping at 42 and 53 days from planting). They observed that 40 kg. nitrogen per hectare gave significant increase in the yield. The maximum percentage of oil (0.64%) and highest oil yield (19.2 kg. per hectare) were obtained when plants topped at 42 days after planting. The higher uptake of nitrogen resulted with increased levels of nitrogen.

Vijakumar and Shanmugavelu (1978) studied the effect of nitrogen and phosphorus on flowering and yield of *Chrysanthemum indicum* L. They observed that the yield of flowers was increased by the highest levels of nitrogen (100 kg. N per hectare). The highest levels of nitrogen and phosphorus gave the significant increase in the weight, the diameter of the flower and stalk length.
More recently, Bharadwaj et al. (1979) worked out the effect of different levels of nitrogen on herb yield and essential oil content in *Mentha* species. They found that 225 kg. nitrogen per hectare gave the highest herbage yield in *M. piperita* and *M. spicata* whereas *M. citrata* required 300 kg. of nitrogen per hectare for optimum yield. It was also observed that there was not any specific effect of different levels of nitrogen on essential oil content in these three species of *Mentha*.

Choudhary et al. (1979) conducted field experiment to study the effect of nitrogen on the growth and yield of *Solanum khasianum* Clarke, var. RRL-SL-6. They studied the response of new spineless strain of *S. khasianum* to different levels of nitrogen (40, 80 and 120 kg. per hectare) in the form of urea. They observed that application of 120 kg. nitrogen per hectare gave the highest yield of berries. Under heavy fertilization a marginal increase in the solasodine content was recorded.

Singh and Duhan (1979) noted the effect of six nitrogen levels (0, 40, 80, 120, 160 and 200 kg. per hectare) on plant height, dry matter accumulation, fresh herbs and oil content of spearmint (*Mentha spicata* L.) at Tarai region of Nainital. The plant height and plant dry matter increased with increased
rates of nitrogen up to 120 kg. per hectare. The fresh herb yield was increased significantly by each level of nitrogen except 40 kg. nitrogen per hectare over control. Application of 120 kg. nitrogen per hectare gave the maximum oil content (0.112%).

Singh and Singh (1979) studied the effect of varying levels of nitrogen on the growth and nitrogen metabolism of Japanese mint (Mentha arvensis L. var. piperascens Holmes). The plants were treated with different nitrogen fractions (0.0, 0.25, 0.50, 1.0, 2.0, 4.0, 8.0, 16.0, 32.0 and 64.0 me per l. of NO₃⁻). They observed that plants receiving 0.0 to 4.0 me per l. of NO₃⁻ showed reduced percentage of essential oil (2.4%) with mild to severe nitrogen deficiency symptoms. Normal growth of bushy appearance with the maximum essential oil content (2.8%), was recorded with nitrate levels of 8.0 and 16.0 me per l. In another study on the nitrogen metabolism they concluded that growth and essential oil content increased with increase in all the nitrogen fractions in the first phase of 0.0 to 4.0 me per l. of NO₃⁻ but little affected in the second phase included 8.0 and 16.0 me per l. and adversely affected by increase in all the nitrogen fractions except insoluble-N. In the third phase consisted of 32.0 and 64.0 me per l. of NO₃⁻.
Mitra et al. (1979) worked out the effect of nitrogen and phosphorus application and bulb size on growth and flowering of tuberose (*Polyanthes tuberosa*). They noted that 75 kg. nitrogen per hectare in combination with 10 kg. *H₂O₅* per hectare applied in two split doses increased vegetative growth, yield and quality of flowers under field conditions. Better plants with higher yield were produced by larger bulbs (2.5 x 3.4 cm.) as compared to smaller ones (1.5 x 2.4 cm.).

The response of *Jasminum grandiflorum* L. to nitrogen and phosphorus application was studied by Natrajan and Rao (1979). They applied four levels of nitrogen (0, 30, 60 and 120 g. per plant per year) and four levels of *H₂O₅* (0, 60, 120 and 240 g. per plant per year). It was observed that 60 g. *N* and 120 g. *P₂O₅* per plant per year gave better flower yield and essential oil production. The oil content was higher at 120 g. *N* with 240 g. *P₂O₅* per plant per year.

Wasiuddin (1979) studied the effect of four levels of basal nitrogen (0, 10, 20 and 30 kg. nitrogen per hectare) and four levels of basal phosphorus (0, 20, 30 and 40 kg. *H₂O₅* per hectare) on growth of root and shoot and yield characteristics of Kasondi (*Cassia occidentalis* L.). The experiments were based on simple randomized block design. It was observed that 30 kg. *N* per hectare and 30 kg. *P₂O₅* per hectare was optimum.
dose for various characteristics studied. In another experiment he studied the effect of five levels of nitrogen (0, 30, 60, 90, and 120 kg. N per hectare) on the growth of root and shoot of Kasni (Cichorium intybus L.) at vegetative and reproductive stages of growth. He found that 90 kg. N per hectare was optimum dose for all the characteristics. He also studied the effect of nitrogen and phosphorus individually and in combination on growth characteristics at two stages and yield of fennel (Foeniculum vulgare Mill.). He observed that 90 kg. N per hectare proved optimum for all the growth characters at both stages of growth and 60 kg. P2O5 per hectare gave the best results for growth characters at both stages of growth except dry matter and number of branches, which were maximum in the control.

2.2.5 Effect of Potassium on growth, yield and oil quality on medicinal and aromatic plants

It has been found that supra-optimal doses of potassium do not have much beneficial influence on oil-crops like other ones, and there is not much return from the economic point of view. Though it is essential for normal and healthy growth of the plant (Pould, 1963). The effect of potassium on growth and glycoside content in leaves of Digitalis lantana was studied by Gresslov (1962). He found that potassium chloride and potassium
sulphate had significant effect for proper growth and glycoside content in leaves of this plant. More recently, Wasiuddin (1975), while working on the fennel (*Foeniculum vulgare Mill.*) found that potassium significantly increased the length, number of branches, fresh weight and dry weight with 90 kg. K₂O per hectare as optimal dose. It seems that few studies have been done to observe the effect of the application of potassium alone.

2.2.6 Effect of combined dose of nitrogen, phosphorus and potassium (NPK)

Traditionally crops are grown with combined dose of nitrogen, phosphorus and potassium for ideal development. Judicious application of these nutrients has been studied on the number of crops. The effect of these fertilizers on medicinal and aromatic plants has not been studied in detail as compared to other plants. Some important work in relation to medicinal and aromatic plants has been reviewed below:

Salm (1970) studied the response of *Rauwolfia serpentina* to the application of nitrogen, phosphate and potash and it was found that the yield of root was 26.8, 12.1 and 7.1% higher respectively over the controls. A combination of 90 kg. N per hectare and 45 kg. per hectare each of P₂O₅ and K₂O was noted to be optimum for the crop.
Chandra (1973) observed the effect of nitrogen, phosphorus and potassium on herbage and oil yield of Cymbopogon winterianus at Lucknow. He found a combination of 200 kg. per hectare of nitrogen, 100 kg. per hectare of phosphorus and 50 kg. per hectare of potassium as significant dose for good herbage and oil yield under local conditions. It was observed that herb yield, giving by 160 kg. nitrogen per hectare was comparable with that of N$_{180}$ : P$_{60}$ : K$_{120}$. Thus nitrogen was the main requirement of the crop. Moreover, NPK mixture was essential in the interest of balanced nutrition for the crop.

The application of nitrogen, phosphorus and potassium on the growth and yield characteristics of Indian belladonna (Atropa acuminata) has been studied by Gulati et al. (1977). The plants were supplied with a basal dose of 25 kg. nitrogen, 60 kg. of phosphorus and 30 kg. of potash per hectare. The sources of nitrogen, phosphorus and potassium were urea, di ammonium phosphate and muriate of potash respectively. They suggested that soil should be mixed with the organic manure at the rate of 2.5 ton per hectare before planting. The top dressing of nitrogen at the rate of 30-45 kg. per hectare was also recommended.

Husain (1977) studied the fertilizer requirements of hyrothrum (Chrysanthemum cinerariaefolium). It was found that 40 kg. nitrogen, 50 kg. phosphorus and 50 kg. potash should be applied as a basal dose at the time of planting while 50% of nitrogen should be applied as basal dose and the rest in split doses.
Maravana et al. (1977) recommended the application of basal
dose of 20 kg. N, 30 kg. P₂O₅ and 30 kg. K₂O per hectare
for Catharanthus roseus G. Don. It was also suggested that
a top dressing with 20 kg. N per hectare should be given
in two equal split doses during the season. This inorganic
nutrients practice was recommended when organic manure was
not applied.

Singh (1977) studied the effect of nitrogen, phosphorus and
potassium on the oil yield of Mentha arvensis. He observed
that 40 kg. N per hectare, 60-70 kg. P₂O₅ per hectare and
20-25 kg. K₂O per hectare were optimum dose for the yield of
oil. He also suggested that fertilizer should be applied in
furrows.

Singh (1977) noted the fertilizer requirements of Eucalyptus
(Eucalyptus citriodora Hook). It was suggested that a
combination of 20 kg. nitrogen, 30 kg. phosphorus and 30 kg.
potassium per hectare should be applied as basal dose at the
time of ploughing in the irrigated areas. He also suggested
that three top dressings of nitrogen should be given at the
rate of 20 kg. per hectare in the form of urea or ammonium
sulphate preferably after every harvest.

Singh and Atal (1977) studied the fertilizer requirements of
Buckwheat (Fagopyrum) in the plains. They noted that application
of nitrogen, phosphorus and potassium were applied as a basal
dose while nitrogen in the split doses, 50% at the sowing time
and rest within a month.

Singh et al. (1977, a) studied the effect of nitrogen, phosphorus
and potash on the herb yield of *Mentha piperita*. Phosphorus and
potassium were applied as a basal dose and nitrogen in split
doses. They noted that 120 kg. nitrogen per hectare gave signi-
ficantly higher yield of herb over other treatments. Application
of 60 kg. each of *P*₂*O₅* and *K*₂*O* per hectare resulted in a
slight increase in yield over the 0 and 30 kg. per hectare level.
Urea, superphosphate and muriate of potash were the sources of
nitrogen, phosphorus and potassium respectively.

In another study Singh et al. (1977, b) further observed the
effect of nitrogen, phosphorus and potassium on Jammu lemongras-
(Symbopogon pendulus Wats var. FHCL-16). They supplied a dose of
80 kg. *P*₂*O₅* per hectare as superphosphate, and 120 kg. of *K*₂*O*
per hectare, as muriate of potash at planting time. They noted
that the crop had a high requirement of nitrogen (250 to 340 kg.
per hectare) in 3 to 5 split doses. It was also observed that the
effect of phosphorus and potassium being most marked under
treatments *N*₁₈₀:*P*₆₀:*K*₁₂₀ and *N*₁₈₀:*P*₆₀:*K*₁₆₀ over control.
Singh and Sharma (1977) estimated the nutritional requirements of henbane (Hyoscyamus muticus). They found that nitrogen, phosphorus, and potassium in the ratio of 50: 40: 20 per acre was beneficial for increasing the quality and quantity of the herb.

Virmani et al. (1977, a) worked on lemongrass (Cymbopogon flexuosus (Staud.) Rats.) in relation to mineral nutrients. It was suggested that lemongrass was a soil-exhausting crop. In order to give encouraging results, it was preferable to use spent lemongrass in the form of compost at the rate of 2.5 tonnes and wood ash at the rate of about 2.00 tonnes per hectare per year. It supplied sufficient amount of nitrogen and potash, the most important growth factors in the crop. It was also recommended to apply 30 kg. N, 30 kg. P₂O₅, and 30 kg. K₂O per hectare as basal dose at the time of planting. He further recommended that 50 kg. N per hectare could be applied in 3-4 split doses as top dressing during the growing season.

Virmani et al. (1977, b) also studied the fertilizer requirements of palmarosa grass (Cymbopogon martini Stapf var. motia). A mixed dose of 20 kg. N, 50 kg. P₂O₅, and 40 kg. K₂O per hectare was recommended as basal dose in deficient soils. This basal dose was supplemented with 40 kg. N per hectare in three split doses. It was also suggested that the mixture of nitrogen,
phosphorus and potassium should be repeated at the time of appearance of fresh leaves each year.

The effect of fertilizers and moisture content in soils on the crude drug yield of herb and roots, alkaloid content, and dynamics of nutrient uptake of Atropa belladonna L. has been studied by Colez et al. (1978, a). They found that increased doses of nitrogen, phosphorus and potassium maintained at quantitative ratios of $\text{N:P:K} : 20$ as $1:0.8:1.0$, gave the significant increase in crude drug yield. The percentage of total alkaloid contents was not affected by increased doses of fertilizers and moisture content in the soil.

Colez et al. (1978, b) also studied the effect of nitrogen, phosphorus and potassium fertilizing on crude drug crop and uptake of nutrients by majoram (Origanum majorana L.). He also included calcium in the mixture and found that the maximum yield of crop was obtained in combination of the quantitative ratios of $\text{N:P:K:Ca} : 20$ equal to $1:0.09:0.81:0.64$. He further noted that the ammonium sulphate resulted significant decrease in crude drug mass in comparison to other fertilizers used. The plant showed the maximum nitrogen and potassium requirement, less of calcium and least of phosphorus.

Husain et al. (1978) studied the fertilizer application on
mint (Mentha arvensis L.). They noted 20 kg. N, 50 kg. P₂O₅ and 100 kg. K₂O per hectare as basal dose for a good crop at the time of planting in deficient soils. However, they recommended that nitrogen dose could be decreased in the presence of green manure or farmyard manure. They also suggested that mint responded very well to the application of organic fertilizers: farmyard manure or compost and yields were much better as compared to artificial fertilizers.

Prasad (1976) observed the effect of nitrogen, phosphorus and potassium on the yield of lemongrass (Cymbopogon citratus). He found a significant increase in the yield of first cut, third cut and total yield with the application of nitrogen. A pronounced increase in yield was obtained in first cut only with the increasing levels of nitrogen. The effect of phosphorus and potassium on yield was not significant. The interaction effect of nitrogen x potassium and phosphorus x potassium was also not significant.

Aini et al. (1978) studied the effect of nitrogen, phosphorus and potassium on rose cv. 'Super Star'. They applied the fertilizer doses 50 and 75 kg. each of nitrogen, phosphorus and potassium during 1973-74 and 75 and 100 kg. per plant during 1974-75. It was noted that there was no significant effect of different fertilizer combinations on the flower-stem length, flower
production and bloom size. Thus, they concluded that 50 g. each of nitrogen, phosphorus and potassium was optimum dose which could meet the requirement of 3-year old rose bushes.

Grivastava (1978) studied the fertilizer requirements of Rauvolfia serpentina. He recommended the use of organic manures and inorganic fertilizers. The response was more to chemical fertilizers than to organic manures. He further noted that nitrogenous fertilizers induced more vegetative growth followed by organic manure. Nitrogen seemed to have stunting effect on the root. But the combination of nitrogen either with farmyard manure or phosphate resulted in the better root growth. Phosphate and farmyard manure induced greater root growth than nitrogen. However, a mixed treatment of organic manure, nitrogen and phosphate resulted in the highest growth of roots and shoots as well. He also suggested to apply a basal dose of 20 kg. N and 30 kg. each of P2O5 and K2O per hectare before planting. The application of two top doses of nitrogen at the rate of 20 kg. per hectare were also recommended during the growing season every year.

El-Shafie (1979) studied the effect of different fertilization rates on the growth and yield of roselle in Egypt. The application of 56 kg. N per hectare, 500 kg. P2O5 per hectare and 254 kg. K2O per hectare was noted to be optimum for roselle. The
sources of nitrogen, phosphorus and potassium were ammonium sulphate (33% N), calcium superphosphate (15% P₂O₅) and potassium chloride (60% K₂O) respectively.

Golez and Kordana (1977) studied the effect of nitrogen, phosphorus and potassium fertilizers on crude drug crop and uptake of mineral nutrients for Trigonella foenum-graceum L. They also included magnesium and calcium in the mixture. It was noted that the crop was mainly influenced by nitrogen, phosphorus and potassium, whereas the effect of magnesium and calcium was not so significant. They observed that the mineral contents on the day of harvest were as follows:

Nitrogen (3.38-5.15%) and phosphorus (0.52-0.67%) in seeds; potassium (3.35-4.87) in legumes; and calcium (1.25-2.73%) and (1.43-2.57%) in herb and legumes respectively. It was concluded that plants showed the highest requirement for nitrogen, lower for potassium and calcium; and the lowest for phosphorus. They also observed that maximum height was attained with nitrogen, phosphorus, potassium and calcium in the ratio of 1.0, 0.71, 1.01, 0.71 respectively.

Vaharan et al. (1979) studied the effect of nutrient elements on the growth and yield of Roselle plants, Hibiscus sabdarifica L. The application of nitrogen at the level of 200 kg. of ammonium
sulphate, phosphorus at the rate of 100 kg. of calcium
superphosphate and potassium at the level of 50 kg. of
potassium sulphate per feddan gave the highest growth, yield
and active constituents of roselle.

Asiuddin (1979) conducted a field experiment to investigate
the effect of nitrogen, phosphorus and potassium on growth
characteristics at two stages and yield of fennel (*Foeniculum vulgare
Mill.*). He noted that 90 kg. N, 60 kg. P<sub>2</sub>O<sub>5</sub> and 50 kg. K<sub>2</sub>O per
hectare were the optimum doses at both stages of growth. Thus,
N<sub>90</sub>P<sub>60</sub>K<sub>90</sub> was the best combination for length, number of branches
and fresh weight; but for seed yield, N<sub>45</sub>P<sub>45</sub>K<sub>90</sub> proved the best
combination.

2.2.7 Foliar Nutrition

It is well known that an adequate and efficient use of fertilizer
by a crop promote its production. Foliar application of mineral
nutrients is an economic and efficient method through which the
appropriate quantities of required nutrients is supplied to the
leaves in the form of dilute solution to the standing crops.
This method is also known as "non-root" feeding of crops.
According to Bould (1963), this technique was first used by
Forsyth in 1803. Another early report on the foliar absorption
Foliar nutrition has many advantages over soil application. It has been reported that up to about 50% of the applied nitrogen remains unutilized by crop plants (Anonymous, 1971). Moreover, 'fixation' of applied phosphorus (up to about 70%) in the soil is also very common (Russell, 1970). Therefore, alternative methods for the application of fertilizers have been suggested to overcome these nutrient losses, e.g., feeding the plant through foliage in the form of dilute solutions of the nutrient. The use of foliar sprays as an emergency treatment to check deficiencies of nutrients helps to avoid heavy losses. Some crops remove large quantities of fertilizers during early vegetative growth and need fertilizer supplements at later stages of growth when soil application is not easily feasible. The micronutrients needed by the crop in very small amounts are the best bet for foliar application. Boron and copper compounds seem to be readily absorbed and transported in all the crop plants investigated thus far, and one or two dilute sprays give satisfactory responses in a single year (Boynton, 1954). The convenience afforded by this technique, together with the saving of the nutrients, makes it attractive and cheap for supply of nutrients at the proper stage of growth. This method can also be beneficial in dry land farming where high doses of solid fertilizers may result in harmful osmotic effect on roots. Finally, under hilly,
water-logged, sandy and porous or highly alkaline and acidic
soil conditions, the foliar feeding is advantageous over soil
application.
The effect of leaf applied nutrients has been reported to be
more than that of soil application. Because foliar absorption
is coupled with plant metabolism within a very short time, the
foliar applied nutrients are absorbed, translocated and
utilized by the plants in the way similar to that applied through
roots (Wittwer and Teubner, 1959). Plants absorbed nutrients
through the foliage often several times faster than from
supplemented soil application (Silberstein and Wittwer, 1951;
Thorne and Watson, 1953; Fisher and Walker, 1955; Wittwer et al.,
1957).
The ready uptake of the applied nutrients and translocation
from the leaves is observed by changes in colour, composition,
growth and yield of various organs of the plants following
foliar spray. It is an 'active' process dependent on
temperature, light and oxygen. It is largely irreversible,
occurring against a concentration gradient and is influenced
adversely by inhibitors.
Success of foliar sprays as a suitable method of fertilization
has been noted for fruit trees in U.S.A (Tukey, 1970), for
sugarcane in Hawaii (Burr et al., 1956), for sugarbeet in U.S.S.R. (Klechkovski, 1956), for paddy, wheat, barley and mustard in India (Narayanan and Vasudevan, 1957; Samiullah, 1971, Qaseem, 1975; Naqvi, 1975; Afridi and Samiullah, 1977; Afridi et al., 1977; Inam, 1978). Foliar nutrition is therefore, an effective, convenient and economical technique to ensure nutrient supply at the proper stage of growth for a wide variety of crops.

Considerable work has been done at Aligarh also on the foliar nutrition of various crops by Afridi and associates. Samiullah (1971) and Afridi and Samiullah (1973) working with barley, Khalique (1975) with barley, maize and lettuce; Qaseem (1977, and Afridi et al. (1976, b) with barley and wheat; Afridi et al. (1976, a), Naqvi (1976) and Naqvi et al. (1977), Farvaiz (1977), with mustard and Inam (1978), Abbas (1980), with triticale, have established the significance and advantage of foliar nutrition as a supplement to regular soil application of mineral nutrients. These workers also recommended proper stage of crop for foliar application of nutrient solution of balanced concentration.

Regarding foliar nutrition of medicinal and aromatic plants, very little work has so far been done. The available literature pertaining to the foliar application of mineral nutrients of
these plants is meagre and is reviewed below:
Bains et al. (1977) compared the application of nitrogen in soil and leaves of Mentha arvensis. They found that soil application produced more herb yield under both the levels of nitrogen (75 kg. N and 150 kg. N per hectare). The herb yield was adversely affected particularly during second year when higher levels of nitrogen (150 kg. N per hectare) was applied through foliage. On the other hand Singh and Sharma (1977) studied the effect of foliar spray of urea on henbane (Hyoscyamus niger) seedlings. They reported that foliar application of a 2% aqueous solution of urea greatly accelerated the growth of 8 week old seedlings.
Mohan and Sharma (1978) reported the foliar feeding as the best tool for higher oil yield from the late planted Japanese mint (Mentha arvensis sub sp. haplocalyx var. piperosens). They noted that the oil yield was successfully increased by foliar application of urea as 3% concentration at 15 days interval.
Lawande et al. (1980) conducted an experiment to study the efficacy of foliar application of urea in fenugreek (Trigonella foenum-graecum L.). They applied foliar sprays of urea (0.25, 0.50, 1.0, 1.25 and 1.50%), replicated four times in a randomized block design. The highest yield was
obtained with 0.25% urea spray and an increase in the urea concentration resulted in a gradual decrease in the yield of fenugreek.

It is thus evident from this brief review of the available literature that there is need to conduct an intensive study of the nitrogen, phosphorus and potassium (NPK) requirements of some species of *Cymbopogon*. It would also be interesting to investigate if fertilizer economy can be brought about by using the technique of foliar spray in these species of this valuable grass.

The foliar application of nitrogen on medicinal and aromatic plants as reviewed above showed some inconsistent results. Keeping this view in mind, the stage and concentration of spray solution should be taken into consideration at the time of the application of foliar treatments.
Chapter 3

MATERIAL AND METHODS
Chapter. 3

MATERIAL AND METHODS

3.0 Introduction

It is proposed to carry out four field trials on the selected species of *Cymbopogon* obtained from J.I.M.A., Lucknow, (U.P.). The aim of the experiments will be to observe the effect of different doses of nitrogen, phosphorus and potassium applied to the soil, and/or leaf on the various growth and yield characteristics and nitrogen, phosphorus and potassium content at developmental stages of the crop. The study will help to establish the optimum soil applied fertilizer doses for different species of this crop under local conditions. In addition, the feasibility of fertilizer economy will be tested by the application of these nutrients in the form of solution on the leaves. Finally an optimum dose of spray solution containing the different combinations of nitrogen and phosphorus will be worked out.

The experiments will be conducted in small plots measuring 2 x 2 meters at Fort of the Aligarh Muslim University, Aligarh. The experiments will be laid out statistically and the soil of the field will be analysed for various physico-chemical properties before the start of each experiment. Meteorological data will be noted during the experimental period. The preparation and lay out of plots, planting of slips, irrigation, weeding and harvesting will be done according to standard agricultural
practices.
The statistically designed experiments will be performed as per details given below:

3.1 Experiment 1
The first experiment will be conducted on three selected species of *Cymbopogon* viz.,
1. Citronella grass (*C. winterianus*)
2. Palmarosa grass (*C. martinii*)
3. Lemon grass (*C. flexuosus*)
The aim of this experiment will be to observe the response of these species in relation to growth and yield characteristics to various levels of nitrogen dressings. The optimum basal dose of nitrogen will be established for each species. The experiment will be based on a factorial randomized block design, according to the scheme given below:

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Treatments</th>
<th>N kg. per hectare</th>
<th>P₂O₅ kg. per hectare</th>
<th>K₂O kg. per hectare</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>N₁ K₁</td>
<td>150</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>2.</td>
<td>N₂ K₂</td>
<td>200</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>3.</td>
<td>N₃ K₃</td>
<td>250</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>4.</td>
<td>N₄ K₄</td>
<td>300</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>5.</td>
<td>N₅ K₅</td>
<td>350</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>6.</td>
<td>N₆ K₆</td>
<td>400</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>7.</td>
<td>N₇ K₇</td>
<td>150</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>8.</td>
<td>N₈ K₈</td>
<td>500</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>
Urea will be used as a source of nitrogen, monocalcium superphosphate as the source of phosphorus and muriate of potash as the source of potassium. Thus, in all there will be 24 treatments each replicated thrice. The phosphorus and potassium at the rate of 75 kg. and 40 kg. per hectare respectively will also be given to maintain the soil fertility. The total number of plots will be 72. The number of slips will be kept at 14 per plot. A spacing of 0.50 x 0.50 metre between the plants and rows will be maintained.

3.2 Experiment 2

The experiment will be conducted like experiment 1 on the same three species of *Cymbopogon* with the same planting procedure. The aim of experiment will be to find out the optimum basal dose of phosphorus for growth and yield characteristics of each species. The experiment will be designed according to factorial randomized block design and will be performed on the basis of the following scheme:

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Treatments</th>
<th>N kg. per hectare</th>
<th>P₂O₅ kg. per hectare</th>
<th>K₂O kg. per hectare</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>NPK</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2.</td>
<td>NPRk</td>
<td>150</td>
<td>25</td>
<td>40</td>
</tr>
</tbody>
</table>
The sources of fertilizers will be the same as used in experiment 1. Thus, in all there will be 2 treatments each replicated thrice and the total number of plots will be 72. The nitrogen and potassium at the rate of 150 kg. and 40 kg. per hectare will also be given to maintain the fertility of soil.

### 3.3 Experiment 3

This experiment will also be conducted like experiment 1 and 2 on the three species of *Cymbopogon* with the same planting procedure. The aim of the experiment will be to establish the optimum basal dose of potassium for each species. This experiment will also be based on a factorial block design according to the following scheme:

<table>
<thead>
<tr>
<th>c. No.</th>
<th>Treatments</th>
<th>N kg. per hectare</th>
<th>P2O5 kg. per hectare</th>
<th>K2O kg. per hectare</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>NPK</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2.</td>
<td>NPK</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3.</td>
<td>NPK</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
3.3 Treatments

<table>
<thead>
<tr>
<th>No.</th>
<th>Treatments</th>
<th>N kg. per hectare</th>
<th>P2O5 kg. per hectare</th>
<th>K2O kg. per hectare</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.</td>
<td>NPK1</td>
<td>150</td>
<td>75</td>
<td>60</td>
</tr>
<tr>
<td>3.</td>
<td>NPK2</td>
<td>&quot;</td>
<td>&quot;</td>
<td>80</td>
</tr>
<tr>
<td>4.</td>
<td>NPK3</td>
<td>&quot;</td>
<td>&quot;</td>
<td>120</td>
</tr>
<tr>
<td>5.</td>
<td>NPK4</td>
<td>&quot;</td>
<td>&quot;</td>
<td>160</td>
</tr>
<tr>
<td>6.</td>
<td>NPK5</td>
<td>&quot;</td>
<td>&quot;</td>
<td>200</td>
</tr>
<tr>
<td>7.</td>
<td>NPK6</td>
<td>&quot;</td>
<td>&quot;</td>
<td>240</td>
</tr>
<tr>
<td>8.</td>
<td>NPK7</td>
<td>&quot;</td>
<td>&quot;</td>
<td>280</td>
</tr>
</tbody>
</table>

The sources of fertilizers for this experiment will also be the same as used in experiment 1 and 2. Thus, in all there will be 21 treatments each replicated thrice, and total number of plots will be 72. The nitrogen and phosphorus at the rate of 150 kg. and 75 kg. per hectare respectively will also be included in the trial to maintain the fertility of soil.

3.4 Experiment 4

The optimum basal doses of nitrogen, phosphorus, and potassium will be noted from experiment 1, 2 and 3 respectively for three species of *Cymbopogon*. On the basis of their performances, the best species will also be selected and with the help of knowledge gained from the previous experiments the final field trial will be conducted. The aim of this experiment will be to investigate fertilizer economy with sub-optimal basal combined
dose of nitrogen, phosphorus and potassium (NPK), supplemented by the foliar spray treatments of nitrogen and/or phosphorus. The scheme of treatments is given below:

<table>
<thead>
<tr>
<th>S.No</th>
<th>Split plot (Spray treatment)</th>
<th>Main plot (Soil dressing)</th>
<th>Sub-optimum dose of NPK</th>
<th>Optimum dose of NPK</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>S0</td>
<td>N₄P₃K₆ plus noN₀</td>
<td>N₄P₃K₆ plus noN₀</td>
<td>Sprayed with deionized water</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>n₁P₀</td>
<td>&quot;</td>
<td>&quot;</td>
<td>n₁P₀ Sprayed with 7 kg. N per hectare only.</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>n₂P₀</td>
<td>&quot;</td>
<td>&quot;</td>
<td>n₂P₀ Sprayed with 10 kg. N per hectare only.</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>n₃P₀</td>
<td>&quot;</td>
<td>&quot;</td>
<td>n₃P₀ Sprayed with 17 kg. N per hectare only.</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>n₀F₁</td>
<td>&quot;</td>
<td>&quot;</td>
<td>n₀F₁ Sprayed with 2 kg. K₂O₇ per hectare only.</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>n₁F₁</td>
<td>&quot;</td>
<td>&quot;</td>
<td>n₁F₁ Sprayed with 5 kg. N and 2 kg. K₂O₇ per hectare.</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>n₂F₁</td>
<td>&quot;</td>
<td>&quot;</td>
<td>n₂F₁ Sprayed with 10 kg. N and 2 kg. K₂O₇ per hectare.</td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>n₃F₁</td>
<td>&quot;</td>
<td>&quot;</td>
<td>n₃F₁ Sprayed with 17 kg. N and 2 kg. K₂O₇ per hectare.</td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td>n₀P₂</td>
<td>&quot;</td>
<td>&quot;</td>
<td>n₀P₂ Sprayed with 4 kg. K₂O₇ per hectare only.</td>
<td></td>
</tr>
<tr>
<td>10.</td>
<td>n₁P₂</td>
<td>&quot;</td>
<td>&quot;</td>
<td>n₁P₂ Sprayed with 7 kg. N and 4 kg. P₂O₅ per hectare.</td>
<td></td>
</tr>
<tr>
<td>11.</td>
<td>n₂P₂</td>
<td>&quot;</td>
<td>&quot;</td>
<td>n₂P₂ Sprayed with 10 kg. N and 4 kg. P₂O₅ per hectare.</td>
<td></td>
</tr>
</tbody>
</table>
3.5 Sampling technique

Sampling will be done at three times as three cuttings. Plants samples will be collected after cuttings to study the growth, development and yield. Random sampling of five plants will be
carried out from each plot. The percentage of essential oil will be determined by the extraction of fresh Cymbopogon leaves with the help of Clevenger apparatus. For the quality of the essential oil the gas liquid chromatography will be done.

3.5.1 Growth characteristics

The following growth characteristics will be studied:

(i) Culm number per plant
(ii) Leaf number per plant
(iii) Shoot length per plant
(iv) Fresh weight per plant
(v) Dry weight per plant

3.5.2 Yield characteristics

After each cutting, the following yield characteristics will be noted:

(i) Herb yield per hectare
(ii) Percentage of essential oil (v/w) in leaves.

3.5.3 Nutrient content

The following data will be obtained by leaf analysis:

(i) Percentage of nitrogen in leaves.
(ii) Percentage of phosphorus in leaves.
(iii) Percentage of potassium in leaves.
3.6 Leaf analysis

Leaf analysis is now an established practice for assessing the nutritional status of the plants (Lundegårdh, 1951). The five sample plants from each treatment, picked randomly, will be cleaned for dust or other adhering materials. The fresh weights of the shoots will be taken. The samples will be dried for 24 hours in an oven maintained at 80 degree centigrade, and their dry weight will be taken thereafter. Fully mature and healthy leaf blades will be powdered fine enough to pass a 75 μm screen. This leaf powder will be kept overnight at 70 degree centigrade before digestion and subsequent analysis for its NPK content according to the method of Lindner (1944), with slight modification as under:

100 mg. of the dried leaf powder of each sample will be carefully transferred to a 50 ml. Kjeldahl flask, and wet ashed in 2 ml. of chemically pure sulphuric acid. Digestion will be continued for about two hours to allow complete reduction of nitrates present in the plant material by the organic matter itself, giving off dense white fumes until the contents turn black. After cooling the flasks for about 15 minutes, 0.5 ml. of chemically pure 30% hydrogen peroxide will be added dropwise and the solution will be heated again till the colour of solution changes from black to light yellow.
After heating for about 30 minutes, the flask will be cooled and for 10 minutes and an additional amount of 3-4 drops of 30% hydrogen peroxide will be added, followed by gentle heating for another 15 minutes, to get clear and colourless extracts. Care will be taken in the addition of hydrogen peroxide because if added in excess there is a possibility that it would oxidise the ammonia in the absence of organic matter. When the contents in the Kjeldahl flasks become perfectly colourless, they will be diluted with double distilled water and transferred with 3 or 4 washings to 100 ml. volumetric flasks and the volumes will be made up. Further analysis of nitrogen, phosphorus, and potassium from the sulphuric acid peroxide digested samples are as summarised below.

3.6.1 Nitrogen

The method of Lindner (1969) will be adopted for the estimation of nitrogen in the samples. Total nitrogen in leaves will be estimated by Nesslerization. A 10 ml. aliquot of the peroxide digested material will be transferred to a 50 ml. volumetric flask. 2 ml. of 2.5 N sodium hydroxide will be carefully added with the help of a microburette to partially neutralize the excess of acids. 1 ml. of sodium silicate solution will also be added to prevent turbidity and then finally the volume will be
made up. A 5 ml. of this solution will be taken in a 10 ml. graduated test tube and 5 ml. of Nessler's reagent will be added dropwise, mixing thoroughly after each installment. After adding distilled water to make up the volume the contents will be allowed to stand for about 5 minutes for maximum colour development. The solution will then be transferred to a colorimetric tube and the optical density will be measured at 525 nm. on a "Spectronic 20" colorimeter. A blank will be run with each sample. Using known dilutions of a standard ammonium sulphate solution, a calibration curve will be obtained from which the amount of nitrogen in the aliquot will be read.

3.6.2 Phosphorus

Total phosphorus will be estimated by the method of Fiske and Subba Row (1925). Briefly, a 5 ml. aliquot will be taken in a 10 ml. graduated tube and 1 ml. molybdic acid (2.5% ammonium molybdate in 10 N H$_2$SO$_4$) will be carefully added followed by 0.4 ml. of 1,2,3-amino napthia-sulphonic acid, which will turn the contents blue. Distilled water will be added to make up the volume up to 10 ml. and the solution will be allowed to stand for about five minutes after mixing thoroughly. It will
be then transferred to a colorimetric tube and the optical
density will be read at 620 nm on a "Spectronic 20" colorimeter.
A blank will be run for each determination. The phosphorus
content of each determination will be obtained by comparing
its optical density with a calibration curve plotted by taking
a standard solution of monobasic potassium phosphate.

3.6.3 Potassium

Potassium will be estimated by using a flame photometer. 1 ml.
 aliquot will be taken and read at 766 nm. A blank will be
 compared with a calibration curve plotted for different dilutions
 of a standard potassium sulphate solution.

3.7 Essential oil extraction and estimation

It will be done with the help of Clevenger apparatus (Anonymous,
1973). This is a continuous distillation apparatus in which the
separated volatile oil is caught in a trap and determined by volume.
The apparatus consists of distillation flask, still head,
graduated measuring tube, levelling tube and return flow tube made
in one piece.

To obtain the maximum yield of oil and facilitate release of
oil, the grass is chopped into shorter lengths. Chopping the grass
has further advantages that more grass can be charged into the
distilling flask and even packing is facilitated. After packing the grass, water is added to the distilling flask and it is then connected to the still head. Before attaching the condenser, water is run into the graduated receiver, keeping the tap open until the water overflows. Air bubbles in the tube are carefully removed by pressing the tube. The tap is then closed and the condenser is attached. The contents of the flask are now heated with mantle heater. The distillation is continued at a rate which keeps the lower end of the condenser cool by the continuous supplying of tap water with the help of rubber tubings. After sometime the steam is formed in distilling flask. The mixture of water vapours and essential oil passes into the condenser. As the distillation proceeds, the distillate collects in the graduated part of the receiver. The oil being lighter than water and insoluble, floats on the top of the receiver.

At the end of specified time, heating is discontinued, the apparatus is allowed to cool for 10 minutes and the tap is opened and the tube is lowered slowly; as soon as the layer of the oil completely enters into the graduated part of the receiver the tap is closed and the volume is read. The measured yield of essential oil is taken to be the content of essential oil in the grass. The content of essential oil is expressed as a percentage v/w.
3.8 **Statistical analysis**

All the experiments will be statistically designed and the data will be subjected to statistical analysis and the significance of the results determined at 5% level of probability.
Chapter 4

REFERENCES
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