EFFECT OF GRADED DOSES OF NITROGEN AND POTASH ON GROWTH, ROOT YIELD AND ALKALOID CONTENT OF PERIWINKLE (*Catharanthus roseus* (L.) G. Don)

BY

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THESIS

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DECLARATION

I hereby declare that this thesis entitled " EFFECT OF GRADED DOSES OF NITROGEN AND POTASH ON GROWTH, ROOT YIELD AND ALKALOID CONTENT OF PERIWINKLE (Catharanthus roseus (L.) G.Don)" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

(B.R. REGHUNATH)

Vellayani,
9-4-1981.

CERTIFICATE

Certified that this thesis, entitled " EFFECT OF GRADED DOSES OF NITROGEN AND POTASH ON GROWTH, ROOT YIELD AND ALKALOID CONTENT OF PERIWINKLE (*Catharanthus roseus*(L.) G.Don)" is a record of research work done independently by Shri. B.R. Reghunath under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

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CONTENTS

LIST OF TABLES

LIST OF TABLES (Contd.)

LIST OF FIGURES

LIST OF PLATES

Plate Number

INTRODUCTION

INTRODUCTION

Catharanthus roseus (L.) G.Don commonly known as Madagascar periwinkle has been variously designated as *Vinca rosea* L. and *Lochnera rosea*(L.) Reichenbach. It belongs to the botanical family Apocynaceae. The genus *Catharanthus* is often confused with the genus *Vinca* and in trade *Catharanthus* is continued to be referred to as *Vinca*. However, the genus *Vinca* is a small one and comprises of the Periwinkles of the temperate zone which are native of Europe and naturalized in North America. *Catharanthus* is a tropical genus, probably indigenous to Madagascar, but is now widely distributed throughout the warm regions of the world (Trease and Evans, 1977). It was introduced into India primarily as an ornamental plant and by virtue of its wide adaptation and year round flowering habit it has found wide acceptance. This continued flowering habit earned the plant the vernacular names of 'Nithya Kalyani'(Malayalam), Sadabahar (Hindi) and Sadaphuli (Marathi).

The emergence of *Catharanthus* as one of the most important medicinal plants is relatively recent, though it is one of the very few medicinal plants which has a long history of use. Periwinkle is recorded as far back as 50 B.C. in folk medicine literature of Europe as diuretic, anti-dysenteric, haemorrhagic and wound-healing (Narayana et al. 1977). They were known for use in the treatment of diabetes in Jamaica and India, but the present investigators have not yet accepted this property. It was the chance discovery of Noble et al.(1958) that the extracts of the leaves produced Leukopenic action in rats which earned world wide reputation for the plant. This inspired the research workers to undertake an extensive phytochemical investigation of the plant, which led to the discovery of the two alkaloids from leaves, namely vinblastine and vincristine, having anti-cancerous properties. Vinblastine sulphate is being used mainly for the treatment of generalised Hodgkins disease and choriocarcinoma. Vincristine sulphate is being used principally in the treatment of Leukaemia in children and reticulum cell sarcoma. So far, over 100 alkaloids have been isolated from various parts of this plant (Narayana et al. 1977). Some of the medicinally important alkaloids extracted from the roots of Catharanthus namely ajmalicine (raubasin), serpentine and reserpine are also found to be present in the roots of Rauvolfia serpentina Benth. But the concentration of these alkaloids, especially that of the first two, is found to be lesser in Rauvolfia roots when compared to Catharanthus roots. These alkaloids possess antifibrillic, hypotensive, sedative and tranquillizing properties similar to, but more marked than those of Rauvolfia serpentina (Rajendra Gupta, 1977).

The utility of *Catharanthus* roots as a source of indole alkaloids especially ajmalicine, followed its detection as an adulterant in *Rauvolfia* roots exported from India. Since *Catharanthus* roots as an adulterant proved as valuable as *Rauvolfia* as a source of ajmalicine, it was readily accepted as an alternate source for the alkaloid. According to Arens *et al.*(1978) 3500 kg of ajmalicine is isolated per annum from *Rauvolfia* and *Catharanthus* by the pharmaceutical industries of the world. Our annual export earning of this crude drug is around Rs.10 million (Rajendra Gupta, 1977). The export of roots and leaves of *Catharanthus* from India has also been continuing for the last ten years in considerable quantities. Recently, the demand seems to have increased much. For exporting, the plant was purely extracted from wild sources. At this rate of extraction, the plant may become extinct very soon and further, the supply would not meet even a small fraction of the demand. Anticipating the possible danger of extermination of *Catharanthus roseus* and to meet the increasing demand for this plant as a source of life saving drugs, commercial cultivation of the crop was undertaken in India in about 1,500 hectares (Rajendra Gupta, 1977). Commercial cultivation of this plant is becoming more and more popular in India, especially in the Southern States namely

Tamil Nadu, Karnataka and Andhra Pradesh, with maximum area in Tamil Nadu. In Tamil Nadu the crop is mainly confined at Ramanathapuram, Tirunelveli and Madurai districts.

The commercial crop raised generally for its roots is a mixture of the commonly available floricultural types namely pink, white and white with pink orifice. The cultivation practices being adopted by farmers are largely their innovations. Eventhough advances have been made in the chemical and pharmacological studies, very little work has been done on the cultivation aspects of *Catharanthus* as a field crop. Hubay (1966) was perhaps the first to investigate into the nutritional requirement of *Vinca rosea* under commercial cultivation. He has reported that fertilizer application increased herbage yield and that, best results were obtained with 525 kg of peat salt (CAN), 350 kg of superphosphate, 350 kg of potassium salt and 17.5 tonnes of peat mulch per hectare. Neczypor (1969) obtained increase in plant alkaloids with increased nitrogen application. From the available literature on this aspect of periwinkles and other medicinal plants it seems that only N has got any favourable effect in enhancing root growth and alkaloid content, whereas P, NP and K have no such favourable effects and at times even have reducing effects (Prasad, 1944; Tsao *et al*.1961; Russel, 1962; Varma and Sharma, 1963; Neczypor, 1969; Kondarenko, 1975).

No work has so far been reported in this country on the investigation of nutritional requirements and the effect of nutrients on the alkaloid content of Catharanthus roseus, except a report from CIMAP (Anonymous, 1979) that there was increase in total dry matter production due to application of fertilizers and the highest yield was obtained with 160 kg N, 120 kg P_2O_5 and 100 kg K_2O per hectare. Such works have been done on other alkaloid yielding medicinal plants like Dioscorea sp., Papaver sp., Solanum sp. etc. (Gangadhara Rao, et al. 1975; Ramanathan and Ramachandran, 1973; Choudhary et al. 1979). This emphasises the need for a similar study in Catharanthus roseus.

In view of these facts, experiments were conducted to study the influence of two major nutrients, nitrogen and potash and their combinations at varying levels, on growth, yield and alkaloid content of Catharanthus roseus at different stages of growth.

This thesis embodies the observations and results of these experiments.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Distribution

Catharanthus roseus (L.)G. Don is a tropical medicinal plant. Taylor and Farnsworth (1973) reviewing its world distribution reported that it belongs to the old world tropics, namely Madagascar and India and it naturalizes so readily that it now has a pantropical range. Narayana et al. (1977) reported the distribution of Catharanthus roseus in India, based on geographic and climatic features. The cosmopolitan distribution of the plant shows that it has no specific soil and climatic requirements. However, the growth is much better in tropical areas. In India Tamil Nadu, Kerala, Karnataka, Andhra Pradesh, Gujarat and Madhya Pradesh States are ideally suited for the growth of the plant. The plant also grows in subtropical areas of Northern India, but the growth is slow due to low temperature during the winter.

Climatic and soil requirements

Narayana et al. (1977) stated that Catharanthus roseus has no specific climatic and soil requirements. However it prefers a tropical and subtropical belt with a well-distributed rainfall of 100 cm or more. It can grow on any type of soil except those which are highly saline, alkaline or water logged. However, it prefers light soils which are rich in humus.

Factors affecting plant growth and alkaloid content

It is evident that the plant growth and alkaloid content varies considerably in different localities. This may be due to the difference in climate and geographic conditions, difference in the time of cultivation and variation in chemical constitution of soils at different places and also there may be distinct genetic variation in the plants used for cultivation. The information available on these aspects and also on the cultural aspects of this plant is meagre. Hence, a review of the work done on Periwinkles and also on some important alkaloid yielding medicinal plants is presented.

Influence of climatic factors on plant growth and alkaloid content

1. Season

Season has a profound influence on the total alkaloid content and its potency in several medicinal plants. Hojkova et al. (1961) noticed highest alkaloid content in *Vinca minor* during autumn and lowest during winter season. Taylor and Farnsworth (1973) reported two peaks in the total alkaloid production of *Vinca minor* in an year and these were during April-May and August-September.

2. Temperature

Temperature has been found to influence the production and potency of alkaloids in several plants. Elzenga, Smeets and

Derbruyn (1956) found that plant development and alkaloid content in one year old Atropa belladonna were higher when grown at 23°C than either at 26°C or at 20°C. Il'inskaya and Yosifova (1956) reported that an optimum temperature during the first half of the growth period of opium poppy encouraged morphine formation, while lower temperature induced codeine formation.

3. Rainfall

Taylor and Farnsworth (1973) found that high rainfall was ideal for the growth of Vinca minor, provided that there was no water logging. Narayana (1977) reported that a rainfed crop needs a rainfall of at least 100 cm per year for ideal growth.

4. Humidity

Taylor and Farnsworth (1973) during the course of growth experiments found that a humid woodland habitat is necessary for the growth of Vinca minor and the growth was ideal when the humidity was high.

5. Light intensity and duration

Reda et al.(1978) found that the growth of stem, roots, buds and flowers of Catharanthus roseus was reduced at lower intensities of light (less than 45 per cent). Total alkaloid content in the leaves and stem was pronouncedly increased on treatment under 60 per cent sun light during flowering and fruiting stages. Taylor and Farnsworth (1973) reported similar trends in Vinca minor.

Influence of soil factors on plant growth and alkaloid content

In general, the physical and chemical characteristics of soils have marked influence on plant growth, alkaloid content and its potency in alkaloid yielding medicinal plants.

Narayana et al.(1977) reported that Catharanthus roseus has no specific soil requirements and it can grow on any type of soil except those which are highly saline, alkaline or water logged. He however reported that, light soils which are rich in humus content are preferable for large scale cultivation. Nagy (1970) has observed as high as 40 to 80 per cent higher yield in Vinca minor when grown on forest soils, compared to arable soil. Taylor and Farnsworth (1973) reported that Vinca minor requires a loose soil rich in humus content and high water holding capacity with a pH ranging between 6.5 and 7.5. Talha et al.(1975) reported that under soil moisture deficit conditions, a marked reduction in the fresh and dry weig ht as well as moisture content of various parts of Vinca rosea was observed. He also observed that there was an increase in the per cent alkaloid content of leaves, stem and roots when the SMD per cent was increased from 25 to 50.

Sokolov (1959) reviewing Russian works on the effect of soil moisture status on alkaloid content of medicinal plants, concluded that excess moisture has an adverse effect while Dovrat and Goldschmidt (1978) observed significant decrease in total root and alkaloid yields in Catharanthus roseus under a dry soil moisture regime, when compared with plants grown under high soil moisture conditions.

Influence of propagating material and age of the plant on its growth, yield and alkaloid content

Narayana et al.(1977) found that there are about 835 seeds in a gram of Catharanthus seeds and that the seeds do not maintain viability for long. Hence, he recommended the use of only fresh seeds for sowing in a nursery for transplantation or for direct sowing in the field. Mitrev (1976) recorded 98 per cent germination in the case of fresh seeds and it was reduced to 95, 53, 28 and 0 per cent after 1, 2, 3 and 4 years of storage respectively. Bhandari et al.(1971) observed that when Catharanthus roseus seeds were soaked prior to sowing in aqueous extracts of Ocimum sanctum leaves early flowering was induced, internodes were shortened and the number of leaves were increased.

The plants could be either propagated by seeds or cuttings. Buger and Sarkany (1973) compared C.roseus propagated from seeds and by cuttings. Plants propagated by cuttings over wintered successfully and flowered earlier than plants from seeds. They also recommended that for root and drug production the plant should be grown from seed and for seed production from cutting.

Anonymous (1977) reported that cuttings of 10-13 cm length can be successfully used for propagation and the cuttings from the tip portions were found to be better for propagation. It is further reported that the cuttings were found to flower in 2 to 3 weeks while the seedlings take 6 to 8 weeks.

Reda (1978) observed that in Catharanthus roseus the highest concentration of alkaloids was found in the roots at the start of flowering. Similar trend was reported in Vinca minor (Mermerska et al. 1976) and in Rauvolfia serpentina (Nandi and Chatterjee, 1975a).

Dovrat and Goldschmidt (1978) reported maximum root and alkaloid yields in Catharanthus roseus plants of seven months growth. However, in Vinca minor and Rauvolfia serpentina the total alkaloid content was found to be increasing with the age of the plant (Mermerska et al.1976; Nandi and Chatterjee, 1975). Rajendra Gupta (1977) repprted that from about 50 days of plant growth when the roots grow to a depth of 5 to 25 cm and give out a number of lateral roots, the crop is ready for harvest.

However, he stated that the commercial crop is usually harvested only at the end of 6 to 8 months.

Alkaloid occurrence and distribution

All parts of the plant, particularly the bark of the roots contain alkaloids (Table 1). Pillay *et al.*(1957) have investigated *C.roseus* and *C.roseus* albus plants for the alkaloids in the root bark. They found that the root bark of pink flowering plants yielded about 9 per cent of total alkaloids, while the white flowering plants yielded only about half of this quantity. They have also noted that the stem of pink flowering plants

Table 1

Alkaloid content of different parts of *Lochnera rosea* (=*Catharanthus roseus*)

Parts of the plant	Pink flowered variety (per cent)	White flowered variety (per cent)
Roots (Jammu)	1.08	1.34
Leaves (")	0.82	0.74
Stems (")	0.36	0.17
Roots (")	1.18 a	..
Roots (Travancore)	1.22 a	..
Root bark (")	9.00	4.50

a - Variety not identified

Source - Wealth of India - Raw materials Vol.VI, C.S.I.R.

usually has a pink pigmentation, the pigment being roughly proportional to the amount of red or pink pigment in the flower, while white flowering plants are usually devoid of this pigment in the stem.

Cowley and Bennet (1928) utilizing different types of C.roseus plants grown in Australia, found that pink flowering plants yielded 0.85 per cent leaf alkaloids while glabrous forms of white flowering plants yielded only 0.59 to 0.62 per cent of leaf alkaloids. Noble et al.(1958) observed that C.roseus plants grown as annuals in England had only about one-fourth as much Vincaleukoblastine as in plants collected from perennial sources of West Indies. In addition, they found that leukopenic activity was present in roots, stems and leaves but not in the seeds and flowers. Sarin et al.(1977) observed that the C.roseus roots contain 0.020 to 0.0224 per cent ajmalicine, whereas stems contain about 0.009 per cent. Arens et al.(1978) screened C.roseus plants for root alkaloids and reported that plant containing as high as 0.5 per cent ajmalicine and as high as 1.2 per cent serpentine could be found. The alkaloids were detected by Radio Immuno Assay method. They also found that these alkaloids were concentrated in the root and not in the aerial parts of the plant. Within the root, the highest concentration of both alkaloids is observed close to the base. Detailed experiment showed that the alkaloids are found in the root bark and not in the woody central cylinder. Dovrat and Goldschmidt (1978) reported that ajmalicine was mainly concentrated in the roots 0 to 5 cm below the soil surface and in the 0 to 5 cm stubble. Khan (1977) reported that of the 100 different alkaloids which the plant contains, vincristine is the lowest among any medicinally important alkaloids isolated on commercial basis (0.00025 per cent).

The yield of root, stem and leaves are found to be varying depending upon the locality, methods of cultivation, irrigation and age of the plant. Rajendra Gupta (1977) has reported that the yield of root varies from 2 to 2.5 tons per hectare from irrigated crop to about 1.5 tons per hectare from rainfed crop. Narayana et al.(1977) have reported for a 12 months old crop, an yield of 3.6 tons of leaves 1.5 tons of stems and 1.5 tons of roots per hectare, on air-dry basis for an irrigated crop and 2, 1 and 0.75 tons of leaves, stem and roots respectively for a rainfed crop. For a 6 to 7 months old crop, an average yield of 0.65 tons of root and 0.25 tons of stem per hectare has been reported (Anonymous, 1979).

Cultural practices

Rajendra Gupta (1977) has stated that the cultivation practices being adopted by farmers are largely their innovations.

However, he reported that the commercial crop is being raised using seeds sown either in a nursery and then transplanted or directly sown in the field. He further stated that some farmers prefer direct sowing in rows 30 cm apart and by thinning out to maintain a plant to plant distance of 20,30 or 45 cm.

Narayana et al.(1977) reported that transplanting is advantageous particularly when seed is scarce, since it requires only about 500g of seeds per hectare whereas direct sowing requires 2.5 kg of seeds per hectare. However he recommended direct sowing particularly when a large area has to be cultivated. He further stated that an area of about 200 sq.m. under nursery gives enough seedlings for transplanting one hectare. He observed that the seeds take about 10 days to germinate and about 60 days to reach transplanting stage. Rajendra Gupta (1977) noted that the seedling grows fast and in 30 to 35 days attains a height of 10 to 15 cm. He recommended the use of seedling with 2 to 3 pairs of leaves for transplanting.

Narayana et al.(1977) recommended for a transplanted crop, a spacing of 45 cm between rows and 30 cm between plants within a row. Anonymous (1979b) reported a spacing of 30 cm between ridges or rows and 25 to 40 cm between plants.

Influence of nitrogen, phosphoric acid and potash on plant development, yield of roots, alkaloid content and allied characters

There is not much of published work on fertilizer application to *Catharanthus roseus*. Hence, a review of work done on the above aspect of periwinkles and also on some important medicinal plants, is presented.

1. Nitrogen

Antarov *et al.* (1957) reported an increase in alkaloid content by 25 per cent in *Atropa belladonna* and *Datura stramonium* with the application of nitrogen alone.

Spilenja (1957) observed that foliar application of two per cent solution of ammonium sulphate increased the rate of growth, yield of roots and seeds, in the case of *Atropa belladonna* and *Datura inermis*.

Schermeister (1958) observed that growth of *Atropa belladonna* was most satisfactory at 20 mg of nitrogen (applied in the form of NO_3-N) and low level of nitrogen stimulated root growth, while high nitrate levels stimulated succulence and nitrate accumulation. Moskov and Tkacenko (1967) found that application of 45 kg per hectare of nitrogen to *Atropa belladonna* at 4-5 leaf stage plus same rate at rosette stage or 90 kg per hectare at rosette stage, in addition to P and K resulted in a good crop in first year. Split application of nitrogen gave the highest yield of air dried leaves and alkaloids which exceeded that of control by 68.4 per cent.

Similarly Neczypor (1969) in pot experiments with periwinkles observed greatest increase in alkaloid content and composition, with nitrogen fertilization.

Nandi and Chatterjee (1975b) studying the effect of fertilization on growth of Dioscorea sp. found that 150 kg of nitrogen per hectare produced maximum extension growth of 372.62 cm whereas that of control was 216.92 cm.

Bernath and Foldesi (1972) revealed that increase in nitrogen supply was accompanied by proportional increase in total plant weight, a limited increase in berry number and a decrease in berry plant weight ratio in Solanum laciniatum.

Reports of both increases and decreases in content of alkaloids as a result of application of nitrogen are available. In hydroponic culture of Papaver somniferum, Costes et al.(1976) observed that NO_3-N was the most effective form of nitrogen with respect to production of fresh weight, dry weight and total morphine content, whereas urea depressed alkaloid production. Nowacki et al.(1976) reported that alkaloids in Papaver somniferum, Vinca perenne, Catharanthus roseus and Datura meteloides were increased by nitrogen fertilization.

Russel (1962) stated that nitrogen manuring, though it mainly affects shoot growth, has also a considerable effect on the growth of root, when the plant is in the field for a long time. He explained this as an indirect effect, due to the increased synthesis and utilisation of carbohydrate in the extra leaf growth produced in early stages.

2. Phosphorus

Prasad (1944) stated that phosphoric acid had influence only on the dry matter content and not on the alkaloid content in *Datura alba* and this too was significant only in the final growth stages. Tsao *et al.* (1961) found that high levels of phosphatic fertilizers significantly reduced leaf growth and total glycoside yield of *Digitalis lanata*. Varma and Sharma (1963) investigating the effect of fertilizers on biological activity of *Digitalis purpurea*, concluded that cardiotonic potency of the drug decreased with the application of superphosphate.

Andries (1957) studying the effect of certain deficiencies on alkaloid biogenesis on *Datura stramonium* found that phosphorus at 0.31 ppm increased alkaloid biogenesis but caused a reduction in total nitrogen. Zoraveleu (1970) observed in pot and field experiments that application of phosphorus increased capsule yield in poppy variety 'Novinka-198' and that application of N increased effectiveness of P, while Neczypor (1969) reported that there was no such improvement of yield by the application of P on periwinkle.

Kondarenko (1975) revealed that row application of granulated superphosphate at the rate of 6-8 kg P_2O_5 per hectare increased the yield of Atropa belladonna with or without irrigation, but it did not increase the stem and leaf alkaloid content.

Coffman and Gentner (1977) reported that the response of green house grown Cannabis sativa to P manifested in the form of increased weight and height of the plant. Costes et al.(1976) concluded that the growth of Papaver somniferum was little influenced by phosphate deficiency but increased phosphate supply was associated with earlier flowering and an increase in capsule number.

Russel (1962) stated that phosphate has no specific action in encouraging root development and the apparent influence noted is only due to its capacity to increase leaf area without adversely affecting the power of leaves to transport carbohydrate to roots.

3. Potash

Prasad (1944) stated that potash had a favourable effect on the height and dry matter content of Datura alba, but it was pronounced only in the last stage of growth. Andries (1957) revealed that K at 117 ppm stimulated alkaloid formation by 34 per cent in Datura stramonium and caused a significant decrease in total N and amino acid. Tsao et al.(1961) found very little effect of potash on leaf growth and total glycoside content of Digitalis lanata while Alov (1961) found that increased rate of potash application gave enhanced yield only in the presence of adequate nitrogen. He also observed that while potash was applied singly it had an unfavourable effect on the percentage content of alkaloid in the leaves of Belladonna. Neczypor (1969) reported no improvement of yield by the application of K on periwinkle. Ramanathan and Ramachandran (1973) observed that application of K in general showed no beneficial effect on the yield of opium and morphine content in opium poppy. Similarly, Pinzaru and Casocarin (1978) reported that K fertilization had no effect on the productivity of opium poppy.

4. Two factor combinations

a. Nitrogen and phosphorus

Prasad (1944) recorded that the interactional effect of nitrogen and phosphorus was not significant with regard to the yield and alkaloid content of Datura alba. Varma and Sharma (1963) found that superphosphate combined with ammonium sulphate or ammonium nitrate did not improve the cardiotonic potency in Digitalis.

Brewer and David Hiner (1950) reported that application of ammonium nitrate with phosphatic fertilizer increased the leaf yield of Atropa belladonna. They also concluded that, the alkaloid content of Hyoscyamus niger was increased by the interactional effect of nitrogen and phosphorus, while nitrogen applied singly tended to decrease the alkaloid content. Dalev (1958) obtained highest yield of leaves in Atropa belladonna fertilized at the rate of 20 tons, of FYM, 200 kg of ammonium nitrate and 400 kg of superphosphate per hectare.

Kurunosov and Pikova (1975) obtained highest yield of solasodine, in Solanum laciniatum plants receiving P_2O_5 as basal dressing at 150 kg per hectare with N at 210 kg per hectare.

Dovrat and Goldschmidt (1978) reported that in C.roseus, P with N was found to be essential for intensive root and shoot growth. Gupta et al.(1977) showed that application of 50-80 kg N and upto 100 kg P_2O_5 per hectare gave significantly higher leaf and pod yields in Cassia angustifolia.

b. Nitrogen and potash

Prasad (1944) reported that the additive as well as interactional effect of nitrogen and potash was significant in all stages in increasing height, yield and dry matter content of Datura alba, but with regard to alkaloid content, the interactional effect of nitrogen and potash alone was marked.

Brewer and David Hiner (1950) concluded that a combination of nitrogen and potash increased the alkaloid content of Atropa belladonna and Hyoscyamus niger. Alov (1961) found that the effect of incremental doses of potash in enhancing yield of Atropa belladonna was operative only in the presence of nitrate nitrogen.

Singh et al.(1973b) obtained a maximum yield of 33.1 tons per ha and a maximum dry matter content of 36 per cent in Dioscorea alata fertilized at the rate of 60 kg of nitrogen per hectare while Sing et al.(1973a) reported that Dioscorea esculenta plants receiving 80 kg of nitrogen and 40 kg of K_2O produced maximum dry matter content.

c. Phosphoric acid and potash

Prasad (1944) stated that the combined application of phosphorus and potash significantly increased the height, dry matter content and yield of Datura alba at all stages of growth. He also observed that it had no additive or interactional effect on the alkaloid content and plant part ratio.

Neczypor (1969) reported no improvement in yield by the application of P and K and by minor elements such as Ca or Mg.

5. Three factor combinations (Nitrogen, phosphoric acid and potash)

Gstirner (1950) stated that complete fertilizer, nitrogen, phosphorus and potash increased the alkaloid content in roots and the whole plant of Atropa belladonna while potash gave highest alkaloid content in leaf alone.

Kinoshita et al. (1959) revealed that Papaver somniferum plants fertilized with 2:1:1 NPK fertilizer mixture resulted in highest whole plant weight and alkaloid yield, while Naumova and Scheberstov (1972) reported that NPK ratio 2:2:1 gave highest yield and increased alkaloid content.

Demaggio (1961) found that 10:10:10 mixture of N, P and K was most effective in increasing the alkaloid content in leaves as well as in roots of Datura stramonium. It also resulted in a maximum growth of 100 cm. He further observed that in field grown plants, reduction of N resulted in reduced alkaloid production in leaves and roots. Czabajski et al. (1974) reported that the growth and herbage yield of Datura were best in plants receiving NPK, but both factors were primarily dependent on the N supply. K_2O deficiency caused the appearance of spots on leaves and leaf fall. He also reported that higher scopalamine and atropine yields were obtained from plants receiving NPK + lime; the NPK ratio being 1:0.19: 1.05. Afaw et al. (1978) showed that 90 kg P_2O_5 per hectare along with 60 kg N and 30 kg K_2O per hectare is optimum for Datura stramonium.

Hubay (1966) obtained higher herbage yields in Vinca minor with 525 kg of peat salt (CAN), 350 kg of superphosphate, 350 kg of potassium salt and 17.5 tons of peat mulch per hectare. For *Catharanthus roseus*, Narayana *et al.*(1977) recommended 5 tons of FYM per hectare and a basal dose of 30 kg of P_2O_5, 30 kg K_2O and 20 kg N per hectare with a top dressing of 20 kg of N per hectare in two equal split doses. Application of fertilizers at the rate of 160 kg N, 120 kg P_2O_5 and 100 kg K_2O per hectare had given the highest herbage yield in *Catharanthus roseus* (Anonymous, 1979a).

In *Solanum khasianum* application of P and K each at 20 kg per hectare and N at 40 kg and 60 kg per hectare resulted in higher yield of berries (Anonymous, 1973). Kaul and Zutshi (1976) recommended application of fertilizers in two split doses at 50 kg each of superphosphate, potash and urea per hectare for better yields in *Solanum khasianum*. The first dose is to be applied at the time of crop establishment and the second dose at the time of flowering.

In sand culture experiments on *Solanum laciniatum* and *Solanum avicular*, Crush (1973) observed that all levels of N, P and K enhanced solasodine content compared with no fertilizer, the optimum NPK ratio being 14:12:12. In *Rauvolfia serpentina* alkaloid synthesis was reported to be greatest when N, P and K were all applied (Nandi and Chatterjee, 1975a). Karnick (1977) reported that the yield of rhizome and the active principles in the rhizome were increased with the application of NPK in Hemidesmus indicus.

Johnson and Nunez Melendez (1942) found that increase in alkaloid content of wild Stramonium due to complete fertilizer application was much less than the increase obtained by applying them alone. Rauson and Henderson (1943) and Stillings and Lauric (1944) also reported similar trend in Atropa belladonna.

Enin (1952) reported that Valerian gave mildly active drug with a dose of phosphorus and potash or no manure, while it became biologically inactive when complete fertilizer was applied.

MATERIALS AND METHODS

MATERIALS AND METHODS

A. Experiment

The experiment to study the effect of different levels and combinations of nitrogen and potash on the growth, yield and alkaloid content of *Catharanthus roseus* (L) G. Don was conducted at the Department of Horticulture, College of Agriculture, Vellayani from August 1979 to March 1980.

I. Experimental site

A plain land having uniform fertility status was selected as the experimental site.

a. Soil

The soil of the experimental site belongs to red loam type. Soil samples were collected for analysis, from a depth of 0 to 30 cm from four randomly selected spots, before starting the experiment. The soil was analysed for total and available nitrogen, phosphorus and potash, following the standard procedures. The average values for the four samples are furnished in Appendix I.

b. Climate

Seeds were sown on 4th August 1979 and the crop was finally harvested on 7th March 1980. The meteorological data for the above period is given in Appendix II.

II. Experimental details

a. Treatments

Two major plant nutrients namely nitrogen and potash and their combinations at various levels were tried. In addition to this, one treatment without nitrogen and potash (control) was also tried seperately, for comparison. The levels of nutrients employed are given below.

Nutrients	Levels of nutrient (kg/ha)			
	I	II	III	IV
Nitrogen	50	100	150	200
Potassium	50	100	150	..

b. Design and layout

The experiment was laid out in factorial Randomised Complete Block Design (RCBD). The procedure followed for allocation of various treatments for different plots was in accordance with Cochran and Cox (1965). The details of the lay out are furnished below.

Total number of plots under treatment (Besides 12 plots were laid out, in addition, as control for comparison)	36
Total number of plots	48

Net plot size	4.05 x 2.1 sq.m.
Spacing: Row to row	45 cm
Plant to plant	30 cm
Number of plants per plot	63
Number of replications	3

III. Materials

1. Seed

Seeds were collected from private growers of *Catharanthus roseus* (L.) G.Don from Sivakasi District, Tamilnadu. Fully mature seeds collected during July 1979 were obtained and dried well under sun before sowing. Seeds were tested for germination, the percentage of germination was 72.2.

Variety

The variety used for the experiment was the pink flowered variety, which is common under cultivation.

2. Manures and Fertilizers

Cattle manure and fertilizers with the following nutritive value were used for the experiment.

Cattle manure

Nitrogen (Total)	0.4127%
Phosphoric acid (Total)	0.3216%
Potash (Total)	0.2008%

Fertilizers

Urea	...	46% nitrogen
Single superphosphate		16% phosphoric acid
Muriate of potash		60% potash

IV. Cultivation

1. Nursery

a. Preparation of beds: Raised beds of very fine tilth were prepared and cattle manure at the rate of 5,000 kg per hectare was incorporated in the soil.

b. Sowing: Sowing of seeds was done on 4th August 1979. The seeds were mixed with dry sand and sown 1.5 - 2 cm apart in lines spaced 12-15 cm apart and a thin layer of sand was spread over the seed bed. Watering was done daily using a rose can.

c. Germination: Seed germination extended from one to three weeks. Majority of the seeds germinated 10 days after sowing.

d. Lifting seedlings: The seedling attained transplantable stage after 2 months growth in the nursery (1st week of October, 1979). The seedlings of uniform growth and size were gently lifted with the roots intact. A light root pruning was given to make the length of the roots uniform.

2. Experimental plots

a. Preparation of land: The land was ploughed twice, levelled and weeds removed. It was divided into required number of blocks and plots.

b. Application of manures and fertilizers

Manures: Cattle manure was applied prior to the digging of plots at the rate of 5,000 kg per hectare and incorporated with the soil by digging.

Fertilizers: All the three major nutrients were applied in the form of straight fertilizers, nitrogen in the form of urea, phosphorus in the form of single superphosphate and potassium in the form of muriate of potash.

The quantity of nitrogen, potash and phosphorus applied in terms of urea, muriate of potash and single superphosphate respectively to each plot, are given below.

Fertilizers	Levels of fertilizer (g/plot)			
	I	II	III	IV
Urea	92.44	184.88	277.32	369.76
Muriate of potash	70.87	141.74	212.61	..
Single superphosphate	Uniform dose at the rate of 531.56 g/plot			

Stages of application

Prior to planting, full phosphorus as single superphosphate and full potash as muriate of potash were applied as basal dressing. Nitrogen in the form of urea was applied in two equally split doses. The first dose was applied 70 days after sowing and the second dose 100 days after sowing.

c. Transplanting: Sixty days old healthy seedlings of uniform growth, were transplanted to the main field at a spacing of 45 cm between rows and 30 cm between plants. Planting was done on 3rd and 4th of October 1979. Subsequently gaps were filled up.

d. Irrigation: For a week after transplanting, irrigation was given twice a day and afterwards once in two days for another two weeks. Further irrigation was done depending on the rainfall and soil moisture status.

e. After cultivation: The plots were kept free of weeds by weeding at regular intervals.

f. Pests and diseases: (i) A leaf blight disease caused by *Fusarium oxysporum* was noticed on plants in all the replications during October-November, 1979. From the available literature it seems that this is the first report of the disease from India. After confirming the pathogenicity, the disease was duly reported. The disease was effectively controlled by spraying 'Thiride' at the rate of 2.5g per litre of water. The recurrence of the disease in January was controlled by a second spraying of the same fungicide, after which the crop was given a prophylatic spraying of 'Thiride' every month to prevent further incidence of the disease.

g. Harvest: The crop was harvested on 7th and 8th of March,1980, after completing seven months of growth including the period in the nursery. Dovrat and Goldschmidt (1978) have established that roots of approximately seven months old plant contained more alkaloids than of older plant. The plants were carefully dug out after copious irrigation, with thick and thin roots intact, cleaned and fresh weight recorded seperately for root, stem and leaves. They were dried under the sun for a few hours and then under shade for 10-15 days and the dry weight recorded.

V. Sampling technique

Sampling procedures adopted for taking plant samples for character study and chemical analysis are presented below:-

Samples for plant character study

Out of the total nine rows of plants in a plot, four rows were set apart for periodical uprooting for plant character study. Leaving the border rows and border plants, two plants were uprooted from the rows which were earmarked for sampling. Thus, in total for a particular treatment six plants were uprooted at each stage of observation.

Rest of the rows of plants in a plot, were left as such till the fourth and final uprooting, when all the plants except those in the border rows were uprooted for yield assessment.

VI. Characters studied

Plant samples were uprooted for character study at monthly intervals during the crop period of seven months, commencing from the 70th day after transplanting which roughly coincided with four growth phases of the plant ie., peak flowering, seed setting, seed ripening and declining stages.

The various characters studied at each observation are given below:

1. Height of plants

This observation was recorded in cm from the ground level to the tip of the tallest branch.

2. Girth of stem

The girth of the stem portion 5 cm above the ground level was recorded in cm for this purpose.

3. Yield of shoot

i. Fresh shoot yield: Fresh weight of the shoot in grams was recorded for this purpose. The shoot consisted of aerial portion devoid of flowers, fruits, leaves and 5 cm of stem length above the ground level.

ii. Dry shoot yield: Dry weight of shoot in grams was recorded after drying the shoots at 60°C in a hot air oven for two to three days till same concurrent weights were obtained.

iii. Percentage dry matter content: This was calculated from fresh and dry weights of shoot .

4. Leaf yield

i. Fresh leaf yield: All the leaves in a plant were stripped and fresh weight recorded in grams.

ii. Dry leaf yield: Drying of the leaves was done at all strippings, in a hot air oven at 60°C for two to three days till same concurrent weights were obtained and the weight was recorded in grams.

iii. Percentage dry matter: This was calculated from fresh and dry weights of the leaves.

5. Length of root

As per the commercial practices the root length was measured from 5 cm above the ground level to the tip of the tap root and it was recorded in cm.

6. Girth of root

i. Tap root: Girth at three fixed points from the top to the bottom of the tap root were recorded in cm for each of the observational plants and the mean value recorded.

ii. Secondary roots: Maximum girth of each representative secondary root from each category of root thickness, except very thin fibrous ones, was recorded in cm for each of the observational plants and the mean value recorded.

7. Number of roots per plant

Total number of secondary roots of all categories of thickness except very thin fibrous ones, was recorded for this purpose.

8. Yield of roots

i. Fresh root yield: As per the commercial practices, root yield consisted of underground portion with 5 cm of stem length above the ground level. The observational plants were carefully uprooted, after copious irrigation, taking maximum care for not breaking any of the roots. The root portion, consisting of both the thick root and the thin secondary roots, was weighed after cleaning in water and the fresh weight recorded in grams.

ii. Dry root yield: Dry root yield was recorded in grams after drying roots in a hot air oven at 60°C for 2 to 3 days till same concurrent weights were obtained.

iii. Percentage dry matter: This was calculated from fresh and dry weights of root.

9. Total crude alkaloid content (roots)

i. Estimation of total crude alkaloid content: This was done by column chromatography method as outlined by Central Institute of Medicinal and Aromatic plants.

Procedure: Power of 5g of roots of the plant, containing about 30-50 mg equivalent of total alkaloids was stirred with 2N sodium carbonate solution. The paste was treated with a small quantity of silica jel till the mixture was well dried. About 5 to 6 g of silica jel was sufficient. The mixture was placed in the chromatographic column and packed well. It was eluted with chloroform (one drop per second) till a solution of the residue (obtained from 5 ml of elute) in 0.1N hydrochloric acid gave negative results with iodine solution or Meyers reagent. Totally about 200 to 300 ml elutant was collected. The chloroform was evaporated to dryness over water bath. The residue was dried thoroughly. It was dissolved in 25 ml of water free glacial acetic acid and titrated against standard

0.1N perchloric acid in water free acetic acid, using 0.1N crystal violet solution in acetic acid as an indicator. The total alkaloid content in the sample was calculated by using the following conversion factor.

1 ml of 0.1N perchloric acid = 75 mg of total alkaloids

ii. Percentage alkaloid content in roots: This was calculated by converting the amount of alkaloid present in 5g of sample into percentage.

iii. Total alkaloid content per plant: This was calculated by converting the alkaloid content of the sample (5g) to alkaloid yield per plant (root only).

iv. Total alkaloid yield per hectare: This was calculated by multiplying total alkaloid yield per plant with total number of plants per hectare.

10. Statistical interpretation of data

Statistical interpretation was done by following Fisher's analysis of variance technique (Snedecor and Cochran, 1976). The results were compared at 5 per cent level of significance.

RESULTS

RESULTS

An investigation was carried out at the Department of Horticulture, College of Agriculture, Vellayani during 1979-80 to study the effect of graded doses of nitrogen and potash on growth, yield and alkaloid content of <u>Catharanthus roseus</u> (L.) G. Don. The various observations recorded were statistically analysed and the salient features of the results are presented below.

A. <u>Effect on growth characters</u>

1. <u>Height of plant</u>

The data on the plant height at different growth stages as influenced by the application of different levels of nitrogen (N) and potash (K) and their combinations are presented in Table 2 and Fig.1.

The data showed that N alone had a set pattern of significant response on plant height at all stages of growth from 70th day after planting to 160th day after planting (DAP) whereas K and various combinations of N and K failed to show so.

Different levels of N showed significant response at all stages of plant growth. Application of N at the rate of 150 kg per hectare recorded increased plant height at all stages except at 70th DAP. At 70th DAP the plant height was more with N at 200 kg per hectare than N at 150 kg per hectare. N at 100 kg

Table 2

Effect of nitrogen and potash and their combinations at various levels on height of plant in cm at different stages of growth

Treatments	70 DAP	100 DAP	130 DAP	160 DAP	Treatments	70 DAP	100 DAP	130 DAP	160 DAP
N levels					NK combinations				
0	40.00	60.17	68.17	71.83	00	40.00	60.17	68.17	71.83
1	59.72	71.56	73.89	83.44	11	54.00	67.33	71.00	84.00
2	66.56	75.56	79.50	88.50	12	61,50	75.67	78.33	87.83
3	67.94	79.94	87.89	93.94	13	63.67	71.67	72.33	78.50
4	71.17	79.39	86.67	92.06	21	63.67	75.67	80.17	86.83
					22	67.33	75.33	80.50	90.00
C.D.	4.96*	3.31*	4.30*	5.54*	23	68.67	75.67	77.83	88.67
K levels	..	..	..	..	31	65.33	76.00	85.00	91.67
0	40.00	60.17	68.17	71.83	32	62.83	78.83	84.17	93.33
1	62.36	73.79	80.13	87.79	33	75.67	85.00	94.50	96.83
2	65.92	77.63	82.83	90.08	41	66.50	76.17	84.33	88.67
3	70.75	78.42	83.00	90.58	42	72.00	80.67	88.33	89.17
	..	..	..	..	43	75.00	81.33	87.33	98.33
C.D.	4.30	2.87	NS	NS	CD	NS	NS	NS	NS

* Significant at 5 per cent level   NS - Not Significant   DAP - Days after planting

FIGURE 1

Component bar diagram showing the height of plant in cm at different growth stages as influenced by:

NK fertilization:

M_1 - No fertilizer

M_2 - N 50 kg per hectare

M_3 - N 100 kg per hectare

M_4 - N 150 kg per hectare

M_5 - N 200 kg per hectare

M_6 - K_2O 50 kg per hectare

M_7 - K_2O 100 kg per hectare

M_8 - K_2O 150 kg per hectare

DAP - Days after planting

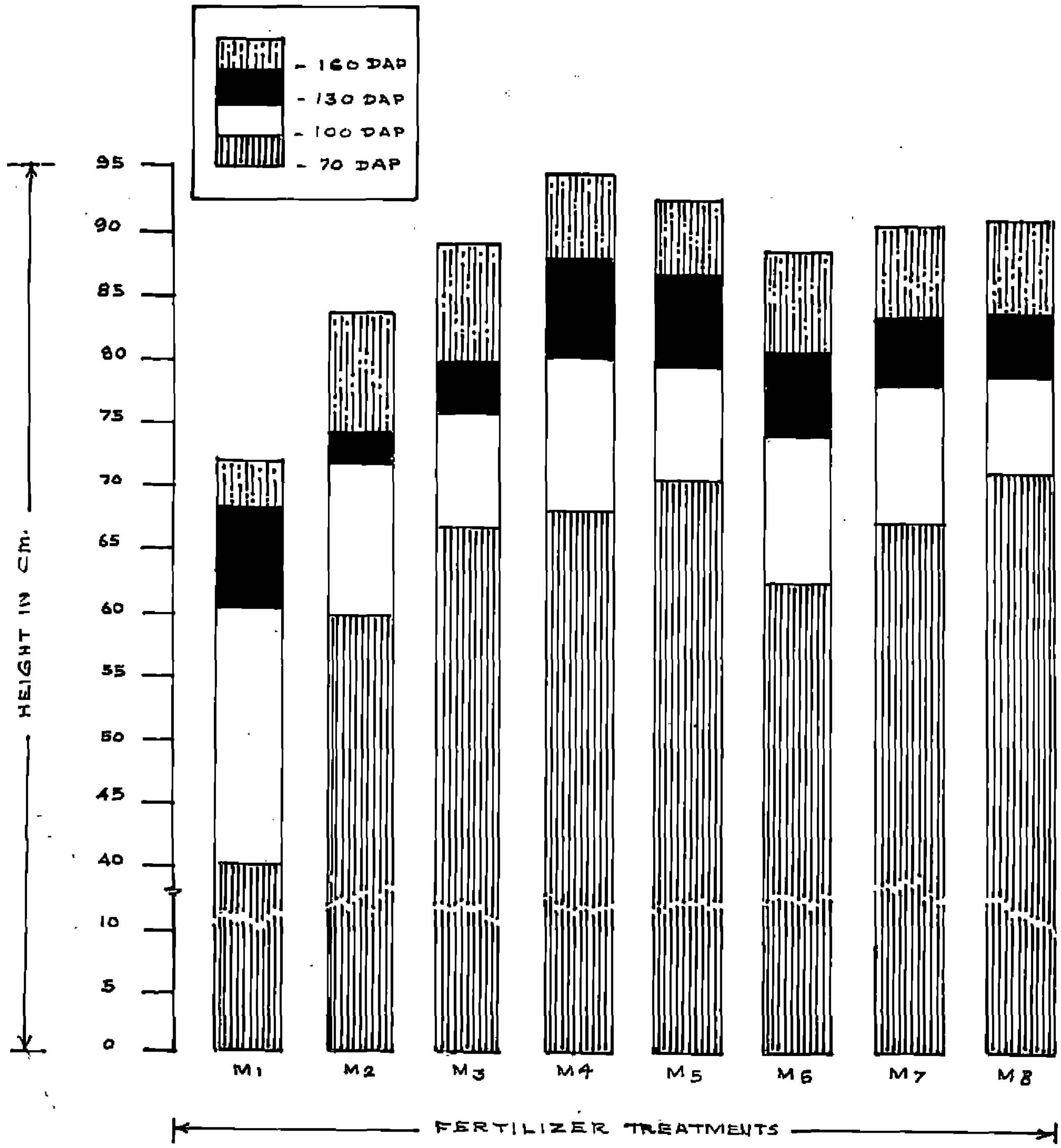

FIG. 1 HEIGHT OF PLANT AT DIFFERENT STAGES OF GROWTH

per hectare showed significant response on the plant height at all stages when compared to N at 50 kg per hectare and control.

The effect of K on plant height was significant only at the early stages ie. at 70th and 100th DAP. Increased plant height on both periods were recorded by the application of 150 kg K per hectare and its effect was on par with K at 100 kg per hectare at 100th DAP. At 130th and 160th DAP also, increased plant height was recorded by the application of K at 150 kg per hectare.

The interaction effect of N and K was not significant at any stage of growth. However the treatment combination n_3k_3 recorded increased plant height at all stages except at 160th DAP on which n_4k_3 recorded maximum plant height (98.33 cm) followed by n_3k_3 (96.83 cm).

Plant height was found to be progressively increasing at all growth stages under all treatments and the maximum plant height (98.33 cm) was recorded at 160th DAP.

2. <u>Girth of stem</u>

The data on the stem girth at different growth stages as affected by the application of various levels of N and K and their combinations are presented in Table 3 and Fig.2.

The data revealed that N had significant response on stem girth at different stages of growth while K and combinations of N and K had no such effect at any stage.

Table 3

Effect of nitrogen and potash and their combinations at various levels on girth of stem in cm at different stages of growth

Treatments	70 DAP	100 DAP	130 DAP	160 DAP	Treatments	70 DAP	100 DAP	130 DAP	160 DAP
N Levels					NK combi-nations				
0	1.70	2.42	2.70	2.76	00	1.70	2.42	2.70	2.76
1	2.56	2.75	2.91	3.13	11	2.42	2.54	2.86	3.10
2	2.89	3.02	3.22	3.31	12	2.75	2.63	3.12	3.17
3	3.07	3.16	3.47	3.48	13	2.52	3.07	2.75	3.13
4	3.15	3.19	3.42	3.43	21	2.65	3.14	3.44	3.17
	..	..	..	..	22	3.07	3.10	3.03	3.44
C.D.	0.18*	0.10*	0.23*	NS	23	2.94	2.81	3.20	3.31
K levels	..	..	..	..	31	2.89	3.10	3.05	3.40
0	1.70	2.42	2.70	2.76	32	3.15	3.08	3.88	3.40
1	2.80	2.96	3.19	3.24	33	3.15	3.30	3.49	3.65
2	3.06	2.99	3.32	3.36	41	3.25	3.12	3.41	3.28
3	2.89	3.13	3.27	3.42	42	3.26	3.14	3.23	3.41
	..	..	..	..	43	2.94	3.33	3.62	3.59
C.D.	0.15*	NS	NS	NS	C.D	NS	0.34*	0.41*	NS

* Significant at 5 per cent level NS - Not significant DAP - Days after planting

FIGURE 2

Curves showing the girth of stem and tap root in cm as affected by:

NK fertilization:

M_1 - No fertilizer

M_2 - N 50 kg per hectare

M_3 - N 100 kg per hectare

M_4 - N 150 kg per hectare

M_5 - N 200 kg per hectare

M_6 - K_2O 50 kg per hectare

M_7 - K_2O 100 kg per hectare

M_8 - K_2O 150 kg per hectare

DAP - Days after planting

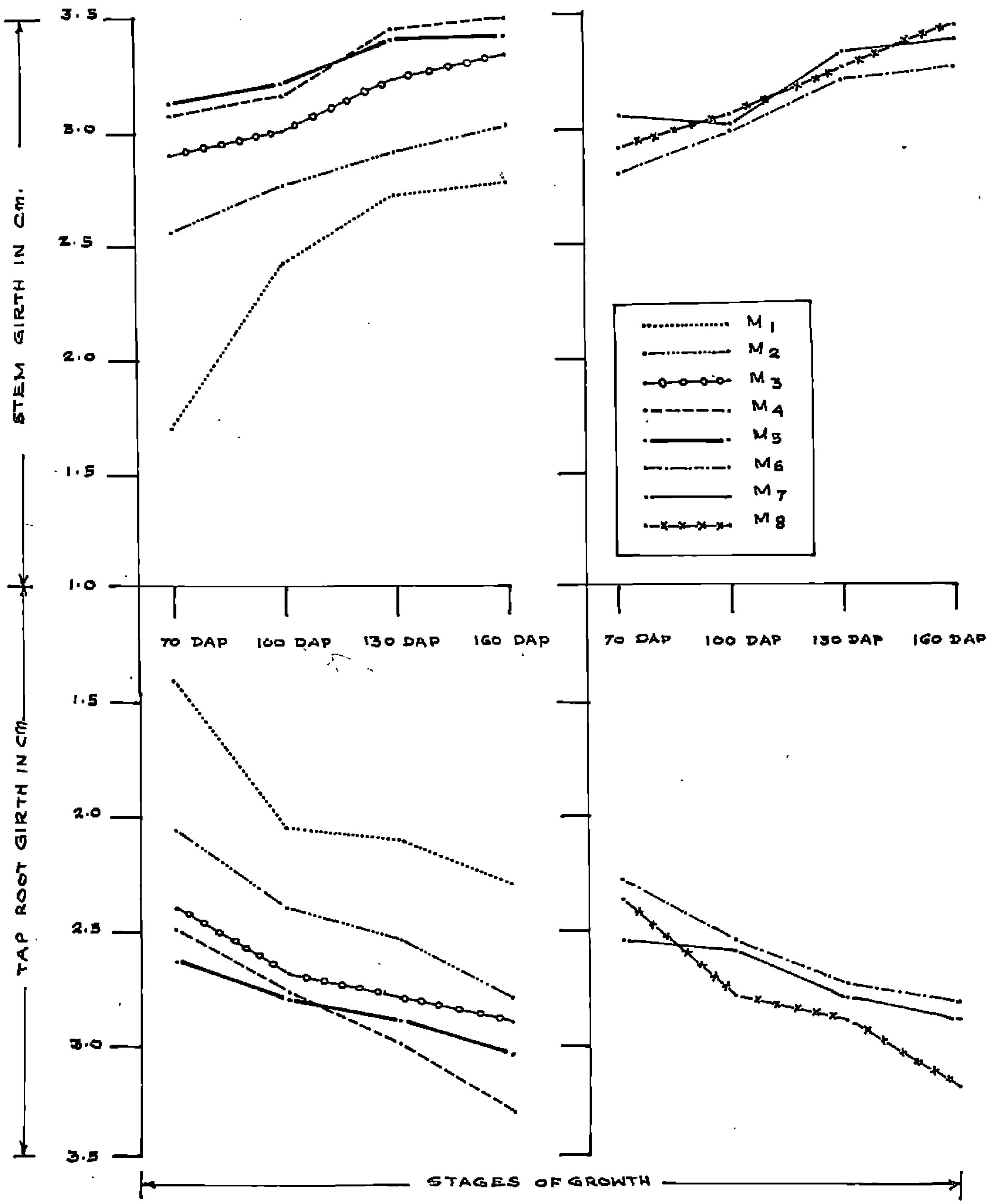

FIG: 2. GIRTH OF STEM AND TAP ROOT AT DIFFERENT STAGES OF GROWTH

Response of stem girth to different levels of N was significant at all stages except at 160th DAP. Application of N at 200 kg and 150 kg per hectare resulted in increased stem girth at all stages and both the treatments were on par in effect. N application at 100 kg per hectare showed significant response at all stages except 160th DAP, over N at 50 kg per hectare and control.

The effect of K on stem girth was not significant at any stage except at 70th DAP. However at 70th and 130th DAP K application at 100 kg per hectare resulted in increased stem girth. At 100th and 160th DAP application of K at 150 kg recorded increased stem girth.

The effect of NK interaction on stem girth was not significant at any stage of growth. However the maximum stem girth (3.88 cm) was attributed by the treatment combination n_3k_2 at 130th DAP.

Stem girth was found to be non-consistent at different stages of growth and the maximum stem girth (3.88 cm) was recorded at 130th DAP.

3. Length of tap root

The data on the length of tap root was influenced by different levels of N and K and their combinations at various growth stages are furnished in Table 4 and Fig.3.

Table 4

Effect of nitrogen and potash and their combinations at various levels on length of tap root in cm at various stages of growth

Treatments	70 DAP	100 DAP	130 DAP	160 DAP	Treatments	70 DAP	100 DAP	130 DAP	160 DAP
N levels					NK combinations				
0	10.33	14.07	14.40	15.37	00	10.33	14.07	14.40	15.37
1	10.06	10.83	11.68	11.87	11	10.63	11.47	11.77	11.75
2	11.31	12.48	13.18	13.57	12	9.33	9.67	11.50	11.73
3	11.09	11.71	12.83	13.76	13	10.20	11.37	11.77	12.15
4	11.50	12.72	15.06	16.22	21	10.87	11.40	12.10	12.53
	..	..	..	..	22	10.17	11.97	12.97	13.27
C.D.	1.09*	1.16*	1.37*	1.28*	23	12.90	14.07	14.47	14.90
K levels	..	..	..	..	31	11.00	12.03	13.00	13.40
0	10.33	14.07	14.40	15.37	32	10.40	11.07	11.50	12.60
1	10.92	11.88	12.52	12.75	33	11.87	12.03	14.00	15.27
2	10.05	10.97	12.88	13.43	41	11.17	12.60	13.20	14.83
3	12.00	12.97	14.17	15.01	42	10.30	11.17	15.53	16.10
	..	..	..	..	43	13.03	14.40	14.63	17.73
C.D.	0.94*	1.00*	1.18*	1.11*	C.D.	NS	NS	NS	NS

* Significant at 5 per cent level   NS - Not significant   DAP - Days after planting

FIGURE 3

Curves showing the length of tap root in cm as influenced by:

NK fertilization:

M_1 - No fertilizer

M_2 - N 50 kg per hectare

M_3 - N 100 kg per hectare

M_4 - N 150 kg per hectare

M_5 - N 200 kg per hectare

M_6 - K_2O 50 kg per hectare

M_7 - K_2O 100 kg per hectare

M_8 - K_2O 150 kg per hectare

DAP - Days after planting

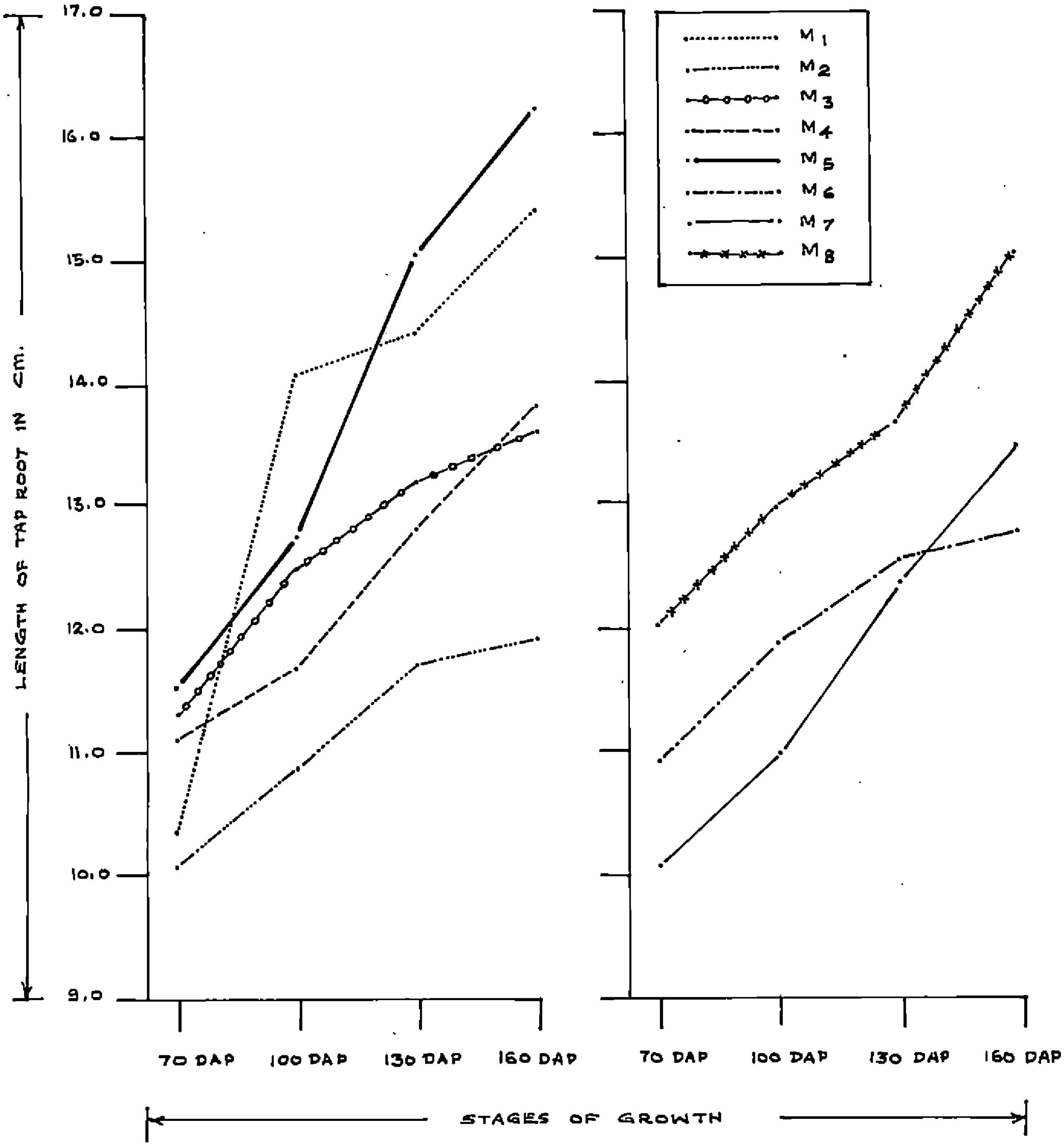

FIG: 3. LENGTH OF TAP ROOT AT DIFFERENT STAGES OF GROWTH

It is observed from the data that only lower levels of N affected significantly the length of tap root. At 100th DAP increased tap root length was recorded by control. At 130th and 160th DAP the effect due to N application at 200 kg per hectare and control was found to be on par.

The effect of K on tap root length was similar to that of N. Increased tap root length at all stages except at 70th DAP, was resulted by control.

The effect of NK interaction on tap root length was not significant at any stage of growth. However increased tap root length was recorded by n_4k_3 at all stages.

The length of tap root was found to be progressively increasing at all the stages under all treatments and the maximum root length (17.73 cm) was recorded at 160th DAP.

4. Girth of tap root

The data on the influence of N and K at different levels and their combinations on the girth of tap root at different stages of growth are presented in Table 5 and Fig.2.

It is evident from the data that N at higher levels had no significant influence. However tap root girth showed an increasing trend with increased levels of N except at 130th and 160th DAP.

Table 5

Effect of nitrogen and potash and their combinations at various levels on mean girth of tap root in cm at different stages of growth

Treatments	70 DAP	100 DAP	130 DAP	160 DAP	Treatments	70 DAP	100 DAP	130 DAP	160 DAP
N Levels					NK combinations				
0	1.43	2.05	2.10	2.29	00	1.43	2.05	2.10	2.29
1	2.06	2.38	2.54	2.83	11	1.88	2.29	2.44	2.68
2	2.42	2.69	2.78	2.88	12	2.37	2.32	2.66	2.74
3	2.51	2.76	3.00	3.28	13	1.92	2.52	2.52	3.06
4	2.62	2.80	2.93	3.04	21	2.18	2.63	2.84	2.84
	..	..	..	..	22	2.56	2.80	2.80	2.89
C.D.	0.16*	0.25*	0.24*	0.30*	23	2.52	2.64	2.72	2.91
K Levels	..	..	..	..	31	2.39	2.59	2.74	2.84
0	1.43	2.05	2.10	2.29	32	2.67	2.65	2.97	3.13
1	2.31	2.58	2.74	2.87	33	2.48	3.03	3.30	3.88
2	2.55	2.63	2.81	2.93	41	2.80	2.79	2.94	3.12
3	2.35	2.76	2.90	3.23	42	2.59	2.76	2.80	2.95
	..	..	..	..	43	2.48	2.84	3.06	3.05
C.D.	0.14*	NS	NS	0.26*	C.D.	0.29*	NS	NS	NS

* Significant at 5 per cent level NS - Not significant DAP - Days after planting

The effect of K on tap root girth was significant at 70th and 160th DAP. At all the stages except 70th DAP, application of K at 150 kg per hectare recorded increased tap root girth. Girth of tap root showed an increasing trend with increased levels of K except at 70th DAP.

The influence of NK interaction on girth of tap root was not significant at any stage. However increased tap root girth was resulted by the treatment n_3k_3 at all stages except at 70th DAP.

In general, tap root girth showed a progressively increasing trend at all stages of growth and the maximum (3.88cm) was recorded at 160th DAP.

5. Girth of lateral roots

The data presented in Table 6 revealed that neither N, K nor NK interaction had any significant influence on the girth of lateral roots at any stage of growth. However, higher levels of N and K recorded geater lateral root girth than their lower levels at all stages of growth except at 70th DAP.

Girth of lateral roots was found to be non-consistent at different stages of growth. An increasing trend was observed only upto 130th DAP afterwards it declined slightly. Maximum lateral root girth (1.80 cm) was observed at 130th DAP.

Table 6

Effect of nitrogen and potash and their combinations at various levels on girth of lateral roots in cm at different stages of growth

Treatments	70 DAP	100 DAP	130 DAP	160 DAP	Treatments	70 DAP	100 DAP	130 DAP	160 DAP
N Levels					NK combinations				
0	0.56	0.97	1.02	1.07	00	0.56	0.97	1.02	1.07
1	0.88	1.17	1.32	1.15	11	0.86	1.12	1.18	1.13
2	1.13	1.36	1.44	1.26	12	0.91	1.18	1.31	0.95
3	1.19	1.45	1.54	1.28	13	0.86	1.20	1.47	1.39
4	1.08	1.38	1.62	1.35	21	1.14	1.29	1.42	1.26
	..	..	..	..	22	1.07	1.52	1.54	1.26
C.D.	0.15*	NS	NS	NS	23	1.18	1.26	1.34	1.26
K Levels	..	..	..	..	31	1.07	1.26	1.38	1.31
0	0.56	0.97	1.02	1.07	32	1.33	1.48	1.54	1.28
1	1.06	1.22	1.35	1.27	33	1.18	1.62	1.70	1.23
2	1.10	1.33	1.51	1.20	41	1.16	1.23	1.44	1.36
3	1.05	1.46	1.58	1.31	42	1.10	1.16	1.63	1.31
	..	..	..	..	43	0.99	1.74	1.80	1.36
C.D.	NS	NS	NS	NS	C.D.	NS	NS	NS	NS

* Significant at 5 per cent level NS - Not significant DAP - Days after planting

6. Number of roots per plant

It is clear from the data presented in Table 7 that application of different levels of N or K or their combinations, had no significant influence on the production of roots in the plant. However application of N at 100 kg per hectare resulted in more number of roots per plant at all stages except at 70th DAP than N at higher levels. Similarly application of K at 50 kg per hectare produced more number of roots per plant than K at higher levels, at all stages. Treatment n_2k_1 recorded maximum number of roots per plant (30.0) at 160th DAP.

Number of roots per plant was found to be increasing with the age of the plant and maximum number (30.0) was obtained at 160th DAP.

B. Effect on yield attributes

1. Leaf yield

The data on the effect of different levels of N and K and their combinations on the leaf yield and percentage dry matter content at different stages of growth are given in Table 8, 9 and 10 and Fig. 4 and 6.

In general, the effect of N was significant at all stages in increasing fresh leaf yield, dry leaf yield and percentage dry matter content.

Table 7

Effect of nitrogen and potash and their combinations at various levels on the production of roots (expressed as number of roots per plant) at different stages of growth

Treatments	70 DAP	100 DAP	130 DAP	160 DAP	Treatments	70 DAP	100 DAP	130 DAP	160 DAP
N Levels					NK combinations				
0	9.00	11.67	14.33	14.50	00	9.00	11.67	14.33	14.50
1	17.39	18.72	22.94	23.83	11	18.33	19.33	24.67	24.67
2	15.78	20.39	26.17	28.11	12	20.17	21.00	24.83	25.00
3	13.61	17.83	22.11	25.33	13	13.67	15.83	19.33	21.83
4	16.17	19.17	22.56	25.78	21	18.67	24.33	26.67	30.00
	..	..	..	..	22	14.33	20.17	27.00	28.00
C.D.	NS	NS	NS	NS	23	14.33	16.67	24.83	26.33
K Levels	..	..	..	..	31	15.17	19.83	24.67	27.50
0	9.00	11.67	14.33	14.50	32	12.83	19.33	22.17	25.33
1	16.33	20.08	24.21	26.96	33	12.83	14.33	19.50	23.17
2	15.25	19.08	23.75	25.42	41	13.17	16.83	20.83	25.67
3	15.63	17.92	22.38	24.92	42	13.67	15.83	21.00	23.33
	..	..	..	..	43	21.67	24.83	25.83	28.33
C.D.	NS	NS	NS	NS	C.D.	4.78*	4.74*	NS	NS

* Significant at 5 per cent level NS - Not significant DAP - Days after planting

Table 8

Effect of nitrogen and potash and their combinations at various levels on the fresh weight of leaves in g per plant at different stages of growth

Treatments	70 DAP	100 DAP	130 DAP	160 DAP	Treatments	70 DAP	100 DAP	130 DAP	160 DAP
N Levels					NK combinations				
0	13.667	15.667	12.500	25.000	00	13.667	15.667	12.500	25.000
1	36.222	33.944	20.278	36.472	11	35.833	28.500	15.000	39.333
2	71.167	43.000	27.389	31.833	12	34.167	27.167	21.500	33.167
3	86.833	54.444	33.944	37.333	13	38.667	46.167	24.333	36.917
4	84.444	55.167	33.111	34.444	21	70.667	53.000	28.667	24.667
	..	..	..	..	22	71.833	41.833	30.333	42.833
C.D.	10.590*	9.564*	8.411	NS	23	71.000	34.167	23.167	28.000
K Levels	..	..	..	..	31	73.833	45.333	32.833	29.833
0	13.667	15.667	12.500	25.000	32	95.667	59.000	41.500	42.833
1	64.375	45.917	23.542	30.625	33	91.00	59.000	27.500	39.333
2	72.542	43.708	31.208	37.208	41	77.167	56.833	17.667	28.667
3	72.083	50.292	31.292	37.229	42	88.500	46.833	31.500	30.000
	..	..	..	..	43	87.667	61.833	50.167	44.667
C.D	NS	NS	NS	NS	C.D.	18.343*	16.566*	14.569*	12.296*

* Significant at 5 per cent level. NS - Not significant DAP - Days after planting

Table 9

Effect of nitrogen and potash and their combinations at various levels on the dry weight of leaves in g per plant at different stages of growth

Treatments	70 DAP	100 DAP	130 DAP	160 DAP	Treatments	70 DAP	100 DAP	130 DAP	160 DAP
N Levels					NK combinations				
0	2.633	3.250	2.810	5.653	00	2.633	3.250	2.810	5.653
1	7.062	7.368	4.596	8.454	11	6.980	6.153	3.377	9.187
2	13.986	9.346	6.212	7.403	12	6.653	5.867	4.880	7.700
3	17.153	11.646	7.996	8.816	13	7.553	10.083	5.530	8.477
4	16.766	11.954	7.506	8.371	21	13.873	11.547	6.647	5.660
	..	..	..	..	22	14.110	9.103	6.723	9.917
C.D.	2.076*	2.074*	2.027*	NS	23	13.973	7.387	5.267	6.633
K Levels	..	..	..	..	31	14.557	9.883	7.507	7.147
0	2.633	3.250	2.810	5.653	32	18.873	12.417	10.263	10.057
1	12.683	9.940	5.396	7.178	33	18.030	12.637	6.217	9.243
2	14.300	9.423	7.270	8.714	41	15.323	12.177	4.053	6.717
3	14.242	10.872	7.066	8.892	42	17.563	10.307	7.213	7.183
	..	..	..	..	43	17.410	13.380	11.250	11.213
C.D.	NS	NS	NS	1.323*	C.D.	NS	3.592*	3.512*	2.646*

* Significant at 5 per cent level NS - Not significant DAP - Days after planting

Table 10

Effect of nitrogen and potash and their combinations at various levels on the dry matter content of leaves (expressed as percentage) at different stages of growth

Treatments	70 DAP	100 DAP	130 DAP	160 DAP	Treatments	70 DAP	100 DAP	130 DAP	160 DAP
N Levels					NK combinations				
0	19.25	20.75	22.40	22.52	00	19.25	20.75	22.40	22.52
1	19.51	21.68	22.66	23.19	11	19.47	21.60	22.51	23.36
2	19.64	21.73	22.70	23.30	12	19.52	21.61	22.69	23.23
3	19.75	21.44	23.33	23.67	13	19.53	21.83	22.78	22.97
4	19.85	21.69	22.76	24.53	21	19.62	21.79	23.20	22.99
	..	..	..	..	22	19.62	21.76	22.47	23.15
C.D.	0.08*	NS	NS	NS	23	19.67	21.63	22.73	23.77
K Levels	..	..	..	..	31	19.72	21.81	23.14	23.93
0	19.25	20.75	22.40	22.52	32	19.72	21.07	24.23	23.46
1	19.67	21.66	22.95	22.44	33	19.81	21.43	22.61	23.63
2	19.68	21.61	22.99	23.46	41	19.86	21.46	22.94	23.47
3	19.72	21.63	22.65	24.12	42	19.84	21.98	22.87	24.02
	..	..	..	..	43	19.86	21.64	22.48	26.11
C.D.	NS	NS	NS	NS	C.D.	NS	NS	NS	NS

* Significant at 5 per cent level NS - Not significant DAP - Days after planting

FIGURE 4

Bar diagram showing the dry weight of leaves in g per plant at different growth stages as influenced by:

NK fertilization:

M_1 - No fertilizer

M_2 - N 50 kg per hectare

M_3 - N 100 kg per hectare

M_4 - N 150 kg per hectare

M_5 - N 200 kg per hectare

M_6 - K_2O 50 kg per hectare

M_7 - K_2O 100 kg per hectare

M_8 - K_2O 150 kg per hectare

M_9 - N 50, K_2O 50 kg per hectare

M_{10} - N 50, K_2O 100 kg per hectare

M_{11} - N 50, K_2O 150 kg per hectare

M_{12} - N 100, K_2O 50 kg per hectare

M_{13} - N 100, K_2O 100 kg per hectare

M_{14} - N 100, K_2O 150 kg per hectare

M_{15} - N 150, K_2O 50 kg per hectare

M_{16} - N 150, K_2O 100 kg per hectare

M_{17} - N 150, K_2O 150 kg per hectare

M_{18} - N 200, K_2O 50 kg per hectare

M_{19} - N 200, K_2O 100 kg per hectare

M_{20} - N 200, K_2O 150 kg per hectare

DAP - Days after planting

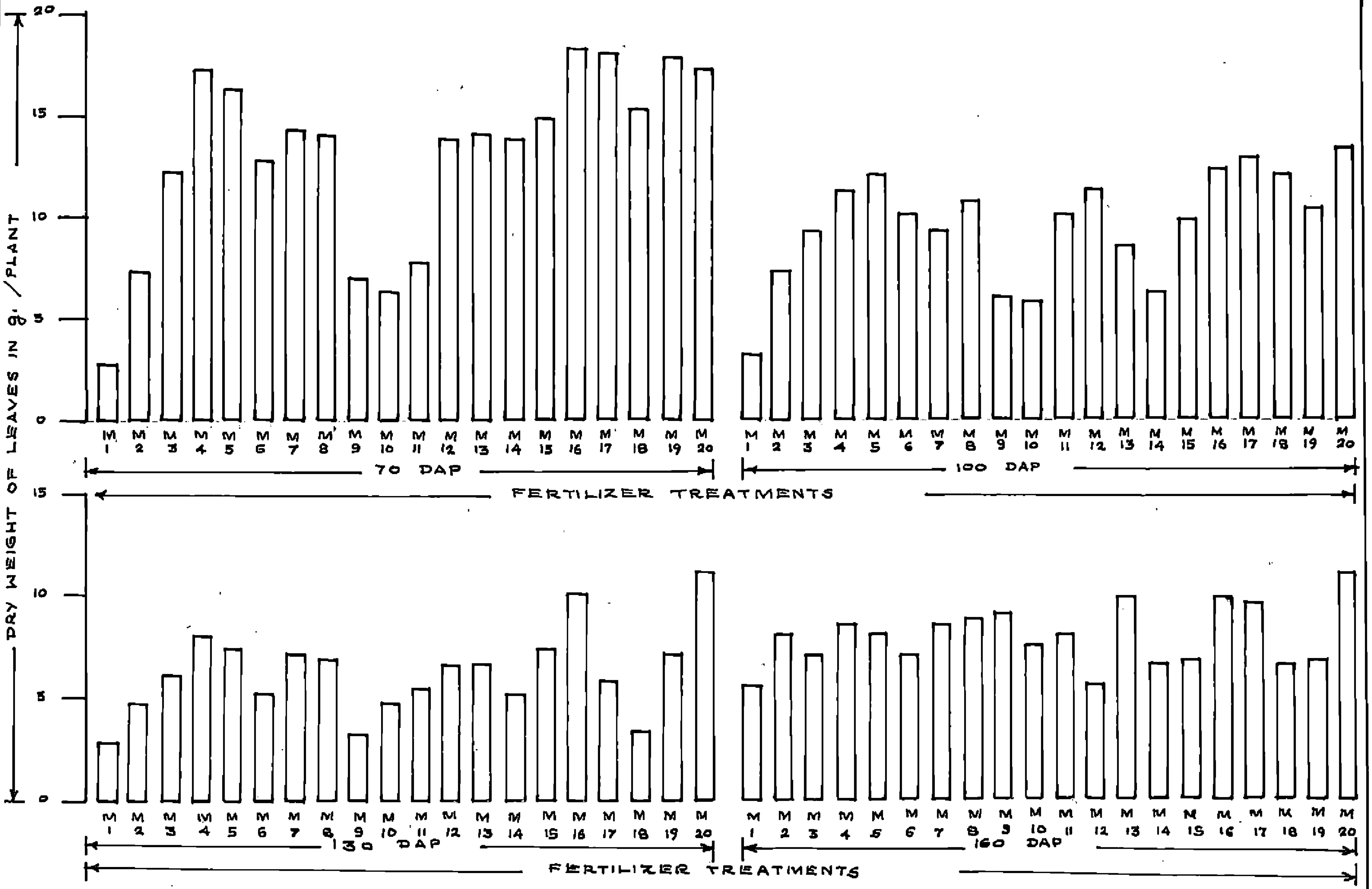

FIG: 4 DRY WEIGHT OF LEAVES AT DIFFERENT STAGES OF GROWTH

Application of N at 150 kg per hectare resulted in increased fresh and dry weight of leaves at all stages except at 100th DAP. The higher levels of N significantly increased fresh and dry leaf yield at all stages except at 160th DAP, when compared to N at 50 kg per hectare and control. The higher levels of N also resulted in slightly increased dry matter content at all stages as compared to N at lower levels. Application of N at 200 kg and 150 kg per hectare recorded increased dry matter content at all stages except at 100th DAP.

Application of K had no significant effect on fresh and dry leaf yield and percentage dry matter content. However application of increased doses of K resulted in increased fresh leaf yield, dry leaf yield and percentage dry matter content in most cases. Application of K at higher levels ie. at 150 kg and 100 kg per hectare recorded increased fresh and dry leaf yield except at 100th DAP. Effect of K on the dry matter content of leaves did not show a set pattern. At 130th and 160th DAP K application at 100 kg and 150 kg per hectare respectively gave increased dry matter percentage.

Interactions of NK, in general, showed significant effect on fresh and dry leaf yield. Treatment n_4k_3 recorded increased fresh and dry leaf yield at all stages except at 70th DAP. However the maximum fresh and dry leaf yield per plant

(95.667 g and 18.873 g respectively) was obtained at 70th DAP by the treatment n_3k_2. The interaction effect of N and K did not influence the percentage dry matter content in leaves significantly. Increased dry matter percentage was attributed by treatments n_4k_1, n_4k_2 and n_4k_3 respectively at 70th, 100th and 160th DAP.

Fresh and dry leaf yield at different stages of plant growth showed a decreasing trend with the age of the plant. Maximum fresh and dry leaf yield per plant (95.667g and 18.873g respectively) was recorded at 70th DAP afterwards it declined gradually upto 130th DAP. At 160th DAP a slight increase from that of 130th DAP was noted. The percentage of dry matter content of leaves showed slightly increasing trend at different stages from 70th DAP to 160th DAP and the maximum (26.11 per cent) was recorded at 160th DAP.

2. <u>Shoot yield</u>

The data presented in Table 11 and 12 and Fig.5 showed that application of N and K had significant influence on the fresh and dry shoot yield whereas NK interaction had no significant effect.

The higher levels of N (200 kg and 150 kg per hectare) and K (150 kg and 100 kg per hectare) significantly increased the fresh and dry shoot yield at all stages, when compared to

Table 11

Effect of nitrogen and potash and their combinations at various levels on the fresh weight of shoot in g per plant at different stages of growth

Treatments	70 DAP	100 DAP	130 DAP	160 DAP	Treatments	70 DAP	100 DAP	130 DAP	160 DAP
N Levels					NK combinations				
0	11.500	32.166	36.833	39.166	00	11.500	32.166	36.833	39.166
1	42.722	57.833	69.389	76.250	11	36.833	46.833	63.833	71.250
2	66.944	78.667	86.417	85.056	12	41.167	59.000	71.500	76.583
3	80.556	90.722	114.611	108.611	13	50.167	67.667	72.833	80.917
4	81.556	102.500	107.500	111.889	21	57.667	77.333	90.333	78.833
	..	..	..	..	22	60.167	84.500	90.167	93.833
C.D.	9.284*	12.924*	16.733*	17.365*	23	83.000	74.167	78.750	82.500
K Levels	..	..	..	..	31	68.833	84.667	89.333	98.333
0	11.500	32.166	36.833	39.166	32	80.667	86.333	114.667	115.167
1	59.292	77.458	87.708	90.771	33	92.167	101.167	139.833	112.333
2	66.333	82.958	95.875	98.729	41	73.833	101.000	107.333	114.667
3	78.208	86.875	99.854	96.854	42	83.333	102.000	107.167	109.333
	..	..	..	..	43	87.500	104.500	108.000	111.667
C.D.	8.041*	NS	NS	NS	C.D.	NS	NS	NS	NS

* Significant at 5 per cent level NS - Not significant DAP - Days after planting

Table 12

Effect of nitrogen and potash and their combinations at various levels on the dry weight of shoot in g per plant at different stages of growth

Treatments	70 DAP	100 DAP	130 DAP	160 DAP	Treatments	70 DAP	100 DAP	130 DAP	160 DAP
N Levels					NK combinations				
0	2.763	8.180	9.676	10.413	00	2.763	8.180	9.676	10.413
1	10.678	14.997	18.189	21.344	11	9.157	12.167	16.920	20.023
2	16.513	20.204	22.784	23.933	12	10.293	15.310	18.713	21.480
3	19.881	23.420	30.708	30.374	13	12.583	17.513	18.933	22.530
4	20.107	26.474	28.254	29.717	21	14.280	19.967	23.927	22.040
	..	..	..	..	22	14.860	21.550	23.907	26.490
C.D.	2.249*	3.297*	4.443*	4.798*	23	20.400	19.097	20.520	23.270
K Levels	..	..	..	..	31	16.897	21.810	23.807	27.380
0	2.763	8.180	9.676	10.413	32	20.157	22.173	30.863	32.313
1	14.665	19.997	23.253	25.828	33	22.590	26.277	37.453	31.347
2	16.458	21.323	25.452	26.205	41	18.327	26.043	28.357	33.870
3	19.261	22.502	26.248	26.973	42	20.523	26.260	28.323	24.537
	..	..	..	..	43	21.470	27.120	28.083	30.745
C.D.	1.948*	NS	NS	NS	C.D.	NS	NS	NS	NS

* Significant at 5 per cent level NS - Not significant DAP - Days after planting

FIGURE 5

Bar diagram showing the dry weight of shoot and root in g per plant at different stages of growth, as affected by:

NK fertilization:

M_1 - No fertilizer

M_2 - N 50 kg per hectare

M_3 - N 100 kg per hectare

M_4 - N 150 kg per hectare

M_5 - N 200 kg per hectare

M_6 - K_2O 50 kg per hectare

M_7 - K_2O 100 kg per hectare

M_8 - K_2O 150 kg per hectare

DAP - Days after planting

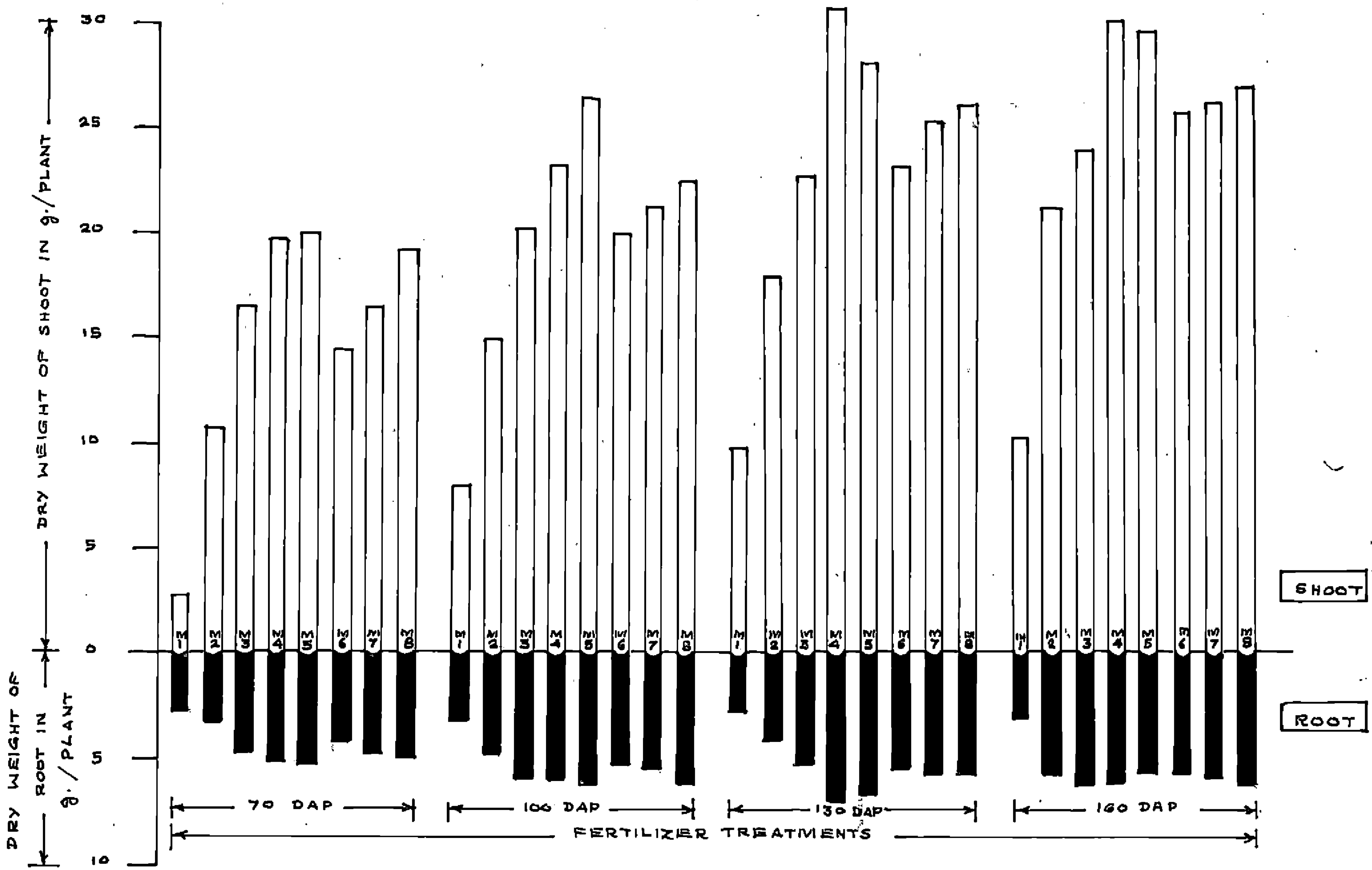

FIG:5 DRY WEIGHT OF SHOOT AND ROOT AT DIFFERENT STAGES OF GROWTH lower levels and control. Increased fresh and dry weight of shoot was recorded by the application of 150 kg N per hectare and 150 kg K per hectare, at 130th and 160th DAP in the case of N and at all stages with respect to K. Still higher fresh shoot yield was obtained, particularly at the early growth stages, with the application of N at 200 kg per hectare. Amongst the various combinations of N and K, treatment n_4k_3 and n_3k_3 attributed increased fresh and dry shoot yield at all stages except at 160th DAP. At 160th DAP n_4k_1 and n_3k_2 resulted in maximum fresh shoot yield (114.667 g and 115.167 g respectively) and dry shoot yield per plant (33.870 g and 32.313 g respectively).

From the data presented in Table 13 and Fig.6 it was seen that different levels of N and K and their combinations showed significant influence on per cent dry matter content of shoot, only at the final stage of growth (160 DAP). The lower levels of N (100 kg and 50 kg per hectare) recorded increased dry matter content in shoot at all stages and at 160th DAP it was significantly superior to N at 150 kg and 200 kg per hectare. Similarly, application of K at 50 kg per hectare resulted in increased dry matter content in shoot when compared to 150 kg per hectare, at all stages of growth except at 100th DAP and the effect was significant at 160th DAP.

FIGURE 6

Pie diagram showing the percentage dry matter content of leaves, shoot and root (at 160th DAP), as influenced by:

NK fertilization:

M_1 - No fertilizer

M_2 - N 50 kg per hectare

M_3 - N 100 kg per hectare

M_4 - N 150 kg per hectare

M_5 - N 200 kg per hectare

M_6 - K_2O 50 kg per hectare

M_7 - K_2O 100 kg per hectare

M_8 - K_2O 150 kg per hectare

DAP - Days after planting

Table 13

Effect of nitrogen and potash and their combinations at various levels on the dry matter content of shoot (expressed as percentage) at different stages of growth

Treatments	70 DAP	100 DAP	130 DAP	160 DAP	Treatments	70 DAP	100 DAP	130 DAP	160 DAP
N Levels					NK combinations				
0	24.03	25.43	26.10	26.53	00	24.03	25.43	26.10	26.53
1	24.97	25.97	26.14	28.00	11	24.85	26.02	26.19	28.12
2	24.71	25.73	26.36	28.11	12	24.99	25.99	26.19	28.04
3	24.69	25.80	26.79	27.96	13	25.06	25.89	25.92	27.84
4	24.65	25.82	26.30	26.57	21	24.82	25.82	26.48	27.96
	..	..	..	..	22	24.69	25.51	26.53	28.22
C.D.	NS	NS	0.43*	0.44*	23	24.62	25.88	26.06	28.14
K Levels	..	..	..	..	31	24.53	25.77	26.06	27.55
0	24.03	25.43	26.10	26.53	32	24.99	25.67	26.91	28.13
1	24.76	25.85	26.44	28.37	33	24.55	25.97	26.77	27.90
2	24.82	25.72	26.51	26.74	41	24.82	25.78	26.44	29.54
3	24.69	25.92	26.20	27.85	42	24.61	25.74	26.42	27.55
	..	..	..	..	43	24.52	25.96	26.03	27.51
C.D.	NS	NS	NS	0.38*	C.D.	NS	NS	NS	0.77*

* Significant at 5 per cent level NS - Not significant DAP - Days after planting

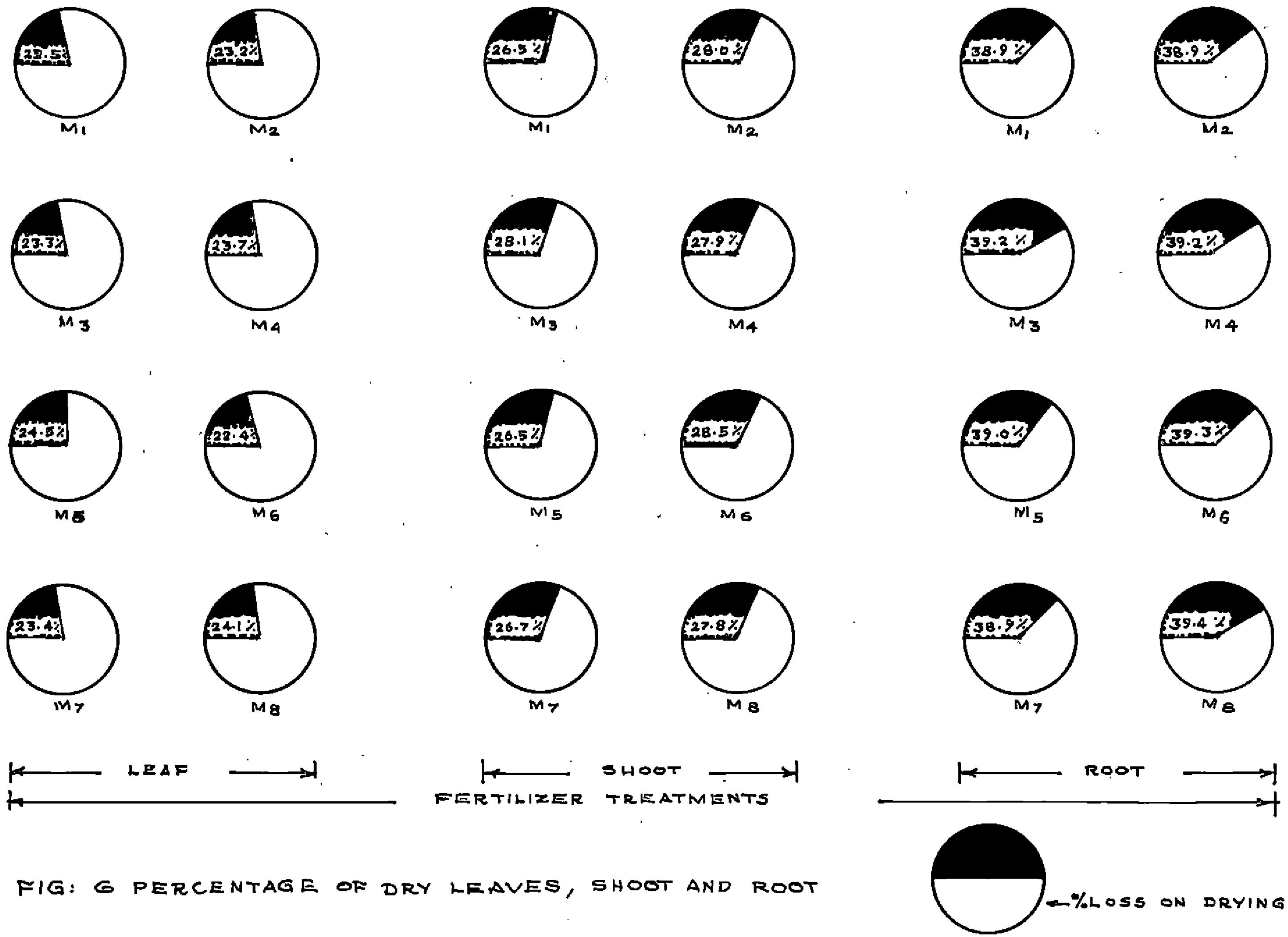

FIG: 6 PERCENTAGE OF DRY LEAVES, SHOOT AND ROOT

The interaction effect of NK on the dry matter percentage in shoot, though not significant, increased with decrease in the levels of N and K. Excepting the final two stages, n_1k_1 and n_2k_2 recorded increased dry matter percentage. However the maximum percentage (29.54) was attributed by n_4k_1 at 160th DAP and it was significantly superior to other treatment combinations.

In general, fresh shoot yield showed an increasing trend from 70th DAP to 130th DAP and at final stage (160th DAP) declined slightly. Maximum fresh shoot yield per plant (139.853 g) was obtained at 130th DAP. The dry shoot yield showed a general trend of progressive increase with increase in the age of plant. However the maximum dry shoot yield per plant (37.453g) was noted at 130th DAP. The dry matter percentage in shoot was also found to be increasing with the age of plant and the maximum percentage (29.54) was recorded at 160th DAP.

3. Root yield

It is evident from the data presented in Table 14 and 15 and Fig.5 that the application of N and K had significant influence on the fresh and dry root yield whereas NK interaction generally had no significant effect.

Applicationof N at higher levels (200 kg, 150 kg and 100 kg per hectare) had significant influence in the fresh and dry root

Table 14

Effect of nitrogen and potash and their combinations at various levels on the fresh weight of root in g per plant at different stages of growth

Treatments	70 DAP	100 DAP	130 DAP	160 DAP	Treatments	70 DAP	100 DAP	130 DAP	160 DAP
N Levels					NK combinations				
0	6.167	9.000	6.667	8.167	00	6.167	9.000	6.667	8.167
1	9.056	12.389	11.000	14.472	11	8.833	10.667	10.333	14.167
2	12.667	16.222	13.556	15.750	12	9.833	10.667	13.000	13.667
3	14.278	15.889	18.722	15.722	13	8.500	15.833	9.667	15.583
4	14.611	16.222	17.056	14.944	21	10.833	15.667	15.667	13.833
	..	..	..	..	22	13.333	17.167	13.333	15.750
C.D.	1.634*	2.065*	2.949*	NS	23	13.833	15.833	11.667	17.667
K Levels	..	..	..	..	31	11.67	15.333	17.167	13.167
0	6.167	9.000	6,667	8.167	32	16.333	13.833	20.167	17.833
1	11.583	14.125	14.500	14.208	33	15.333	18.500	18.833	16.167
2	13.375	14.458	15.458	15.354	41	15.500	14.833	14.833	15.667
3	13.000	16.958	15.292	16.104	42	14.000	16.167	15.333	14.167
	..	..	..	..	43	14.333	17.667	21.000	15.000
C.D.	1.415*	NS	NS	NS	C.D.	2.831*	NS	NS	NS

* Significant at 5 per cent level NS - Not significant DAP - Days after planting

Table 15

Effect of nitrogen and potash and their combinations at various levels on the dry weight of root in g per plant at different stages of growth

Treatments	70 DAP	100 DAP	130 DAP	160 DAP	Treatments	70 DAP	100 DAP	130 DAP	160 DAP
N Levels					NK combinations				
0	2.263	3.437	2.590	3.183	00	2.263	3.437	2.590	3.183
1	3.333	4.654	4.254	5.631	11	3.243	4.017	4.003	5.523
2	4.673	6.128	5.223	6.199	12	3.590	4.037	5.050	5.323
3	5.260	5.969	7.147	6.157	13	3.167	5.910	3.710	6.047
4	5.396	6.109	6.590	5.840	21	4.007	5.887	6.007	5.493
	..	..	..	..	22	4.920	6.533	5.143	6.127
C.D.	0.605*	0.779*	1.130*	NS	23	5.093	5.963	4.520	6.977
K Levels	..	..	..	..	31	4.130	5.647	6.617	5.167
0	2.263	3.437	2.590	3.183	32	6.007	5.210	7.570	6.993
1	4.285	5.276	5.582	5.591	33	5.643	7.050	7.253	6.310
2	4.923	5.478	5.917	5.983	41	5.760	5.553	5.700	6.180
3	4.788	6.392	5.913	6.296	42	5.177	6.130	5.903	5.490
	..	..	..	..	43	5.250	6.643	8.167	5.850
C.D.	0.524*	0.674*	NS	NS	C.D.	1.048*	NS	NS	NS

* Significant at 5 per cent level NS - Not significant DAP - Days after planting

yield at all stages, over N at 50 kg per hectare and control, particularly at early stages of growth. However the higher levels of N were found to be on par in effect with each other.

Application of K at different levels also showed a similar trend as that of N. The higher levels of K (150 kg and 100 kg per hectare) significantly increased the fresh and dry root yield at all stages of growth. Increased fresh root yield of 16.958 g and 16.104 g per plant was obtained at 100th and 160th DAP respectively with the application of K at 150 kg per hectare. Similarly, increased dry root yield of 6.392 g and 6.296 g per plant was obtained at 130th and 160th DAP respectively with the application of K at 150 kg per hectare.

The interaction effect of N and K was not significant at any stage. However treatments n_4k_3 and n_3k_2 generally resulted in increased fresh and dry root yield. Treatment n_4k_3 recorded maximum fresh and dry root yield of 21 g and 8.167 g per plant respectively at 130th DAP.

The data on per cent dry matter content in root presented in Table 16 and Fig.6 indicated that application of N and K had no significant influence on it at any stage of growth except at final stage.

The lower levels of N (100 kg and 50 kg per hectare) recorded slightly increased dry matter content in root at

Table 16

Effect of nitrogen and potash and their combinations at various levels on the dry matter content of root (expressed as percentage) at different stages of growth

Treatments	70 DAP	100 DAP	130 DAP	160 DAP	Treatments	70 DAP	100 DAP	130 DAP	160 DAP
N Levels					NK combinations				
0	36.64	37.60	38.03	38.90	00	36.64	37.60	38.03	38.90
1	36.82	37.63	38.66	38.91	11	36.73	37.66	38.75	38.97
2	36.92	37.76	38.55	39.40	12	36.50	37.91	38.84	38.94
3	36.86	37.65	38.23	39.18	13	37.22	37.31	38.39	38.81
4	36.92	37.66	38.60	39.05	21	37.00	37.55	38.34	39.71
	..	..	..	..	22	36.90	38.06	38.57	38.91
C.D.	NS	NS	NS	0.35*	23	36.88	37.68	38.74	39.56
K Levels	..	..	..	..	31	36.98	36.83	38.54	39.30
0	36.64	37.60	38.03	38.90	32	36.80	37.71	37.66	39.21
1	36.97	37.38	38.52	39.34	33	36.80	38.11	38.49	39.02
2	36.79	37.90	38.39	38.96	41	37.17	37.48	38.45	39.39
3	36.88	37.67	38.62	39.40	42	36.97	37.93	38.50	38.76
	..	..	..	..	43	36.62	37.58	38.86	39.00
C.D.	NS	NS	NS	0.30*	C.D.	NS	NS	NS	NS

* Significant at 5 per cent level NS - Not significant DAP - Days after planting

all stages. However the higher levels of N were on par with lower levels of N in effect. Minimum dry matter percentage was recorded by control at all stages.

With respect to application of K, the lower levels (ie.100 kg and 50 kg per hectare) gave increased dry matter content of root, only at the early stages (70th and 100th DAP) whereas application of K at 150 kg per hectare resulted in maximum root dry matter content of 38.62 and 39.40 per cent at 130th and 160th DAP respectively.

The effect of NK interaction on per cent dry matter content of root, though not significant, increased with decrease in the levels of N and K. Treatments n_1k_3 and n_2k_1 recorded maximum dry matter percentage at 70th and 160th DAP respectively and a maximum of 39.71 per cent was noted at 160th DAP with n_2k_1 application. Except for 70th DAP, combinations of higher levels of N and K ie. n_4k_2 and n_3k_2, resulted in minimum percentage of dry matter content in root.

The fresh and dry root yield generally showed an increasing trend from 70th DAP to 100th DAP and then it slightly declined at 130th DAP and again showed an increasing trend by 160th DAP. However maximum fresh and dry root yield per plant (21 g and 8.167 g respectively) was recorded at 130th DAP but under the influence of the treatment combination of highest dose of N and K. The dry matter percentage in root was found to be increasing with the age of plant and the maximum percentage (39.71) was obtained at 160th DAP.

4. Total alkaloid content

The data showing the influence of N and K at different levels and their combinations on the total crude alkaloid content of plant (root only) at different growth stages are presented in Table 17 and Fig.7.

The data revealed that application of different levels of N and K and their combinations had no significant effect on the percentage of total alkaloid content of the plant.

However application of N and K at higher levels resulted in slightly increased percentage of alkaloid content of plant when compared to their lower levels and control. Application of N at 200 kg and K at 150 kg per hectare gave increased alkaloid percentage at all stages.

Among the different NK combinations n_4k_1 recorded increased percentage of alkaloid in the plant at 100th DAP and 130th DAP. At 70th DAP and 160th DAP n_4k_3 and n_4k_2 respectively recorded increased percentage of alkaloid content.

The percentage of total alkaloid content of the plant was found to be varying at different growth stages. The maximum

Table 17

Effect of nitrogen and potash and their combinations at various levels on the total alkaloid content **(expressed as percentage) of the plant (root only) at different stages of growth

Treatments	70 DAP	100 DAP	130 DAP	160 DAP	Treatments	70 DAP	100 DAP	130 DAP	160 DAP
N Levels					NK combinations				
0	1.95	1.93	1.71	2.05	00	1.95	1.93	1.71	2.05
1	2.03	1.71	2.03	1.95	11	1.69	1.67	1.92	1.96
2	2.12	1.93	1.99	2.03	12	2.24	1.65	2.02	1.96
3	2.10	1.95	1.85	2.08	13	2.17	1.81	2.17	1.94
4	2.15	2.03	2.16	2.20	21	2.04	1.87	2.04	1.83
	..	..	..	..	22	1.97	1.95	1.75	2.05
C.D.	NS	NS	NS	NS	23	2.34	1.95	2.17	2.22
K Levels	..	..	..	..	31	2.21	1.95	1.84	2.29
0	1.95	1.93	1.71	2.05	32	2.02	1.97	2.04	1.98
1	2.00	1.89	2.01	2.06	33	2.09	1.94	1.68	1.98
2	2.07	1.89	2.01	2.08	41	2.06	2.06	2.23	2.15
3	2.24	1.93	2.01	2.07	42	2.03	2.00	2.22	2.32
	..	..	..	..	43	2.37	2.03	2.02	2.14
C.D.	NS	NS	NS	NS	C.D.	NS	NS	NS	NS

* Significant at 5 per cent level NS - Not significant DAP - Days after planting

** On dry weight basis

FIGURE 7

Bar diagram illustrating the total alkaloid content in root (expressed in percentage on dry weight basis) at different growth stages as influenced by:

NK fertilization:

M_1 - No fertilizer

M_2 - N 50 kg per hectare

M_3 - N 100 kg per hectare

M_4 - N 150 kg per hectare

M_5 - N 200 kg per hectare

M_6 - K_2O 50 kg per hectare

M_7 - K_2O 100 kg per hectare

M_8 - K_2O 150 kg per hectare

DAP - Days after planting

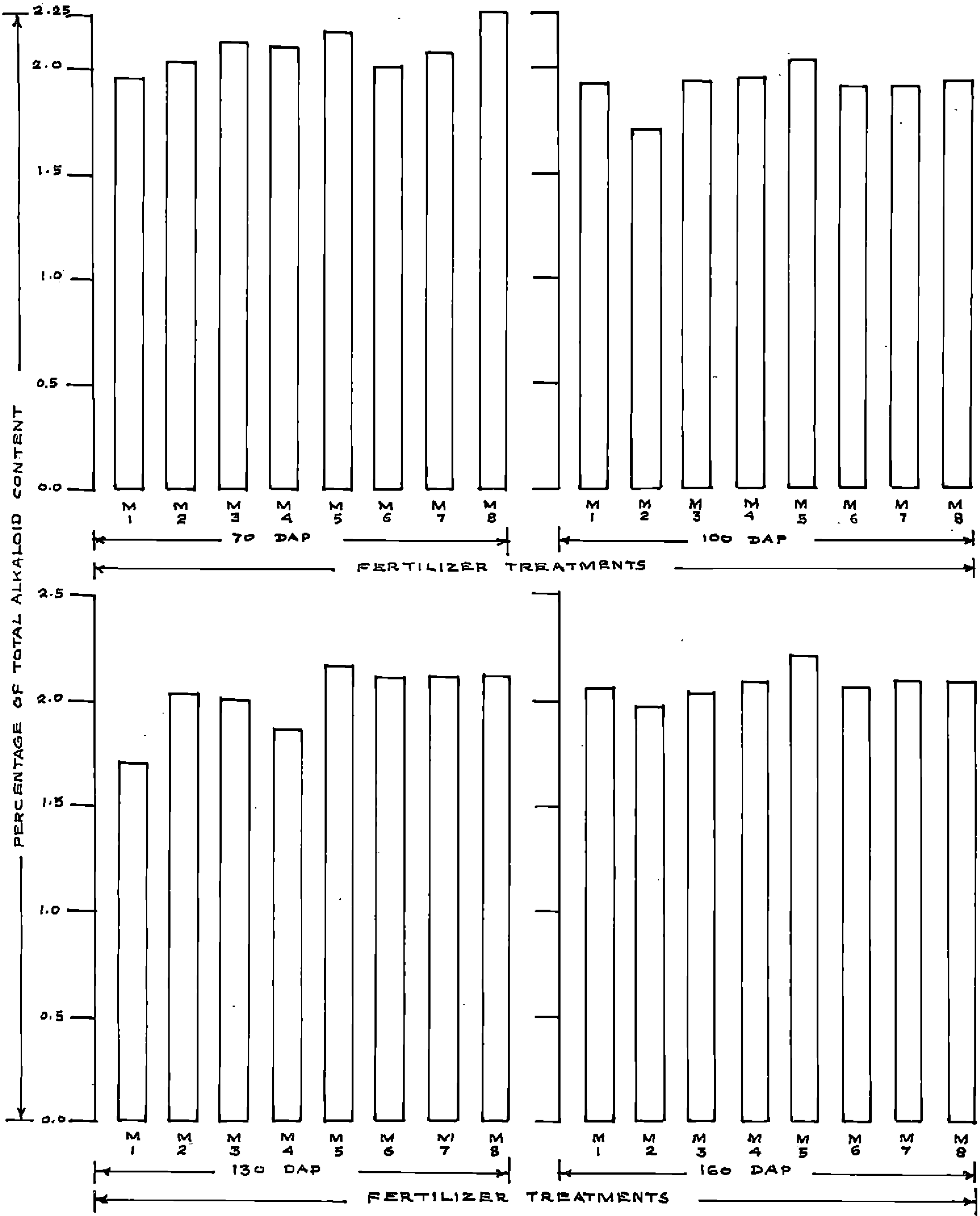

FIG: 7 TOTAL ALKALOID CONTENT IN ROOT

percentage of alkaloid content (2.37) was obtained at peak flowering stage (70th DAP) and it slightly decreased during seed setting stage (70th DAP to 100th DAP) and then gradually increased till the final stage (160th DAP). At 160th DAP the percentage of total alkaloid content in the plant was more or less equal to that of peak flowering stage at 70th DAP.

5. Total alkaloid yield per hectare

The data on total alkaloid yield as influenced by different levels of N and K and NK combinations at various growth stages are given in Table 18 and Fig.8.

It is clear from the data that only N had significant influence on total alkaloid yield per hectare at all stages of growth. Application of K, however, at higher levels in combination with higher levels of N resulted in increased alkaloid yield per hectare.

All the higher levels of N significantly increased total alkaloid yield per hectare at all stages of growth except at 160th DAP, when compared to N at 50 kg per hectare and control. However the higher levels of N were on par with each other in effect at all stages of growth. Increased alkaloid yield was produced by N at 200 kg per hectare followed by N at 150 kg per hectare at all stages except at 100th DAP.

Table 18

Effect of nitrogen and potash and their combinations at various levels on the total alkaloid yield in kg per hectare (on dry weight basis) at different stages of plant growth

Treatments	70 DAP	100 DAP	130 DAP	160 DAP	Treatments	70 DAP	100 DAP	130 DAP	160 DAP
N Levels					NK combinations				
0	3.269	4.914	3.281	4.928	00	3.269	4.914	3.281	4.928
1	4.996	6.007	6.346	8.150	11	4.050	4.950	5.573	8.060
2	7.433	8.801	7.691	9.313	12	5.997	5.067	7.597	7.730
3	8.099	8.621	9.811	9.406	13	4.940	8.003	5.867	8.660
4	8.650	9.190	10.614	9.620	21	6.263	8.280	9.127	7.553
	..	..	..	..	22	7.143	9.390	6.687	9.310
C.D.	1.716*	1.702*	2.642*	NS	23	8.893	8.733	7.260	11.077
K Levels	..	..	..	..	31	6.833	8.150	9.243	8.780
0	3.269	4.914	3.281	4.928	32	8.930	7.593	11.270	10.147
1	6.483	7.475	8.357	8.642	33	8.533	10.120	8.920	9.290
2	7.468	7.786	8.810	9.153	41	8.787	8.520	9.483	10.173
3	7.917	9.203	8.680	9.572	42	7.803	9.093	9.687	9.427
	..	..	..	..	43	9.300	9.957	12.673	9.260
C.D.	NS	NS	NS	NS	C.D.	NS	NS	NS	NS

* Significant at 5 per cent level   NS - Not significant   DAP - Days after planting

FIGURE 8

Bar diagram illustrating the total alkaloid yield (root only) in kg per hectare at different growth stages as affected by:

NK fertilization:

M_1 - No fertilizer

M_2 - N 50 kg per hectare

M_3 - N 100 kg per hectare

M_4 - N 150 kg per hectare

M_5 - N 200 kg per hectare

M_6 - K_2O 50 kg per hectare

M_7 - K_2O 100 kg per hectare

M_8 - K_2O 150 kg per hectare

DAP - Days after planting

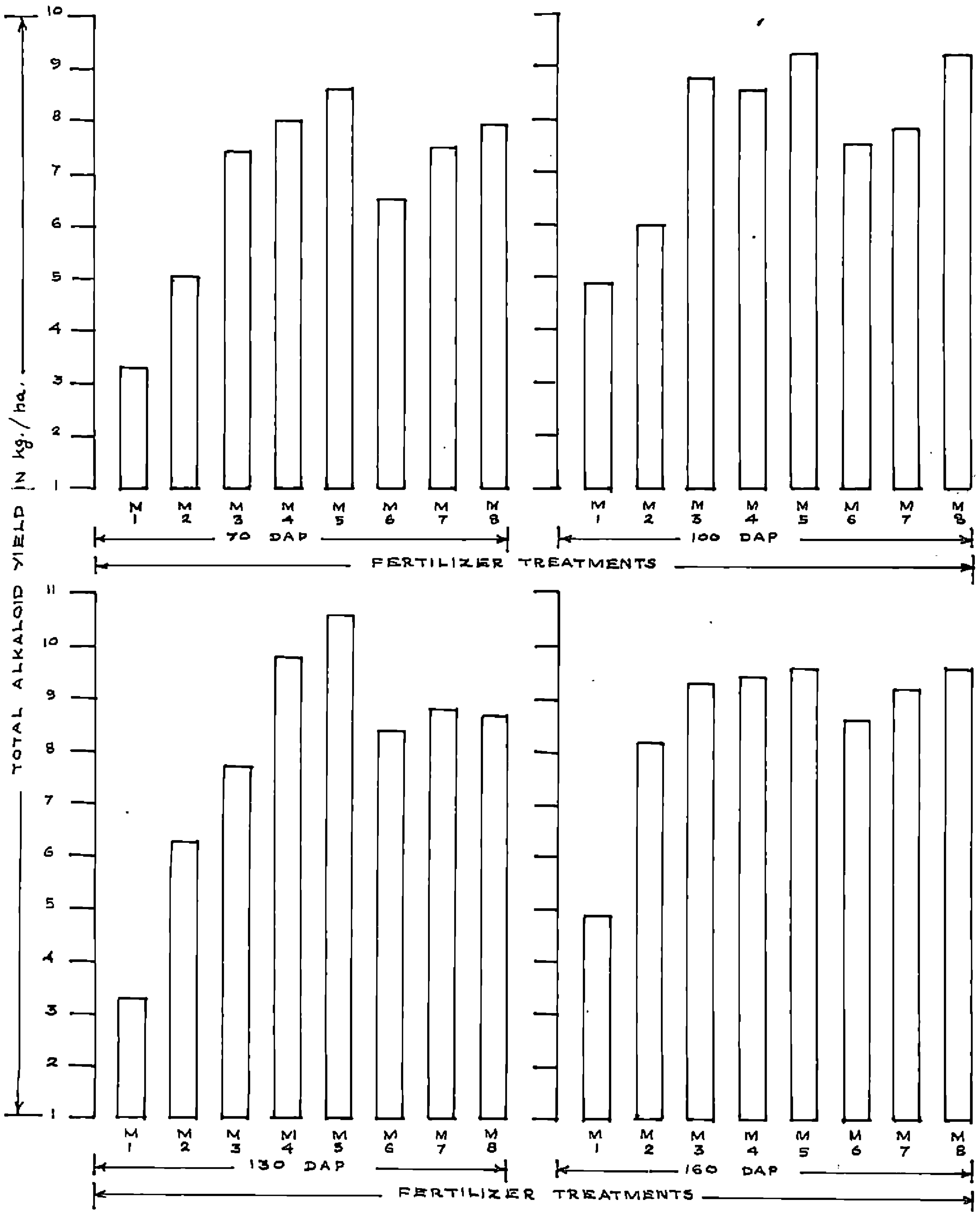

FIG: 8 TOTAL ALKALOID YIELD PER HECTARE

The effect of application of various levels of K was not significant at any stage of growth. However application of K at 150 kg and 100 kg per hectare resulted in higher total alkaloid yield per hectare at all stages compared to K at 50 kg per hectare and control.

The interaction effect of N and K was also not significant at any stage of plant growth. However treatment n_4k_3 recorded maximum alkaloid yield per hectare (12.673 kg) at 130th DAP. At 100th and 160th DAP treatments n_3k_3 and n_2k_3 respectively recorded highest alkaloid yield per hectare (10.120 kg and 11.077 kg respectively). At all stages, minimum alkaloid yield was recorded by control.

The alkaloid yield per hectare was found to be varying at different stages of plant growth. The general trend was an initial increase during the early stages ie. from 70th DAP to 100th DAP and further a decrease till 130th DAP and again an increase towards final stage (160th DAP). However maximum alkaloid yield per hectare (12.673 kg) was obtained at 130th DAP, but under the influence of the treatment combination of highest dose of N and K.

DISCUSSION

DISCUSSION

The isolation of the active principles vinblastine and vincristine from *Catharanthus roseus* (L.) G.Don in 1958 and its great use in modern medical therapy has created a vast demand for its root in the foreign markets. This has given sufficient impetus in India to cultivate *Catharanthus* on a field scale. Information with respect to cultural aspects and manurial requirements of this crop is meagre. Except for a preliminary work at Central Institute of Medicinal and Aromatic Plants, no other systematic work on the effect of manuring on *Catharanthus* has been reported in literature in India.

The results of the present study on effect of graded doses of N and K on the plant as adjudged by plant growth characters and yield characters such as root, shoot and alkaloid yield at various stages of growth are discussed on the following pages.

A. Growth characters

Plant height, as an important growth parameter showed significant response to N at all stages of growth. Application of N at 150 kg per hectare recorded significantly higher plant height at all stages except at 70th DAP. This response of plant height to N even at the later stage (160 DAP) is due to continued vegetative growth as a result of split application of N. It was further observed that the increase in nitrogen level resulted in increased plant height. Similarly Nandi and Chatterjee (1975b) obtained maximum extension growth with N at 150 kg per hectare in Dioscorea sp. It can be explained that larger amounts of N is required for a greater rate of vegetative growth as reflected in the maximum and minimum plant height recorded corresponding to the highest and the lowest quantities of this nutrient applied. Hence it can be concluded that application of N at about 150 kg per hectare was beneficial for this trait.

The plants supplied with graded doses of K showed correspondingly increased plant height at all stages. But the effect of K was significant only at the early stages of growth. This may be due to application of K solely as basal dose during transplanting. Application of higher levels of K (150 kg and 100 kg per hectare) significantly promoted plant height during the early stages of plant growth, when compared to lower level of K (50 kg per hectare), but the effects of both higher levels and lower level were on par, at the later stage. This can be explained that since the nutrient was applied solely as basal dose, it would not have been present in sufficient quantity required for effecting a significantly increased rate of growth at the final stages.

The interaction effect of higher levels of N and K, though not significant, resulted in increased plant height. The treatment combination n_3k_3 promoted plant height at all stages except at 160th DAP. This trait is due to the additive effect of N and K. Similar result was obtained by Prasad (1944) in Datura alba.

Application of higher levels of N significantly increased the girth of stem and increased the girth of tap root and lateral roots at all stages except at the final stage. This can be explained on the basis of accelerated synthetic activities produced by the increased application of nitrogen in building up cells, cell organells and tissues. Abundant supply of nitrogen foods to any actively growing vegetative meristem produces large quantity of protoplasm which encourages growth (Meyer and Anderson, 1952 and Black, 1957).

The effect of K on stem girth, tap root girth and lateral root girth was not significant at any stage of growth, except at the initial stage. However increased doses of K showed an increasing trend of these characters. This also can be attributed to sole basal application of this nutrient. The effect of NK interaction on the above characters was also not significant but their combinations at higher levels recorded an increasing trend of these characters which may be explained as the additive effect of N and K.

Growth characters such as length of tap root and number of roots per plant were found to be increasing with decrease in the levels of N and K. Application of N at 100 kg per hectare and K at 50 kg per hectare stimulated root growth and lateral root production potential. This is in agreement with the findings of Schermeister (1958) in Atropa belladonna.

A study on the growth of Catharanthus roseus at various growth stages, as adjudged by certain growth parameters such as height of plant, girth of stem and length number and girth of roots had revealed that the plant growth was progressively increasing at all stages of growth and the maximum growth with respect to most of the above mentioned characters was obtained at 160th DAP. However, maximum rate of growth was observed at the initial stage (ie., 70th DAP). This may be due to the greater response of younger plants to nutrients and the favourable climatic conditions such as rainfall and high humidity which was present during the month of November (Appendix II). This observation is in conformity with that of Taylor and Farnsworth (1973) in Vinca minor. The slow rate of growth of the plant observed during the rest of the period is attributed to the utilization of metabolic products for seed development and to the dry spell of climate existed during the period. However a slight increase in growth was observed at the harvesting stage and this may be due to the light summer showers obtained during early March (Appendix II).

B. Yield attributes

The effect of N was significant on almost all the yield attributes viz. fresh and dry yield per plant of leaf, shoot and root and total alkaloid yield per hectare. The higher the N applied, the greater the yield obtained.

Application of N at 150 kg and 200 kg per hectare resulted in significantly increased fresh and dry yield of leaves at all stages except 160th DAP when compared to N at 100 kg and 50 kg per hectare. It also resulted in increased dry matter content at all stages. This is due to the increased vegetative growth resulted due to the higher amount of nitrogen applied.

Application of K had no significant effect on fresh and dry leaf yield and percentage dry matter content, whereas NK interactions at higher levels, in general, showed significant effect on fresh and dry leaf yield. This observation is in conformity with the finding of Tsao et al.(1961) in Digitalis lanata that K had very little effect on leaf growth and increased rate of K application gave enhanced yield only in the presence of adequate N.

The higher levels of N (200 kg and 150 kg per hectare) significantly increased the fresh and dry yield of shoot as well as root at all stages when compared to 50 kg N per hectare and control. Similar results were reported on Solanum laciniatum by Bernath and Foldesi (1972) and on Dioscorea sp. by Singh *et al.*(1973a) and Singh *et al.*(1973b). This effect of N even at the later stages of growth is amply substantiated by Russel (1962) that though nitrogen application mainly affects the shoot growth, it has a considerable effect on root growth as well, when the plant is retained in the field for a longer time.

The effect of N on the percentage dry matter content in shoot and root was found to be increasing with the decrease in N levels at all stages of growth. At the time of final harvest, the effect of N at 50 kg and 100 kg per hectare in increasing the dry matter percentage of shoot and root was significantly higher than that of N at 150 kg (n_3) and 200 kg (n_4) per hectare, indicating that higher levels of N such as that of n_3 and n_4 have resulted in increasing slightly the moisture content of the shoot and root tissues. Similarly, Schermeister (1958) observed increased succulence due to high nitrate levels in Atropa belladonna.

The application of K at higher levels (150 kg and 100 kg per hectare) significantly increased the fresh and dry weight of shoot and root and the dry matter percentage of the plant.

NK combinations, however, were not found to have any influence on these characters. The effect of K was more pronounced at the final growth stage (160th DAP) than at the early stages. This response of K is corroborated by the findings of Prasad (1944) in Datura alba.

Application of N and K at higher levels resulted in an increased percentage of alkaloid content of plant when compared to their lower levels. N at 200 kg per hectare and K at 150 kg per hectare gave increased alkaloid percentage at all stages. NK combination, though not significant, recorded maximum percentage of alkaloid content at 70th DAP(2.37) and 160th DAP (2.32) with their highest levels of combination (n_4k_3 and n_4k_2 respectively). This indicates the predominent influence of the interaction between nitrogen and potash on alkaloid content of the plant in root portion. However, the interactional effect of nutrient combinations mentioned above on the alkaloid content was not evident at all the stages of growth.

Understanding the interactional effect of nutrients on a metabolic product like alkaloid, present in a plant like Catharanthus requires a detailed knowledge of the physiology of the plant, which is lacking at present. However, the interactional effects of nitrogen and potash on alkaloid content of root needs special mention, since the effects are concurrently produced at more or less the same stages of growth viz. the peak flowering stage (70th DAP) and final harvesting stage (160th DAP). Similar interactional influence of N and K has been reported in other medicinal plants (Prasad 1944; Brewer and David Hiner, 1950; Alov, 1961).

The total alkaloid yield per hectare was increased significantly by all the higher levels of N at all stages of growth, except at 160th DAP, when compared to N at 50 kg per hectare and control. This suggests that nitrogen is a very important element for alkaloid biogenesis. Similarly Neczypor (1969) observed maximum increase in alkaloid content and composition with N fertilization in Periwinkles.

The yield of alkaloid per hectare increased due to increased dry matter production by nitrogen application. The crude alkaloid yield was 10.614 kg per hectare at 130th DAP and 9.620 kg at 160th DAP with N at 200 kg per hectare, whereas control recorded 3.281 kg and 4.928 kg per hectare respectively at the two stages. This substantial increase in yield may be attributed to increased alkaloid percentage as well as enhanced dry matter production in roots due to N application. This also explains for the slight reduction of alkaloid yield observed at the harvesting stage due to the reduction in dry root yield resulted by the severe drought period which preceded the harvesting stage (Appendix II). Similarly, Nowacki et al.(1976) have reported an increase in quantum of alkaloids in Papaver somniferum, Vinca perenne, Catharanthus roseus and Datura meteloides by nitrogen fertilization.

The effect of K was not significant at any stage in enhancing alkaloid yield per hectare. However, at higher levels in combination with higher levels of N resulted in increased alkaloid yield per hectare. Treatment n_4k_3 recorded a maximum yield of 12.673 kg per hectare at 130th DAP. This may again be attributed to the interaction effect of N and K on the percentage of alkaloid content of root and the complimentary effect of N over K on the higher dry matter production in root.

In nutshell, the results achieved in the present investigation emphasises the predominent effect of N on various growth and yield characters of Catharanthus roseus. The plants treated with N gave the highest growth and yield attributes, except the percentage dry matter content of various vegetative parts. This is evidently due to the succulent growth of roots in the N applied plants in contrast to the non -succulent fibrous roots of the plants under control. Inspite of the greater succulence of the roots, the dry yield of the produce from N treated plantswas still significantly greater. This is due to the very high total yield obtained for N application.

The effect of K was generally noted only on the root. The effect was significant with higher levels of K on many of the growth and yield attributes but with respect to percentage dry matter content of shoot and root and total alkaloid yield per hectare it was significant only at the final stages of growth. This delayed action of K can be attributed to the influence on the translocation process in the plant. Probably,K influences the translocation of the alkaloid from the aerial portion to root,at the final stages of growth. Probably the same effects are operating when K is applied in conjunction with N and this may explain the higher percentage of alkaloid recorded in root of the plants treated with nitrogenous and potassic combinations of fertilizers.

Study on growth phases of Catharanthus roseus in relation to the alkaloid content and yield of the root,is an aspect of practical bearing as it would help to decide the stage at which the plants are to be harvested to get maximum shoot, root and alkaloid yield.

With respect to leaf yield, both fresh and dry leaf yield was more at the early stage (ie.70th DAP). This may be attributed to the greater response of young plants to nutrients and to the favourable climatic conditions prevailed during the period (Appendix-II). Decrease in the leaf yield at the later stages of growth is due to defoliation and absence of rainfall and lesser relative humidity during the period (Appendix II). The fresh and dry yield of shoot increased during the early two stages and decreased slightly at 130th DAP. An increase was noted again at the harvesting stage (160th DAP). This is due to the dry spell of climate prevailed till 130th DAP and the slight increase at 160th DAP is due to the light summer showers obtained during early March. This also explains the similar trend of fresh and dry yield of root.

The percentage alkaloid content in the root of the plant was found to be more at the initial stage of peak flowering (70th DAP). Similar observations has been reported in Catharanthus roseus, Vinca minor and Rauvolfia serpentina by Reda (1978); Mermerska et al.(1976) and Nandi and Chatterjee (1975a) respectively. The decrease in the percentage alkaloid content in the root during the two middle stages (ie. 100th DAP and 130th DAP) is due to the increased utilization of the metabolic products by the aerial parts for seed setting and seed ripening. The increase in the percentage alkaloid content in the root at the final stage (160th DAP), when the flowering and seed setting were comparitively less, makes the above aspect evident. Similarly, Dovrat and Goldschmidt (1978) reported maximum root and alkaloid yields in Catharanthus roseus plants of seven months growth.

As far as the total alkaloid yield per hectare was concerned, it showed similar trend with that of root yield. The substantial increase in alkaloid yield per hectare at the final stage (160th DAP) may be attributed to increased alkaloid percentage as well as enhanced dry matter production in roots at that stage.

SUMMARY

SUMMARY

An experiment was conducted at the Department of Horticulture, College of Agriculture, Vellayani during 1979-80 to study the effects of graded doses of N and K on growth, yield and alkaloid content of Catharanthus roseus (L.) G.Don. The effects were assessed by studying various growth and yield characters such as the height of plant, girth of stem, length and girth of tap root, girth and number of lateral roots, fresh and dry yield and percentage of dry matter content of leaves, shoot and root. Studies were also made regarding the total alkaloid content in root at different stages of growth.

The salient features of the findings are summarised below.

1. The effect of N on plant height was significant at all stages of growth. Application of N at the rate of 150 kg per hectare recorded increased plant height at all stages except at 70th DAP. Application of K influenced the plant height particularly at the early stages of growth. It promoted plant height at all the growth stages when applied at the rate of 150 kg per hectare. Combined effect of N and K did not influence the plant height. The maximum plant height (96.83 cm) was obtained at 160th DAP.

2. Application of N significantly influenced the girth of stem at all stages of growth except at 160th DAP. Higher levels of N (200 kg and 150 kg per hectare) resulted in increased stem girth at all stages and both the treatments were on par in effect. Influence of K on stem girth was not significant at any stage except at 70th DAP. However, K at 150 kg per hectare recorded increased stem girth towards the final stages of growth. The effect of NK interaction on stem girth was not significant at any stage of growth. Maximum stem girth (3.88 cm) was noted at 130th DAP.

3. Neither the higher levels of N, K nor NK interactions did significantly influence the tap root length.when compared to control. Maximum tap root length (17.73 cm) was observed at 160th DAP.

4. Mean girth of tap root was not significantly influenced by N at higher levels (200 kg and 150 kg per hectare). But N at 100 kg per hectare did significantly increase the mean girth of tap root at all stages of growth except at 160th DAP. Effect of K was significant only at 70th and 160th DAP. At all stages except at 70th DAP, application of K at 150 kg per hectare recorded increased tap root girth and it was significant at 160th DAP. The influence of NK interaction on tap root girth was not significant at any stage of growth. Maximum girth of tap root (3.88 cm) was recorded at 160th DAP.

5. The girth of lateral roots was not influenced by either N, K or NK interaction. However, higher levels of N and K were found beneficial in increasing lateral root girth. Maximum lateral root girth (1.80 cm) was observed at 130th DAP.

6. The effects of N, K and NK were not found significantly increasing the number of roots per plant. However, N and K at lower levels (100 kg and 50 kg per hectare respectively) stimulated the production of more number of roots per plant than at higher levels. Maximum number of roots (30.0) was obtained at 160th DAP.

7. The fresh yield, dry yield and percentage dry matter content of leaves were found significantly influenced by N. Applicationof N at 150 kg per hectare significantly increased the fresh and dry yield of leaves at all stages except 100th DAP. Effect of K was not significant at any stage. However, K at 150 kg per hectare resulted in increased fresh yield, dry yield and percentage dry matter content of leaves. Interaction effects of N and K on dry and fresh leaf yield were significant at all stages except at 70th DAP. Treatment n_4k_3 was found beneficial for increased leaf yield at all stages. Maximum fresh and dry leaf yield per plant (95.667g and 18.873 g respectively) was obtained at 70th DAP while maximum percentage dry matter content (26.11) was recorded at 160th DAP.

8. The effects of N and K on fresh and dry shoot yield were significant at all stages of growth. The higher levels of N (200 kg and 150 kg per hectare) and K (150 kg and 100 kg per hectare) significantly increased the fresh and dry shoot yield at all stages and the effects of these levels were on par. The effect of N and K on percentage dry matter content of shoot was significant only at the last stage of growth. Interaction effects of N and K on the yield characters of shoot was not significant at any stage except in the case of percentage dry matter content. Maximum fresh and dry shoot yield per plant (115.167 g and 33.870 g respectively) and percentage dry matter content (29.54) were recorded at 160th DAP.

9. Application of higher levels of N (200 kg, 150 kg and 100 kg per hectare) and K (150 kg and 100 kg per hectare) had significant influence in the fresh and dry root yield at all stages of growth. But the effects of those levels were on par with each other. The interaction effect of N and K was not significant at any stage. However, treatment n_4k_3 recorded maximum fresh and dry root yield of 21 g and 8.167 g per plant respectively at 130th DAP. As far as the per cent dry matter content of root is concerned, no significant influence was observed by the application of N and K and NK combinations at any stage of growth except at 160th DAP. The maximum percentage of dry matter content in root (39.71) was recorded at 160th DAP.

10. The percentage of total alkaloid content of the plant (root only) was not significantly influenced by the application of either N, K or NK combinations. However, application of N and K at higher levels resulted in slightly increased percentage alkaloid content of plant. Amongst the different NK combinations n_4k_1 recorded increased percentage of alkaloid content in the plant at 100th and 130th DAP. The maximum percentage of alkaloid content (2.37 and 2.32) was obtained at the peak flowering stage (70th DAP) and at the last harvest stage (160th DAP) respectively.

11. Significant increase of total alkaloid yield per hectare was obtained at all stages by the application of N. All the higher levels of N (200 kg, 150 kg and 100 kg per hectare) significantly increased total alkaloid yield per hectare at all stages of growth except at 160th DAP and the effects of those levels were on par with each other. The effect of application of K was not significant at any stage of growth. However, K at higher levels in combination with higher levels of N resulted in increased total alkaloid yield per hectare. Treatment n_4k_3 recorded maximum alkaloid yield per hectare (12.673 kg) at 130th DAP.

The results of the present study reveals that Catharanthus roseus crop requires manuring with complete fertilizer having nitrogen as the predominent constituent, since it has predominent influence on the growth of plant, yield and alkaloid content of root. Potash has beneficial effect in enhancing alkaloid content of root. Phosphorus with nitrogen is reported to be essential for intensive root and shoot growth (Dovrat and Goldschmidt,1978). These facts indicate the necessity and possibility of a detailed study in Catharanthus roseus with respect to its optimum and economical requirement of major and minor nutrients. The proper stage of growth for harvest of the crop in order to get maximum root and alkaloid yield is 160 days after transplanting, ie., six to seven months after sowing seeds.

It is evident from the results of the present study that it is feasible to cultivate Catharanthus roseus as a field crop under the agro-climatic conditions of South Kerala.

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* Original not seen.

PLATES

A general view of the standing crop.

A *Catharanthus roseus* plant

APPENDICES

APPENDIX I

Data of soil analysis
(Experimental site)

pH	5.25
Total nitrogen	0.55%
Available nitrogen	0.0057%
Total phosphorus	0.115%
Available phosphorus	0.00148%
Total potash	0.083%
Available potash	0.0009%

APPENDIX II

Meteorological data for the period of crop growth

Month	Mean tempera-ture(°C)	Relative humidity (%)	Total rain-fall (mm)
August 1979	26.25	93.0	4.0
September "	26.44	92.9	7.5
October "	26.45	90.9	1.3
November "	26.05	94.6	10.8
December "	26.10	94.0	1.4
January 1980	25.90	86.0	..
February "	26.75	84.0	..
March "	27.05	86.0	6.0

Source: Department of Agronomy, College of Agriculture, Vellayani.

APPENDIX III

Height of plant at different stages of growth
(Analysis of variance)

Source	df	MS			
		70 DAP	100 DAP	130 DAP	160 DAP
Total	35				
Block	2	11.03	4.42	7.44	4.78
Treatment					
N	3	209.14*	136.50*	385.49*	191.86*
K	2	212.09*	73.42*	31.26	26.59
NK	6	26.61	22.01	44.24	52.25
Error	22	25.82	11.49	19.36	32.20

* Significant at 5% level.

$F_{2,22} = 3.44$

$F_{3,22} = 3.05$

$F_{6,22} = 2.55$

DAP = Days after planting

APPENDIX IV

Girth of stem at different stages of growth
(Analysis of variance)

Source	df	MS			
		70 DAP	100 DAP	130 DAP	160 DAP
Total	35				
Block	2	0.117	0.120	0.063	0.005
Treatment					
N	3	0.608*	0.372*	0.582*	0.216
K	2	0.207*	0.085	0.048	0.100
NK	6	0.063	0.113*	0.273*	0.029
Error	22	0.036	4.144	0.059	0.073

* Significant at 5% level

$F_{2,22} = 3.44$

$F_{3,22} = 3.05$

$F_{6,22} = 2.55$

DAP = Days after planting

APPENDIX V

Length of root at different stages of growth
(Analysis of variance)

Source	df	MS			
		70 DAP	100 DAP	130 DAP	160 DAP
Total	35				
Block	2	2.79	0.35	1.87	1.77
Treatment					
N	3	3.74*	6.53*	17.68*	35.31*
K	2	11.45*	12.03*	9.04*	16.14*
NK	6	1.13	1.92	2.81	1.07
Error	22	1.05	1.42	1.97	1.72

* Significant at 5% level.

$F_{2,22} = 3.44$

$F_{3,22} = 3.05$

$F_{6,22} = 2.55$

DAP = Days after planting

APPENDIX VI

Girth of tap root at different stages of growth
(Analysis of variance)

Source	df	MS			
		70 DAP	100 DAP	130 DAP	160 DAP
Total	35				
Block	2	0.264	0.041	0.066	0.052
Treatment					
N	3	0.538*	0.325*	0.371*	0.378*
K	2	0.191*	0.107	0.077	0.452*
NK	6	0.101*	0.050	0.064	0.192
Error	22	0.030	0.068	0.061	0.098

* Significant at 5% level.

$F_{2, 22} = 3.44$

$F_{3, 22} = 3.05$

$F_{6, 22} = 2.55$

DAP = Days after planting

APPENDIX VII

Girth of lateral roots at different stages of growth

(Analysis of variance)

Source	df	MS			
		70 DAP	100 DAP	130 DAP	160 DAP
Total	35				
Block	2	0.049	0.019	0.118	0.057
Treatment					
N	3	0.169*	0.130	0.157	0.057
K	2	0.010	0.162	0.157	0.037
NK	6	0.025	0.101	0.037	0.040
Error	22	0.242	0.067	0.074	0.024

* Significant at 5% level

$F_{2,22} = 3.44$

$F_{3,22} = 3.05$

$F_{6,22} = 2.55$

DAP = Days after planting

APPENDIX VIII

Number of roots per plant at different stages of growth

(Analysis of variance)

Source	df	MS			
		70 DAP	100 DAP	130 DAP	160 DAP
Total	35				
Block	2	49.01	2.53	7.30	2.42
Treatment					
N	3	22.30	10.18	30.69	28.27
K	2	3.63	14.11	10.92	13.59
NK	6	40.85*	50.57*	22.25	12.82
Error	22	7.99	7.85	10.20	14.70

* Significant at 5% level.

$F_{2,22} = 3.44$

$F_{3,22} = 3.05$

$F_{6,22} = 2.55$

DAP = Days after planting

APPENDIX IX

Fresh weight of leaves per plant
(Analysis of variance)

Source	df	MS			
		70 DAP	100 DAP	130 DAP	160 DAP
Total	35				
Block	2	115.58	556.92	177.09	251.04
Treatment					
N	3	4901.51*	924.12*	358.84*	53.84
K	2	252.65	134.73	237.69	173.91
NK	6	93.41*	277.86*	273.67*	169.28*
Error	22	117.33	95.70	74.02	52.73

* Significant at 5% level.

$F_{2,22} = 3.44$

$F_{3,22} = 3.05$

$F_{6,22} = 2.55$

DAP = Days after planting

APPENDIX X

Dry weight of leaves per plant
(Analysis of variance)

Source	df	MS 70 DAP	MS 100 DAP	MS 130 DAP	MS 160 DAP
Total	35				
Block	2	5.63	24.05	11.74	9.95
Treatment					
N	3	196.37*	41.58*	20.80*	3.28
K	2	10.09	6.47	12.69	10.66*
NK	6	3.66	12.51*	14.95*	10.34*
Error	22	4.51	4.50	4.30	2.44

* Significant at 5% level.

$F_{2,22} = 3.44$

$F_{3,22} = 3.05$

$F_{6,22} = 2.55$

DAP = Days after planting

APPENDIX XI

Per cent of dry matter content in leaves
(Analysis of variance)

Source	df	MS 70 DAP	100 DAP	130 DAP	160 DAP
Total	35				
Block	2	0.297	0.125	0.655	4.255
Treatment					
N	3	0.198*	0.159	0.879	3.334
K	2	0.009	0.010	0.415	1.789
NK	6	0.002	0.227	0.384	1.605
Error	22	0.008	0.170	0.611	2.380

* Significant at 5% level

$F_{2,22} = 3.44$

$F_{3,22} = 3.05$

$F_{6,22} = 2.55$

DAP = Days after planting

APPENDIX XII

Fresh weight of shoot
(Analysis of variance)

Source	df	MS			
		70 DAP	100 DAP	130 DAP	160 DAP
Total	35				
Block	2	210.51	192.38	16.63	155.74
Treatment					
N	3	2944.39*	3272.17*	3808.09*	2760.41*
K	2	1096.88*	268.53	460.10	207.72
NK	6	60.72	133.81	552.08	103.73
Error	22	90.19	174.66	292.93	315.48

* Significant at 5% level

$F_{2,22} = 3.44$
$F_{3,22} = 3.05$
$F_{6,22} = 2.55$

DAP = Days after planting

APPENDIX XIII

Dry weight of shoot
(Analysis of variance)

Source	df	MS			
		70 DAP	100 DAP	130 DAP	160 DAP
Total	35				
Block	2	14.26	11.23	3.75	9.45
Treatment					
N	3	173.97*	216.60*	283.41*	174.61*
K	2	64.38*	18.85	28.88	4.08
NK	6	3.76	8.96	42.04	34.91
Error	22	5.29	11.37	20.66	24.08

* Significant at 5% level.

$F_{2,22} = 3.44$

$F_{3,22} = 3.05$

$F_{6,22} = 2.55$

DAP = Days after planting

APPENDIX XIV

Per cent dry matter content in shoot
(Analysis of variance)

Source	df	MS			
		70 DAP	100 DAP	130 DAP	160 DAP
Total	35				
Block	2	0.118	0.152	0.967	0.038
Treatment					
N	3	0.184	0.086	0.735*	5.026 *
K	2	0.056	0.120	0.335	8.337*
NK	6	0.096	0.042	0.049	10.229*
Error	22	0.136	0.074	0.201	0.208

* Significant at 5% level.

$F_{2,22} = 3.44$

$F_{3,22} = 3.05$

$F_{6,22} = 2.55$

DAP = Days after planting

APPENDIX XV

Fresh weight of roots
(Analysis of variance)

Source	df	MS 70 DAP	MS 100 DAP	MS 130 DAP	MS 160 DAP
Total	35				
Block	2	24.26	8.34	1.75	0.88
Treatment					
N	3	58.25*	31.40*	108.42*	3.50
K	2	10.72*	28.78*	3.15	10.94
NK	6	7.62*	7.67	20.09	7.17
Error	22	2.79	4.46	9.09	8.04

* Significant at 5% level.

$F_{2,22} = 3.44$

$F_{3,22} = 3.05$

$F_{6,22} = 2.55$

DAP = Days after planting

APPENDIX XVI

Dry weight of roots
(Analysis of variance)

Source	df	MS			
		70 DAP	100 DAP	130 DAP	160 DAP
Total	35				
Block	2	3.203	1.123	0.125	0.155
Treatment					
N	3	7.983*	4.544*	15.477*	0.655
K	2	1.358*	4.245*	0.443	1.497
NK	6	1.031*	1.115	3.016	1.165
Error	22	0.383	0.635	1.337	1.232

* Significant at 5% level.

$F_{2,22} = 3.44$

$F_{3,22} = 3.05$

$F_{6,22} = 2.55$

DAP = Days after planting

APPENDIX XVII

Per cent dry matter content in roots
(Analysis of variance

Source	df	MS			
		70 DAP	100 DAP	130 DAP	160 DAP
Total	35				
Block	2	0.226	0.656	0.802	0.019
Treatment					
N	3	0.024	0.072	0.333	0.388*
K	2	0.092	0.822	0.156	0.453*
NK	6	0.194	0.370	0.339	0.159
Error	22	0.128	0.354	0.221	0.127

* Significant at 5% level

$F_{2,22} = 3.44$

$F_{3,22} = 3.05$

$F_{6,22} = 2.55$

DAP = Days after planting

APPENDIX XVIII

Per cent alkaloid content in root at different stages of growth *
(Analysis of variance)

Source	df	MS			
		70 DAP	100 DAP	130 DAP	160 DAP
Total	35				
Block	2	0.012	0.137	0.039	0.027
Treatment					
N	3	0.023	0.166	0.140	0.099
K	2	0.186	0.007	0.003	0.001
NK	6	0.109	0.009	0.109	0.078
Error	22	0.195	0.078	0.190	0.079

* On dry weight basis

$F_{2,22} = 3.44$

$F_{3,22} = 3.05$

$F_{6,22} = 2.55$

DAP = Days after planting

APPENDIX XIX

Alkaloid yield per hectare
(Analysis of variance)

Source	df	MS			
		70 DAP	100 DAP	130 DAP	160 DAP
Total	35				
Block	2	9.22	8.91	0.19	2.17
Treatment					
N	3	23.20*	18.96*	34.30*	3.93
K	2	6.45	10.19	0.65	2.60
NK	6	2.41	2.19	7.42	3.17
Error	22	3.08	3.03	7.30	4.60

* Significant at 5% level

$F_{2,22} = 3.44$

$F_{3,22} = 3.05$

$F_{6,22} = 2.55$

DAP = Days after planting.

APPENDIX XIX

Alkaloid yield per hectare
(Analysis of variance)

Source	df	MS			
		70 DAP	100 DAP	130 DAP	160 DAP
Total	35				
Block	2	9.22	8.91	0.19	2.17
Treatment					
N	3	23.20*	18.96*	34.30*	3.93
K	2	6.45	10.19	0.65	2.60
NK	6	2.41	2.19	7.42	3.17
Error	22	3.08	3.03	7.30	4.60

* Significant at 5% level

$F_{2,22} = 3.44$

$F_{3,22} = 3.05$

$F_{6,22} = 2.55$

DAP = Days after planting.

EFFECT OF GRADED DOSES OF NITROGEN AND POTASH ON GROWTH, ROOT YIELD AND ALKALOID CONTENT OF PERIWINKLE (*Catharanthus roseus* (L.) G. Don)

BY

B. R. REGHUNATH

ABSTRACT OF THE THESIS

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ABSTRACT

An experiment was conducted at the College of Agriculture, Vellayani during the year 1979-80 to study the effect of four levels of nitrogen (50, 100, 150 and 200 kg per hectare) and three levels of potash (50, 100 and 150 kg per hectare) in RCBD on the growth, yield and alkaloid content of Periwinkle (*Catharanthus roseus* (L.) G.Don).

Application of N, particularly at higher levels, had significant influence on plant height, girth of stem and tap root, fresh and dry yield of leaves, shoot, root and total alkaloid yield per hectare. Increased application of N increased the percentage of alkaloid content in root and at lower levels it increased the length of tap root, girth and number of lateral roots. Effect of K on length of tap root, girth and number of lateral roots, yield of leaves and total alkaloid per hectare and percentage of alkaloid content in root was not significant while it significantly increased the plant height and stem girth at early stages of growth and shoot yield, root yield and percentage dry matter content of shoot at later stages of growth. Interaction effect of NK was not significant on growth and yield characters of the plant except in the case of fresh and dry leaf yield. However, combinations of higher levels of N and K increased the yield of leaves, shoot and root and total alkaloid yield per hectare.

The proper stage of growth for harvest of the crop so as to get maximum root and alkaloid yield is 160 days after planting, ie., six to seven months after sowing seeds. It is evident from the results of the present study that it is feasible to cultivate Catharanthus roseus as a field crop under the agro-climatic conditions of South Kerala.