RESPONSE OF THREE FLOWERING ORNAMENTALS TO VA MYCORRHIZAL INOCULATION

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Affectionately Dedicated to

My Beloved Parents

DEPARTMENT OF AGRICULTURAL MICROBIOLOGY
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BANGALORE

CERTIFICATE

This is to certify that the thesis entitled **"RESPONSE OF THREE FLOWERING ORANAMENTALS TO VA MYCORRHIZAL INOCULATION"** submitted by Ms. Mamatha, K.B. in partial fulfillment of the requirement for the degree **MASTER OF SCIENCE in AGRICULTURAL MICROBIOLOGY** to the University of Agricultural Sciences, Bangalore, is a record of research work carried out by her, under my guidance and supervision and that no part of the thesis has been submitted for the award of any degree, diploma, associateship, fellowship or other similar titles.

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INTRODUCTION

I INTRODUCTION

Floriculture industry is growing at a fast rate and has become a full fledged industry world wide. The returns of investment in floriculture are very high because the gestation period is less in floriculture. Due to varied climatic conditions, cheaper availability of land and labour, floriculture industry has a great potential for development in India. Floriculture in India is growing at a rate of 10-15% annually. In India, Karnataka is a pioneer state in floriculture . Among the various flower crops grown in Karnataka, Chrysanthemum (Chrysanthemum morifolium. L), China aster Callistephus chinensis. Nees) and Marigold (Tagetus erecta. L) are most widely cultivated.

Chrysanthemum is the most popular florists' flower. It is the third most important cut flower cultivated world wide after carnation and rose. It is known as "Queen of East". In India, chrysanthemum occupies place of pride both as a commercial flower crop and as a popular exhibition flower. It is a native of Europe and Asia and belongs to the family Compositae.

China aster is another important commercial crop that is grown for cut flower and as bedding and pot plant. It has very good ornamental look. The genus Callistephus derived its name from two Greek words "Kalistos" meaning most beautiful and "Stephos" meaning a crown; referring to the flower. Asters are free blooming types having short crop duration. It is most widely used in making bouquets, button holes and garlands. It is a native of China and belongs to the family Asteraceae.

Marigold is grown on commercial scale as a cut flower. Marigold gained

popularity among the gardeners and flower dealers on account of its easy cultivation and wide adaptability. Its habit of free flowering, short duration to produce marketable flowers, wide spectrum of attractive colour, size and shape and good keeping quality attracted the attention of flower growers. In India, it is one of the most commonly grown flowers and extensively used during religious and social functions in one form or the other. It is a native of Mexico and belongs to the family Compositae.

For the production of good quality cut flowers, the major nutrients N, P and K must be supplied in adequate quantities. Hence, there is an extensive use of N, P and K fertilizers in floriculture industry. Phosphorus is a major element influencing growth and yield of flowers. Phosphorus deficiency is known to retard flowering and decrease the number of flower buds.

Vesicular arbuscular mycorrhizal (VAM) fungi are unique group of ubiquitous soil microorganisms known to form symbiotic association with roots of economically important crop plants. They are known to colonize the roots of most vascular plants. The importance of this association is well recognised because of the key role of VAM fungi in enhancing the uptake of diffusion limited nutrients such as P, Zn, Cu etc. especially in soils of low nutrient status. The inoculation of unsterile soil with VAM fungi has resulted in significant increase in not only nutrient uptake but also yield of various crops.

Since VAM fungi are obligate symbionts their mass production is not easy. Hence at present it is recommended for use in nursery raised and transplanted crops (Bagyaraj and Varma, 1996). There are a number of horticultural crops which are raised in nursery beds and then planted in the field. Several flowering ornamentals are grown as bedded plants and some of them are grown in small

nursery or containers and then planted in the field.

Chrysanthemum, China aster and Marigold being transplanted crops, it is practicable to raise mycorrhizal seedlings even with small quantity of VAM inoculum before transplanting. The present investigation was carried out by inoculating Glomus mosseae at three levels of phosphorus viz. 100, 75 and 50 per cent of the recommended P and compared with uninoculated plants which were given 100 per cent recommended P. The objectives of the present study are :

1. To study the response of Chrysanthemum, China aster and Marigold to VAM inoculation.
2. To know the possibility of reducing the application of phosphatic fertilizer by VAM inoculation in Chrysanthemum, China aster and Marigold.

REVIEW OF LITERATURE

II REVIEW OF LITERATURE

2.1 Mycorrhizal Association :

A German Botanist, A.B.Frank in 1885 was the first to coin the term "Mycorrhiza" which literally means `Fungus root' to describe the mutualistic symbiosis between roots of higher plants and certain fungi. Based on morphological and anatomical characters, these associations are grouped into two major groups - Ectomycorrhiza and Endomycorrhiza. The ectomycorrhizal fungi are the ones which cover the root surface by a dense mass of mycelium or mantle. From the mantle, fungal strands radiate outside into the soil thus enhancing root surface absorptive area. Hyphae also penetrate through the epidermis into the intercellular spaces of the cortical cells forming an inter connecting network known as "Hartig net". Ectomycorrhizae are common among temperate forest tree species. Ectomycorrhizal fungi can be cultured on laboratory media.

The endomycorrhiza colonize both the intra and inter cellular regions of the root cortex. Endomycorrhiza include orchid mycorrhiza, ericoid mycorrhiza and the most predominant type, vesicular arbuscular mycorrhiza (VAM). Orchid and ericoid mycorrhiza are formed by septate fungi. The association formed by aseptate fungi are called VA mycorrhizal association.

VAM fungi are grouped under the class Zygomycetes under the order Glomales. The order Glomales is divided into two sub-orders-Glominae and Gigasporinae. Glominae has 2 families namely Glomaceae and Aculosporaceae while Gigasporinae has one family Gigasporaceae. The family Glomaceae

includes the genera Glomus and Sclerocystis. Acaulosporaceae includes genera Acaulospora and Enterophospora and Gigasporaceae includes the genera Gigaspora and Scutellospora. VAM fungi are obligate symbionts and hence cannot be cultured on synthetic media. (Mosse and Hepper, 1975). The penetration of the fungal endophyte takes place through root hairs and/or epidermal cells. As many as 21 fungal entry points/mm length of the root tissue have been observed. (Mosse, 1973). Colonization of the cortical cells takes place extensively as a result of hyphal growth coiling in the epidermal cells. Subsequent hyphal growth may be intercellular or intracellular in the cortex, ultimately resulting in the development of short lived haustoria like structures called 'arbuscules' with in the cortical cells. These arbuscules are formed by dichotomous branching from a coarse hyphal base. The arbuscules function as the site of nutrient exchange between the fungus and the host (Cox et al., 1975). With the full establishment of the association between the fungus and the roots, vesicles are formed in the cortical cells. These vesicles are thin walled structures of various size and shape and contain oil droplets; they function as temporary storage organs. A root system colonised by VAM fungi does not show any morphological variation from the normal root system and hence cannot be distinguished visually. The presence of vesicles and arbuscules is the criterion for identifying a VAM fungus in the root.

2.2 Occurrence of VAM fungi :

VAM fungi are almost omnipresent in the natural ecosystem. These are known to colonise the roots of most of the vascular plants (Harley and Smith, 1983; Trappe, 1987). Families not forming VA mycorrhizae include Pinaceae,

Betulaceae, Orchidaceae, Fumeraceae, Commelineaceae,Urticaceae and Ericaceae. Families that rarely form VA Mycorrhizae include Caryophyllaceae, Cruciferae, Chenopodiaceae and Cyperaceae (Hirrel et al., 1978; Bowen, 1987). In addition to the wide spread distribution of VAM fungi through out the plant kingdom, this mutuallistic symbiosis is geographically ubiquitous and occurs in plants growing in arctic, temperate and tropical regions. VA mycorrhizae occur over a board ecological range, from aquatic to desert environments. (Mosse et al., 1981). Fossil records suggest their presence in the subterranean parts of earliest land plants. VA mycorrhizal fungi are believed to have occurred in 'Gondwanaland' even before the continental drift, 125 million years ago. The discovery of arbuscules in Aglaophyton major, an early Devonian land plant, provides unequivocal evidence that the mycorrhizae were established more than 400 million years ago (Remy et al., 1994). The occurrence of VAM fungi in aquatic plants (Soundergard and Leogard, 1977; Bagyaraj et al.,1979), halophytes and xerophytes (Khan, 1974; Raghupathy et al.,1990), in plants growing in sand dunes (Koske and Gemma, 1996) and coal mines (Khan, 1978) has been reported. They however do not develop in nitrogen fixing nodules (Lamnot, 1972) and under anaerobic soil condition (Tester et al., 1987).

2.3. Importance of VAM Fungi :

Improved plant growth due to inoculation of soil with VAM fungus has been demonstrated especially under P deficient conditions (Mosse, 1973; Gerdemann, 1976; Hayman, 1978; Bagyaraj and Padmavathy, 1993). In addition to enhanced P uptake, VAM fungi have been reported to improve the uptake of other elements like Zn and Cu (La Rue et al., 1975; Krishna et al., 1982). K (Powell, 1975),

N and Ca (Ross and Harper, 1970, Pai et al., 1994) and Mn (Krishna and Bagyaraj, 1984). VAM fungi are also known to be involved in increased water uptake (Stamps et al., 1984; Allen, 1982; Grahm and Sylvertsen, 1984). The VA Mycorrhizal association is also known to alleviate water stress in plants (Nelson, 1987; Pai et al., 1993).

VAM fungi are known to protect the plants from root invading organisms such as parasitic nematodes and phytopathogenic fungi since the occupation of root cortex by VAM fungi reduces or prevents colonization of that zone by the pathogens (Bagyaraj, 1984; Price et al., 1990; Huzengiia and Guikangdoni, 1991). In addition, the mycorrhizal plants have shown greater tolerance to toxic heavy metals, saline soils, high soil temperature and to transplant shock than the non-mycorrhizal plants. Because of these attributes, mycorrhizae are now considered important in the reestablishment of plants in inhospitable sites like coal and copper mine wastes, burrow pits and badly eroded locations (Hall, 1980).

Benefits of VAM associations include :

a. improved uptake of diffusion limited macro and micro nutrients.

b. increased tolerance to abiotic and biotic stresses

c. beneficial alterations of plant growth regulators which result from root interactions which are complex and dynamic (Jastfer and Sylvia, 1993).

2.4 Uptake of P and Other Nutrients :

Sanders and Tinker (1971) explained the mechanism of P uptake from soil by VA Mycorrhizae. The hyphae of mycorrhizae form better distributed surface for absorbing P from the soil solution than the roots alone.

Rhodes and Gerdemann (1975) demonstrated that VAM hyphae can

absorb and translocate P from distances of 1.9 to 7 cm from roots which is beyond the nutrient depletion zone around the plant roots. Soil hyphae of VAM fungi allow the root system to exploit greater volume of soil P by (1) extending away from the root and translocating P from as far as 8 cm (Rhodes and Gerdemann, 1975). (2) by exploiting smaller soil pores as VAM fungal hyphae are less than 20% of the diameter of the root hairs (3) by adding surface area to the adsorptive system (O'keefe and Sylvia, 1990)

Hayman and Mosse (1972) observed that mycorrhizal onion plants grown in a range of soils labelled with ^{32}P had taken up more P and grew larger.

The inoculation of subterranean clover with Glomus mosseae resulted in increased PO_4 content in them. At low rates of P, zinc uptake was increased (Pairunan et al., 1980).

Crops like cowpea, cotton and finger millet when inoculated with the VAM fungus Glomus fasciculatum had higher P and Zn content when compared to uninoculated plants (Bagyaraj and Manjunath, 1980).

Experiments with labelled P indicate that hyphae of VAM fungi obtain their extra P from the labile pool rather than by dissolving insoluble P (Raj et al., 1981).

Apple, teak and Tegetus patula plants inoculated with VAM fungi contained more tissue P than uninoculated plants. The concentration of other nutrients, namely N, K, Ca, Cu and Zn in the tissues was enhanced at low P levels when the plants were inoculated with VAM fungus (Plenchette et al., 1983).

According to Zhou and Li (1983), when the seedlings are inoculated with mycorrhizal inoculum, much mycelium appears on the roots and thus it increases the area of nutrient uptake.They demonstrated that in Camellia oleosa plants, more P was taken up by the inoculated plants; also P was absorbed more quickly.

Chrysanthemum morifolium when inoculated with Glomus fasciculatum showed high levels of P in leaves (Johnson et al., 1984). Rooted cuttings of Rosa multiflora inoculated with VAM fungi G. mosseae and G. fasciculatum showed greater K, Zn, Mn, S and Ca uptake (Davies, 1987). Manjunath et al. (1987) showed that inoculation of Leucaena leucocephala with Glomus aggregatum along with rock phosphate significantly increased the uptake of P, Cu and Zn. Lei et al. (1991) suggested that root factors like root exudates and volatiles stimulate P uptake. George et al. (1992) proved that the VA mycorrhizal hyphae have a role in the translocation of PO_4 and N from the soil zones several cm away from roots in cough grass (Agropyron repens) or white clover (Trifolium repens). Johnson et al. (1992) demonstrated the transport of ^{15}N labelled nitrogen by the hyphae of Glomus intraradices in Cucumis sativus.

2.5. Water Uptake :

Safir et al. (1972) for the first time reported the mycorrhizal effect on plant water relation in soybean plants. Mycorrhizal soybean showed lower resistance to water transport than the non-mycorrhizal plants. Sorghum bicolor and Eupatorium odoratum colonised by VAM fungi could withstand water stress in two tropical soils (Sieverding, 1981). Onion plants inoculated with Glomus etunicatum were more drought tolerant than non-mycorrhizal plants (Nelson and Safir, 1982a). Allen (1982) suggested that the increase in water uptake in mycorrhizal plants may be due to increase in surface area provided by the hyphae.

The greater water uptake by mycorrhizal roots is due to enhancement of P uptake through a VAM colonization (Grahm and Sylvester, 1984). Nelson (1987) attributed drought tolerance of VAM associated plants to improved P nutrition in

the plants.

Pai et al. (1993) conducted pot culture experiments to study the effect of moisture stress on growth and water relations of cowpea inoculated with G. fasciculatum at 2 levels of P for non-mycorrhizal and VAM plants and at 3 moisture levels. The results showed that VAM inoculation increased dry matter accumulation at all levels of moisture stress, difference in accumulation of dry matter between VAM and non-mycorrhizal plants being more at drier regimes.

VAM fungi may increase resistance of plants to drought by a number of mechanisms, such as increased water uptake and osmotic adjustment (Ruiz-Lozance and Azcon, 1995). They demonstrated in lettuce that much of the water was taken up by hyphae in VAM plants suggesting that mycorrhizal hyphae can take up water and that there are considerable variations in both behaviour of the VAM fungi and in the mechanisms involved in their effects on plant water relations.

2.6. Interaction of VAM With Other Soil Microorganisms :

With relatively few exceptions, roots form associations with mycorrhizal fungi. The degree of colonization of roots by mycorrhizal fungi and thus the effects of symbiosis may vary depending on total interaction between host, symbiont and environment. In most cases, the evidence is strong that the mycorrhizae may significantly change the host morphology and/or physiology. The net effect is that the other microorganisms in soil are affected by the change and a new rhizosphere balance results (Bagyaraj, 1984). There can be no doubt that the mycorrhizae affect other soil microbes and the other microbes affect mycorrhizal fungi. These interactions can be either direct by metabolic exchange or indirect as mediated by changes in the host plant. Because these

interactions are sure to occur, they must be studied in detail in order to increase the predictability of inoculation with mycorrhizal fungi or other beneficial microbes and there by also increase their consistency of performance and benefit plant growth and development (Paulitz and Linderman, 1991).

Azcon et al. (1976) studied the interaction between VAM fungi and phosphate solublising bacteria. They reported that the plants with mycorrhizal fungi plus bacteria took up more total P than the plants with either VAM fungi or bacteria separately.

Bagyaraj and Menge (1978) studied the interaction between Glomus fasciculatum and Azotobacter and their effects on rhizosphere microflora and plant growth. Larger populations of bacteria and actinomycetes were recovered from the rhizosphere of tomato plants inoculated with mycorrhizal fungi and Azotobacter chroococcum either individually or together, than from those of non-inoculated plants. Inoculation with Azotobacter chroococcum enhanced colonisation and spore production by Glomus fasciculatum. The dry weights of tomato inoculated with both the organisms were significantly greater than non-inoculated plants.

Raj et al. (1981) studied the influence of soil inoculation with G. fasciculatum and a phosphate dissolving bacterium, Bacillus circulans on plant growth and ^{32}P uptake. They recorded a synergistic effect with increased P uptake and dry matter production.

Manjunath et al. (1984) studied the response of Leucaena to dual inoculation with Glomus fasciculatum and Rhizobium. They found that the dual inoculation improved nodulation, mycorrhizal colonization, dry weight, nitrogen and phosphorus content of the plants compared to single inoculation with either

organism.

Poinsettia cuttings inoculated with G. fasciculatum showed greater root colonization in presence of Pythium ultimum than in the soil without the pathogen. Plant height and foliar P content of the mycorrhizal plants were greater than those of non-mycorrhizal plants in Pythium ultimum infested soils. Mycorrhizal plants had lower final P. ultimum populations in rhizosphere soil than non-mycorrhizal plants (Kaye et al., 1984).

Parvathi et al. (1985) observed synergistic effect of Glomus mosseae and Rhizobium on the growth in terms of dry weight, uptake of N and P in groundnut. Similar synergistic effect of VAM fungi and Azospirillum brasilense was observed by Subba Rao et al. (1985) on barley plants.

Champawat (1990) examined the interaction between a VAM fungus and Rhizobium in chickpea and reported that dual inoculation considerably enhanced plant growth and uptake of essential nutrients. Nahid and Gomah (1991) reported that dual inoculation with Azospirillum and VAM fungi increased shoot dry matter of wheat compared to control.

2.7. Mycorrhizal Fungi and Biological Control :

VA mycorrhizal plants are known to exhibit varied resistance towards soil borne and foliar pathogens. The known interactions include a number of mechanisms such as exclusion of pathogen, lignification of plant cell walls, changed phosphate nutrition resulting in altered exudation by roots and formation of inhibitory low molecular weight compounds (Mada and Bagyaraj, 1993 ; Sharma et al., 1992).

Latha Thomas et al. (1994) reported the possibility of using Glomus

fasciculatum in the biological control of damping - off of cardamum caused by Fusarium moniliformae. Plants inoculated with Glomus fasciculatum harboured more organisms in their rhizosphere with properties antaganostic to Fusarium moniliformae which clearly indicated that Glomus fasciculatum can reduce the severity of disease caused by Fusarium moniliformae.

Sundarbabu et al. (1995) reported that incorporating VAM fungi into nursery beds of tomato helped the fungus to colonise tomato roots, before it was transplanted to the main field, there by preventing the penetration and development of the nematodes in the VAM infected plants. Thus, VAM was able to offset the adverse effects of nematodes and increased yield by 90.3%.

Egg plants (Solanum melangena. L) infected with VAM fungus Glomus etunicatum and Gigaspore margarita showed greater tolerance to Verticillium wilt than the non-inoculated plants. (Matsubara et al., 1995).

Inoculation of cotton seeds with VAM fungus improved seedling growth, increased yield of seed cotton and reduced the incidence and disease index of Verticillium wilt. Some new soluble protein were found in the roots and leaves of the cotton colonized with the VAM fungus. More than ten types of new proteins were identified as pathogensis related proteins (PRS). One of the PRS exhibited chitinase activity. Tests in vitro showed that the PRS at certain concentrations were able to retard hyphal growth and kill the conida of Verticillium (Liu et al., 1995).

2.8. Mycorrhizal Dependency :

In 1975, Gerdemann defined mycorrhizal dependency as "the degree to which a plant species is dependent on mycorrhizal condition to produce maximum

growth or yield at a given level of soil fertility".

Plenchette et al. (1983) studied the mycorrhizal dependency of various crops under field condition in both fumigated and non-fumigated soils. They distinguished three groups of plants. Group I included mycorrhizal plants like Tagetus patula, carrot, pea, leek, tomato etc. which grew better in non-fumigated soil. The second group included oak and wheat which were mycorrhizal but grew equally well on fumigated soil as on non-fumigated soil. Group III plants were found to be non-mycorrhizal.

Lin and Hao (1989) reported that there was no correlation between per cent infection and mycorrhizal dependency. They reported that mycorrhizal dependency decreased in the order of grapes > Chinese roses > violets > petunias > lilies > chrysanthemums.

Manjunath and Habte (1992) studied the external and internal P requirements of plant species differing in their mycorrhizal dependency. VAM colonization levels generally decreased with increase in soil solution P concentration; the degree of decrease being more pronounced with decrease in VAM dependency of host species.

De Clerk et al. (1995) studied the mycorrhizal dependency of seven banana cultivars. Inoculated plants had generally greater shoot dry weight and shoot P concentration compared to the non-inoculated plants. They observed great variation in dependency on mycorrhizal colonization among the different banana cultivars.

2.9.a. Response of Ornamental Crops to VA Mycorrhizal Inoculation :

Daft and Okasanya (1973) reported that the mycorrhizal infection stimulated flower production in petunia. They reported that the infection increased the amount of vascular tissue and caused greater lignification of xylem in mycorrhizal plants.

Inoculation of Lilium regale and L. 'Burgundy' with Endogone increased their growth and dry weight of shoot, root and bulb. (Vanderploeg, J.F., 1974).

Infection of Chrysanthemum morifolium with mycorrhizal fungus Glomus fasciculatum increased the plant height, improved flower, stem and root dry weight especially at high N levels (Johnson et al., 1982).

Backhans (1982) reported that the inoculated unrooted cuttings of Chrysanthemum frutescens produced significantly greater mass of new roots than uninoculated cuttings. He also reported that Heliotropium arborascens cv. Marine and Fuchsia x hybrida cv. Beacon plants inoculated with the isolates of VAM fungi produced significantly more flowers and bloomed earlier than the non-inoculated plants.

Total fresh weight and crown spread of Juniperus chinensis var. Sargenti were significantly increased by inoculation with a spore mixture of Gigaspora margarita, Glomus fasciculatum and G. mosseae especially under low fertility conditions (Roncadori and Pokorny, 1982).

Biermann and Lindermann (1983) reported that pre transplant inoculation of soil, peat or vermiculite resulted in larger plants than post transplant inoculation. Geranium plants inoculated with Glomus fasciculatum in potting mix had more uniform growth, greater leaf area leaf weight, and root and shoot weight but lower foliar Mn concentration.

Colonization of Geranimum with Glomus fasciculatum was not extensive and shoot dry weight and P uptake were not increased in soilless media such as peat, bark, perlite or vermiculite, whereas in media containing soil and fertilized at low P levels, the roots were extensively colonized by G. fasciculatum and host shoot growth and P concentration were increased. (Biermann and Lindermann, 1983).

Tagetus patula plants inoculated with Glomus species and grown in calcined montmorillonite clay grew better than the non-inoculated ones even at high P levels (Plenchette et al., 1983).

Sweatte and Davies (1984) reported that Pelargonium plants inoculated with Glomus mosseae or G. fasciculatum acclimatise more efficiently to water stress than the non-mycorrhizal plants. Mycorrhizal plants grown under high moisture had higher P levels than the uninoculated controls while those grown under low moisture had greater shoot growth, more advanced floral formation and greater N uptake.

Inoculation of Tagetus erecta with VAM fungi along with application of fertilizer application increased the shoot length, shoot and flower dry weight. Further increase in fertilizer application depressed mycorrhizal root colonization in marigold (Bagyaraj and Powell, 1985).

In Narcissus poeticus plants (liliflorae) infected with VAM fungi, infections mainly occurred in roots during growth and expansion of foliar parts of the plants while during flowering higher infection occurred in both roots and sheathing leaves. Arbuscules were more numerous during the growing period but during the flowering period, vesicles were found to be more common. The dried sheathing leaves served as inoculum source for new roots (Iqbal and Bareen, 1986).

Kawal et al. (1986) reported that the endomycorrhiza was formed by almost all the ornamental plants examined in Japan with the exception of stock

Plenchette (1986) reported that the fungal development in the roots of marigold depended on internal P concentration when the external P concentration was low and on external P concentration when the internal P concentration was high. All the mycorrhizal fungi tested had beneficial effect on the growth of French Marigold (Tagetus patula).

Rosa multiflora rooted cuttings showed greater plant growth noculated with either Glomus mosseae or G. fasciculatum compared to the uninoculated control plants (Davies Jr., 1987).

Salvias and Asters inoculated with mycorrhiza when transplanted into pots containing worm casts instead of FYM showed greater mycorrhizal root colonization and this had beneficial effect on plant vegetative characteristics and flowering. (Kale et al., 1987).

Camprubi et al. (1987) stated that inoculation of Chrysanthemum coronarium with Glomus mosseae gave better yield than the non-inoculated plants.

Davies Jr. et al. (1987) reported that Rosa multiflora rooted cuttings inoculated with Glomus mosseae and G. fasciculatum had lower transpiration rates and a high diffusive resistance under reduced irrigation regimes.

Raverkar et al. (1991) reported that the natural levels of mycorrhizal infection in most of the ornamental plants is low and suggested that artificial inoculation may prove beneficial.

Wang et al. (1993) reported that symbiosis was established between micropropagated ornamental plants like Gerbera jamensonii and Syngonium within

4-8 weeks of culture in the green house but not during acclimatization. Mortality of the mycorrhizal plantlets was lower than the non-inoculated plantlets 8 weeks after transplanting. Mycorrhizal substrates had a long term benefit of increasing shoot and root dry weight in Gerbera jamensonii and Syngonium.

Seeds of Tagetus erecta and Zinnia elegans when inoculated with VAM fungus Glomus etunicatum showed faster flowering and an increase in the number of flowers, shoot height, shoot and root dry weight when compared to the non-inoculated plants. The reactions of these plants to mycorrhizal infection were shown to be independent of changes in the P, K and Na content of plants (Cazares and Smith, 1996).

2.9.b. Effects of VAM on Ornamental Plants :

Effects of VAM fungi on ornamental plants include enhanced seedling growth (Chang, 1990a); reduced phosphate requirement (Chang, 1992); and increased survival rate and growth of micropropagated plantlets (Chang, 1992; Glaninazzi - Pearson, 1989; Lin, 1996; Ponton et al., 1990).

Mycorrhizal plants of Gerbera, three flowering ornamentals and strawberry flowered and fruited atleast a week before the non-mycorrhizal plants (Backhans, 1983; Chang, 1990b; Cheng, 1989; Wen, 1991).

Gerbera plants colonized by Glomus mosseae produced more flowers which lasted three days longer than the non-mycorrhizal plants in vase (Wen, 1991). The increased vase life of flowers from mycorrhizal plants was attributed to the greater development of water conducting tissues in mycorrhizal plants, than in non-mycorrhizal plants. (Chang, 1992; Wen, 1991).

MATERIAL AND METHODS

III MATERIALS AND METHODS

A green house investigation was conducted at the Department of Agricultural Microbiology, University of Agricultural Sciences, GKVK, Campus, Bangalore to study the response of three commercially important flowering ornamentals, Chrysanthemum (Chrysanthemum morifolium. L), China Aster (Callistephus chinensis. Nees) and Marigold (Tagetus erecta. L) to VA mycorrhizal inoculation.

3.1 Planting Material

Rooted cuttings of Chrysanthemum morifolium var. Red gold were obtained from the Department of Horticulture, University of Agricultural Sciences, Bangalore. Callistephus chinesis var. Ostrich plume and Tagetus erecta var. Double orange seeds were sown in the nursery beds maintained at the Department of Horticulture, University of Agricultural Sciences, Bangalore. One month old seedlings were transplanted.

Planting medium consisted of sand : Soil : FYM in 1 : 2 : 1 proportion which was filled into 19 cm diameter plastic pots (3.5 kg capacity). Sufficient drainage was provided to the pots. The physico-chemical characteristics of the growth medium used in the experiment is shown in Table - I. The growth medium had native VA mycorrhizal population of 50 spores per 50 ml.

Table - I : Physical and chemical properties of the growth medium used in the study

Characteristics	Magnitude	Method of determination
1. a. Texture	Red sandy loam	Bouyounces Hydrometer (Piper, 1966)
b. Mechanical Analysis		
i. Coarse sand	24.33%	
ii. Fine sand	48.25%	
iii. Silt	4.40%	
iv. Clay	22.76%	
2. Physico Chemical Analysis		
i. EC at 25°C	0.003 m.mhos/cm	Conductivity meter (Jackson, 1973)
ii. pH	5.7	pH meter (Jackson, 1973)
iii. Organic carbon (%)	1.08	Walkley and Black method (Jackson, 1973)
iv. Available N (kg/ha)	307.33	Alkaline permanganate method (Subbaiah and Asija, 1956)
v. Available P (P_2O_5 kg/ha)	53.12	Bray's method (Jackson, 1971)
vi. Available K (K_2O kg/ha)	30.00	Flame photometer method (Olsen and Sommers, 1982)

Source : Dept. of Soil Science, UAS, Bangalore.

3.2 **Mycorrhizal inoculum used** : The inoculum of Glomus mosseae maintained at the Department of Agricultural Microbiology, University of Agricultural Sciences, Bangalore, in the sterilized sand : soil mixture (1:1 by volume) using Rhodes grass (Chloris guyana) as host was used in this study The inoculum contained extramatrical hyphae, spores and infected root bits of Rhodes grass and was used at the rate of 25 g/pot.

3.3.a. Method of Planting :

The pots containing sand : soil : FYM mix in 1 : 2 : 1 proportion were watered till field capacity a day before planting. The next day, inoculum of Glomus mosseae at the rate of 25 g/pot was placed in the root zone. i.e., at a depth of 6 cm and then, the planting was done.Only one plant was maintained per pot

3.3.b. Fertilizer recommendation and Application :

The recommended NPK fertilizers for Chrysanthemum, China Aster and Marigold are given below :

Crop	Fertilizer recommendation					
	kg per hectare			g per pot		
	N	P	K	N	P	K
i Chrysanthemum	125	150	100	0.44	0.52	0.350
ii. China Aster	180	120	060	0.63	0.42	0.21
iii. Marigold	225	60	60	0.78	0.21	0.21

The NPK fertilizers were supplied in the form of urea, single super phosphate and muriate of potash respectively. N and k fertilizers were applied at the recommended rate uniformly for all the pots. P fertilizer was added at different levels based on the treatment given under experimental details.

Fifty per cent of N and entire P and K fertilizers were mixed thoroughly with the planting medium before planting. The remaining 50 per cent N fertilizer was applied 15 days after planting.

3.4.a. Experimental Details :

There were four treatments; each treatment having 13 replications in case of chrysanthemum, 10 replications in case of China aster and 12 replications in case of marigold.

The treatment details are as follows :

U P100 - Uninoculated control, 100 per cent recommended P without VAM.

I P100 - Inoculated, 100 per cent recommended P.

I P75 - Inoculated, 75 per cent recommended P.

I P50 - Inoculated, 50 per cent recommended P.

VA mycorrhiza was added as 25 g of Glomus mosseae inoculum per pot.

3.4.b. Maintenance of Experimental Plants :

The treatments were arranged on green house benches in randomised complete block design (RCBD). Plants were grown under natural light till flowering. The plants were watered when ever necessary.

3.4.c. Plant Nutrition :

Chrysanthemum plants were given Ruakura nutrient solution minus P (Appendix I) at the rate of 50 ml/pot twice; once after bud initiation and again at 50 per cent flowering stage.

3.4.d. Plant Protection :

In Chrysanthemum, in the early stages severe incidence of aphids was

noticed which was controlled by spraying monocrotophos at the rate of 1.25 ml/l of water. The growth of China Aster was affected by leaf eating caterpillar which was controlled by spraying methylparathion at the rate of 1.65 ml/l of water. There was no disease incidence in the three crops.

3.4.e. Pinching :

It refers to the removal of terminal shoot buds in order to facilitate side branching. Pinching was done during seventh week in chrysanthemum and in fourth week in case of China aster and Marigold.

3.5. Harvesting :

The flowers of all the three crops were harvested as and when the central whorl of petals were found in opened condition. Two harvests were done in chrysanthemum and marigold and only one harvest in case of China Aster.

3.6. Observations on Plant Growth Parameters :

The first observation with respect to all the growth parameters was recorded one month after planting in all the three crops. The subsequent observations were recorded once in 15 days till harvest.

3.6.a. Plant Height :

The height (cm) was measured from the ground to the tip of main stem.

3.6.b. Number of Branches :

The number of main branches arising from the main stem were counted

and recorded at different intervals.

3.6.c. Dry Matter Production :

After harvest, the harvested plant samples were dried in an oven at 60°C to a constant weight and the dry weight was recorded.

3.7. Observations on Yield and Yield Attributes :

3.7.a. Time Taken for Bud Initiation :

This observation was recorded by counting the days from the date of planting to the stage at which the first flower bud appeared in each treatment.

3.7.b. Time Taken for 50 Per cent Flowering :

The number of days taken for 50 per cent of the plants to produced first flower in each treatment was recorded by counting the days from the date of transplanting.

3.7.c. Total Number of Flowers :

The number of flowers at each harvest was recorded and finally added to get the total number of flowers per plant.

3.7.d. Size of Flowers :

Ten flowers were selected randomly from each pot and the flower diameter was measured with the help of a Vernier callipers and average diameter in cm was determined.

3.7.e. Stalk Length of Flowers :

The stalk length (cm) was calculated by measuring of the distance between the base of the flower and the first pair of leaves. For this purpose, ten flowers were selected randomly from each pot.

3.7.f. Flower Yield :

After recording the total number of flowers, the flowers harvested were weighed after each harvest. The total weight of flowers gave yield per plant.

3.7.g. Vase Life of Flowers :

Studies on vase life was conducted only in case of chrysanthemum. The flowers were harvested along with the stalk when two or three lower most florets were opened. After removing the lower leaves, the stems were cut equally and the stalks were placed in 250 ml 2 per cent sucrose solution in conical flasks.

3.8.a. Mycorrhizal Spore Count in Root Zone Soil :

Extramatricular chlamydospores produced by the VAM fungus in soil was estimated by wet sieving and decanting method (Gerdemann and Nicolson, 1963).

Fifty grams of air dried representative soil sample drawn from each pot after harvest was suspended in 500 ml water and stirred thoroughly. The suspension was allowed to stand undisturbed for one minute and was then passed through a series of sieves of sizes 1 mm, 450, 250, 105 and 45 um arranged one below the other in the same order. The spores on the bottom two sieves were transferred on to a nylon mesh with pore size of 40 um which was then placed in a petriplate and spores were counted under 40 x stereo microscope.

3.8.b. Mycorrhizal Colonization in Roots :

The extent of VAM colonization in roots was determined by grid line intersect method outlined by Giovannetti and Mosse (1980) after staining the roots with Acid fuchsin.

3.8.c. Staining Procedure :

Fresh root samples (300 mg) were cut into 1 cm segments and placed in test tubes to which 10 ml KOH was added to facilitate clearing of root bits which was achieved by placing these tubes in boiling water for 60 minutes. KOH solution was then decanted and the roots were rinsed in tap water to remove the residual KOH. Then, the roots were treated with 10 % HCl for 10 minutes to neutralise the alkali. After 10 minutes, HCl was decanted and the root segments were stained with Acid fuchsin (0.2 %) in lacto glycerol (lactic acid : Glycerol : water in 40 : 40 : 20 v/v) and simmered for 10 minutes. The stain was then decanted and the roots were stored in screw cap tubes in lacto glycerol which helped in removing the excess stain.

3.8.d. Per cent Root Colonization :

The stained roots were examined under stereo microscope (40x magnification) for the presence of VA mycorrhizal colonization by placing them randomly on a glass plate of size 10 cm x 10 cm with 1 cm grid. Both the horizontal and vertical grid lines were scanned to determine the total number of intersections between root segments and grid line. The number of intersections positive for the presence of VAM structures like hyphae, vesicles and arbuscules

were also determined. The per cent root colonization was determined by the formula :

$$\% \text{ VAM Colonization} = \frac{\text{No. of intersecting root bits positive for VAM colonization}}{\text{Total number of intersecting root bits observed}} \times 100$$

3.9.a. Estimation of Phosphorus in Plant Tissues :

The plant samples were dried at 60°C to obtain a constant weight and were then powdered. The phosphorus content was estimated by vanadomolybdate phosphoric yellow colour method (Jackson, 1967). 0.5 g each of the powdered sample was digested in triacid mixture (Nitric acid : Perchloric acid : Sulphuric acid 7 : 3 : 1) in 100 ml conical flasks. The volume of the digested sample was made up to 100 ml with distilled water, 10 ml of aliquot of this solution was taken in a 50 ml volumetric flask and 10 ml vanadomolybdate reagent was added and the volume was made up to 50 ml using distilled water. This reaction mixture was allowed to stand for 20 minutes. The intensity of yellow colour developed due to phospho vanadomolybdate complex was measured at 420 nm using Baucsh and Lomb spectronic-20 spectrophotometer. Phosphorus concentration in the sample was determined by comparing with standard curve developed using KH_2PO_4 as the source of P.

3.9.b. Statistical Analysis :

The data collected in this study was subjected to statistical analysis suitable to RCBD. Duncan's multiple range test was done to separate the treatment means (Little and Hills, 1978)

EXPERIMENTAL RESULTS

IV EXPERIMENTAL RESULTS

The results of the experiment conducted to study the influence of inoculation of the VAM fungus Glomus mosseae at different levels of phosphorus (P) on growth, yield and yield components of chrysanthemum, China aster and Marigold are presented in this chapter.

4.1 Effect of VAM Inoculation at Different P Levels on :

4.1.1. Growth Parameters of Chrysanthemum :

4.1.1.a. Plant Height :

The data on plant height as influenced by inoculation with the VAM fungus at different P levels recorded at 30, 45, 60, 75 and 90 days after planting (DAP) is presented in Table 1.

At 30 and 45 DAP, the plant height observed in inoculated plants at 100 and 75 per cent recommended P was statistically on par with that of uninoculated control plants which were given 100 per cent P. At 60 DAP, plants inoculated with the VAM fungus at 100 per cent and 75 per cent recommended P showed maximum plant height which was significantly greater than that of uninoculated plants and inoculated plants given 50 per cent of the recommended P. At harvest, maximum plant height was observed in plants inoculated with VAM fungus at 75 per cent of the recommended P. However it was on par with inoculated plants and uninoculated plants at 100 P. At all the stages of growth, lowest plant height was observed in inoculated plants given 50 per cent recommended P which differed statistically from the other treatments.

4.1.1.b. Number of Branches per Plant :

The data on number of branches at 30, 45, 60, 75 and 90 DAP is presented in Table 1.

The influence of the VAM fungus on the number of branches was significantly more at 75 and 100 per cent recommended P at all stages of plant growth compared to the uninoculated control. In general, inoculation with the VAM fungus at 75 per cent recommended P resulted in maximum number of branches at 45, 60, 75 and 90 DAP. The number of branches recorded in plants inoculated with the VAM fungus at 50 per cent recommended P was least at all the stages of plant growth but it was statistically on par with uninoculated control plants at 45, 60 and 90 DAP.

4.1.1.c. Plant Spread :

The data on plant spread as influenced by inoculation with the VAM fungus at different P levels observed on 30, 60 and 90 DAP is presented in Table 1.

At all the stages of plant growth, there was no significant difference among plants inoculated with the VAM fungus at 100 and 75 per cent recommended P and uninoculated plants which were given 100 per cent recommended P with respect to plant spread. At 60 and 90 DAP, maximum plant spread was observed in plants inoculated with the VAM fungus and given 75 per cent of the recommended P. At all the stages, plant spread was least in plants inoculated with VAM at 50 per cent recommended P.

Table 1 : Effect of soil inoculation with VAM fungus at different P levels on plant height, number of branches and plant spread of Chrysanthemum

Treatment	Plant height (cm)					Number of branches					Plant spread (cm)		
	Days after planting												
	30	45	60	75	90	30	45	60	75	90	30	60	90
U P 100	18.23a	29.77a	35.54b	49.39a	57.92a	6.85b	7.85b	21.15b	24.62b	33.38b	26.00a	49.15a	49.62a
I P 100	17.92a	31.00a	40.62a	46.77b	58.38a	7.31a	10.23a	27.08a	33.62a	38.15a	25.08a	48.92a	48.73a
I P 75	17.46a	30.23a	40.85a	43.23c	61.15a	7.08b	10.31a	29.77a	33.92a	38.69a	24.42a	50.15a	49.92a
I P 50	13.00b	22.08b	32.23c	38.46d	51.85b	5.00c	6.62b	22.15b	26.23b	30.92b	20.15b	37.46b	37.15b

Means followed by the same superscript within each column do not differ significantly at P = 0.05

U P 100 = Uninoculated, 100 per cent recommended P.

I P 100 = Inoculated, 100 per cent recommended P.

I P 75 = Inoculated, 75 per cent recommended P.

I P 50 = Inoculated, 50 per cent recommended P.

4.1.2. Time Taken for Flower Bud Initiation and 50 per cent Flowering of Chrysanthemum :

The data on the number of days taken for flower bud initiation and 50 per cent flowering is presented in Table 2 and figure 1.

VAM inoculation had significant influence on the number of days taken for flower bud initiation and 50 per cent flowering at all the three levels of P as compared to the uninoculated control plants which were given 100 per cent P. In the inoculated plants bud initiation was hastened as compared to the uninoculated plants. The uninoculated plants took longer period for bud initiation (76.23 days). Plants inoculated with the VAM fungus at 50 per cent recommended P took 75.31 days for bud initiation which differed significantly from the uninoculated plants. While bud initiation was hastened (73.69 days) in plants inoculated with the VAM fungus at 75 per cent recommended P but was statistically on par with that of plants inoculated with the VAM fungus at 100 per cent P both differing significantly from the other two treatments.

The influence of the VAM fungus on days taken for 50 per cent flowering was significant. In general, inoculation with the VAM fungus resulted in stimulation of flowering compared to the uninoculated plants. Inoculation with the VAM fungus at 75 per cent recommended P resulted in less number of days for 50 per cent flowering (118.54) which differed significantly from the other treatments. Inoculation with VAM fungus at 100 per cent P resulted in flowering by 127.08 days which differed significantly from that of plants inoculated with VAM fungus at 50 per cent recommended P (128.55). Maximum number of days (137.54) was

taken by uninoculated plants.

4.1.3 Flower Number and Flower Yield per Plant of Chrysanthemum :

Inoculation with the VAM fungus at 75 per cent recommended P gave maximum number of flowers per plant (135.69) which differed significantly from the other treatments. Plants inoculated with VAM at 50 per cent recommended P gave least number of flowers (99) but it was statistically on par with that of plants inoculated with VAM at 100 per cent P and the uninoculated plants given 100 per cent P.

Flower yield per plant was maximum in plants inoculated with the VAM fungus at 75 per cent of the recommended P (178.72) but it was statistically on par with the yield of plants inoculated with VAM at 100 per cent P. Lowest yield was recorded in plants inoculated with VAM at 50 per cent recommended P (123.35) but it was statistically on par with uninoculated control plants given 100 per cent P (Figure 2).

4.1.4 Flower Characteristics of Chrysanthemum :

The data on the effect of soil inoculation with the VAM fungus at different P levels on flower characteristics is presented in Table 3.

4.1.4.a. Flower Diameter :

There was no significant effect of VAM inoculation on flower diameter compared to uninoculated control plants.

Table 2 : Effect of soil inoculation with VAM fungus at different levels of P on time taken for flower bud initation, 50 per cent flowering, flower number and flower yield of Chrysanthemum.

Treatment	Days taken for flower bud initiation	Days taken for 50 per cent flowering	Number of flowers	Flower yield per plant (g)
U P 100	76.23a	137.54a	105.85b	124.92b
I P 100	73.69c	127.08c	123.54b	164.06a
I P 75	73.69c	118.54d	135.69a	178.72a
I P 50	75.31b	128.85b	99.00b	123.35b

Means followed by the same superscript within each column do not differ significantly at P = 0.05

U P 100 = Uninoculated, 100 per cent recommended P.

I P 100 = Inoculated, 100 per cent recommended P.

I P 75 = Inoculated, 75 per cent recommended P.

I P 50 = Inoculated, 50 per cent recommended P.

UP100 – Uninoculated, recommended P

IP100 – Inoculated, recommended P

IP75 – Inoculated, 75 percent P

IP50 – Inoculated, 50 percent P

☒ No. of days taken for flower bud initiation.

☐ No. of days taken for 50 percent flowering

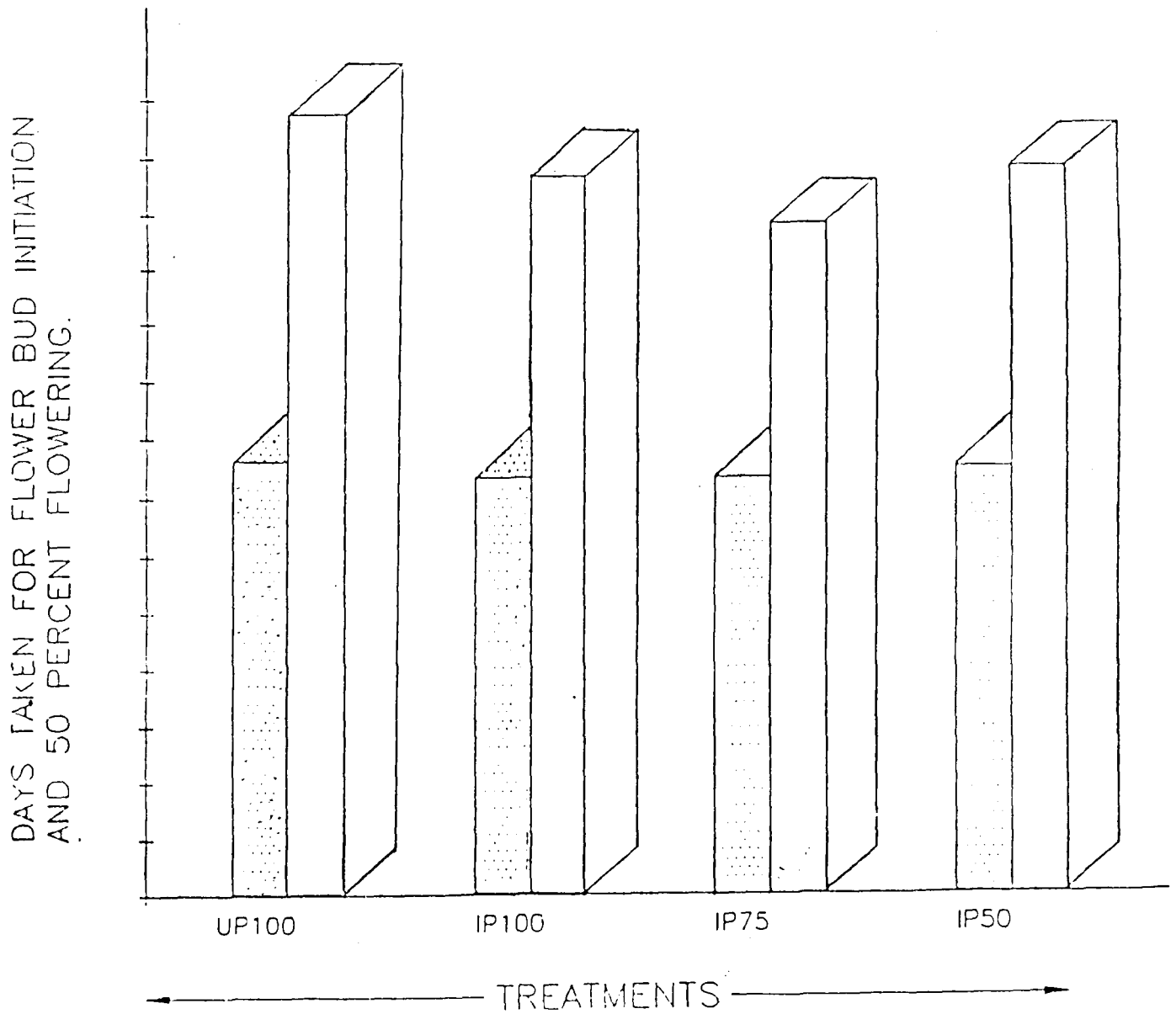

FIG 1 : Effect of soil inoculation with VAM fungus at different P levels on time taken for flower bud initation and 50 per cent flowering of Chrysanthemum.

UP100 – Uninoculated, recommended P

IP100 – Inoculated, recommended P

IP75 – Inoculated, 75 percent P

IP50 – Inoculated, 50 percent P

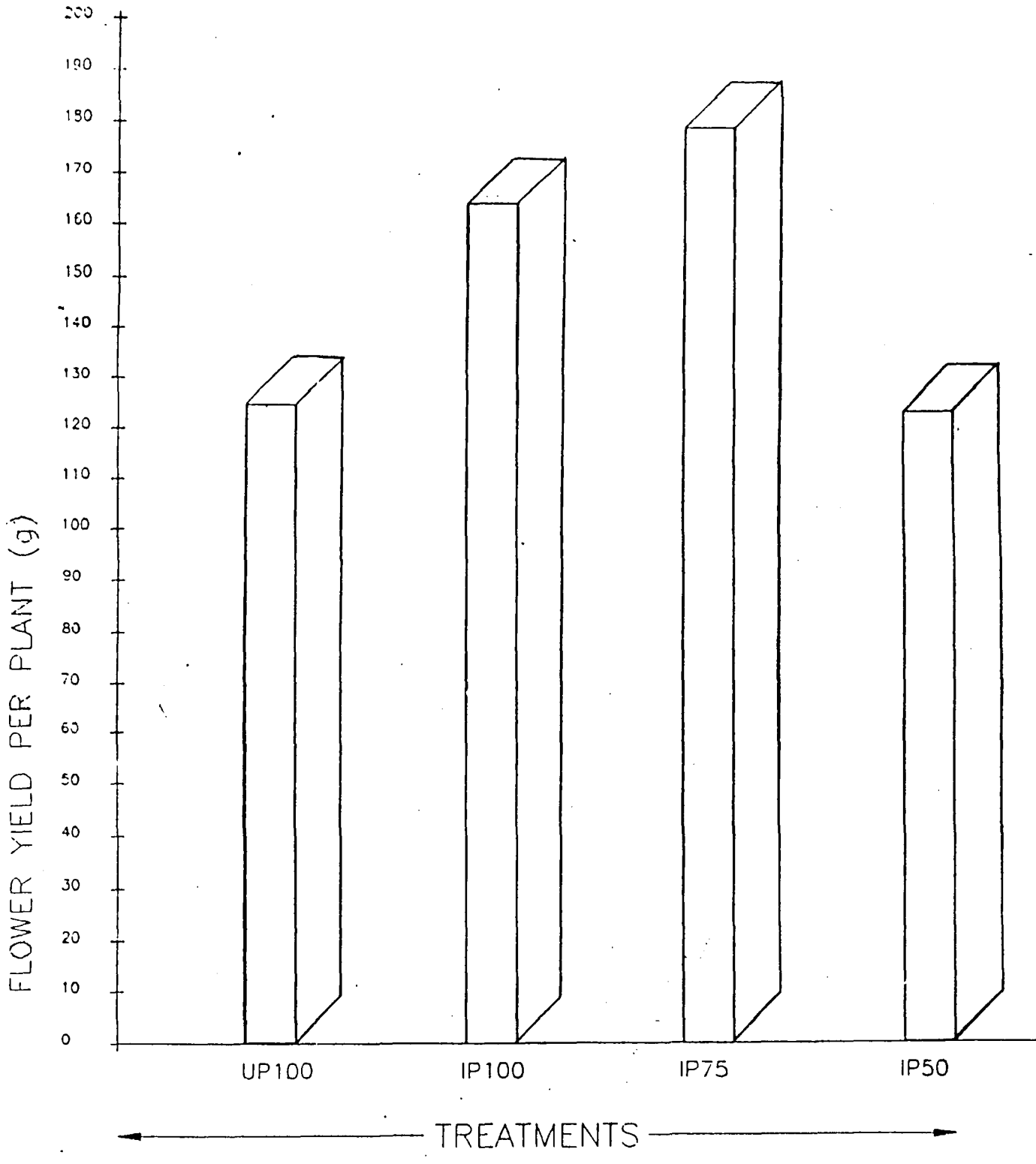

FIG 2 : Effect of soil inoculation with VAM fungus at different P levels on flower yield per plant of Chrysanthemum.

PLATE 1 Response of Chrysanthemum to VA mycorrhizal inoculation

A = Uninoculated, recommended P
B = Inoculated, recommended P
C = Inoculated, 75 percent P
D = Inoculated, 50 percent P

PLATE I

Maximum flower diameter (5.25 cm) was observed in plants inoculated with the VAM fungus at 75 per cent of the recommended P. But, it was statistically on par with that of plants inoculated with the VAM fungus at 100 per cent P. The flower diameter in plants inoculated with VAM fungus at 50 per cent recommended P was on par with uninoculated control plants given 100 per cent P, but had least flower diameter (4.98 cm).

4.1.4.b. Stalk length :

The stalk length of flowers in plants inoculated with the VAM fungus at 75 per cent recommended P was significantly higher compared to all the other treatments. Lowest stalk length was observed in plants inoculated with the VAM fungus at 50 per cent of the recommended P (10.45 cm) but it was statistically on par with stalk length recorded in plants inoculated with the VAM fungus at 100 per cent P and also uninoculated control plants given 100 per cent P.

4.1.4.c. Vase Life of Flowers :

In general, the vase life of flowers from plants inoculated with the VAM fungus was more when compared to the uninoculated control plants in 2 per cent sucrose solution. Maximum vase life (14 days) was recorded in flowers of the plants inoculated with the VAM fungus at 75 per cent recommended P (Table 4). Flowers from uninoculated plants had least vase life (9 days). The flowers from plants inoculated with VAM fungus at 100 per cent P and 50 per cent P had a vase life of 10 and 12 days respectively (Plate-2).

Table 3 : Effect of soil inoculation with VAM fungus at different P levels on flower characteristics of Chrysanthemum.

Treatment	Flower diameter (cm)	Stalk length (cm)	Dry weight (g)
U P 100	4.98b	10.68b	27.84a
I P 100	5.23a	11.28b	27.95a
I P 75	5.25a	11.72a	29.34a
I P 50	5.00b	10.45b	24.11a

Means followed by the same superscript within each column do not differ significantly at P = 0.05

U P 100 = Uninoculated, 100 per cent recommended P.

I P 100 = Inoculated, 100 per cent recommended P.

I P 75 = Inoculated, 75 per cent recommended P.

I P 50 = Inoculated, 50 per cent recommended P.

Table 4 : Effect of soil inoculation with VAM fungus at different P levels on flower logevity of Chrysanthemum in 2 per cent sucrose solution.

Treatment	Flower longevity (days)
U P 100	9c
I P 100	10c
I P 75	14a
I P 50	12b

Means followed by the same superscript within each column do not differ significantly at P = 0.05

U P 100 = Uninoculated, 100 per cent recommended P.

I P 100 = Inoculated, 100 per cent recommended P.

I P 75 = Inoculated, 75 per cent recommended P.

I P 50 = Inoculated, 50 per cent recommended P.

PLATE 2: Effect of VA mycorrhizal inoculation at different P levels on flower longevity of Chrysanthemum.

A = Uninoculated, recommended P.
B = Inoculated, recommended P.
C = Inoculated, 75 percent P.
D = Inoculated, 50 percent P.

PLATE 2

4.1.4.d. Flower Dry Weight :

The influence of VAM fungus on flower dry weight was not significant. Maximum dry weight was observed in plants inoculated with the VAM fungus and given 75 per cent of the recommended P while lowest flower dry weight was observed in plants inoculated with VAM fungus and given 50 per cent P.

4.1.5. Plant Biomass of Chrysanthemum :

The data on the effect of soil inoculation with the VAM fungus on plant biomass is presented in Table 5 and Figure 3.

4.1.5.a. Shoot Biomass :

Maximum shoot biomass was recorded in plants inoculated with the VAM fungus and given 75 per cent P which was significantly higher than the other treatments. The shoot biomass of plants inoculated with the VAM fungus at 100 per cent P was statistically on par with uninoculated plants given 100 per cent P. The shoot biomass of plants inoculated with VAM fungus at 50 per cent recommended P was lowest and it differed significantly from the other treatments.

4.1.5.b. Root Biomass :

Plants inoculated with the VAM fungus at 75 per cent recommended P had maximum root biomass which was statistically on par with that of uninoculated control plants given 100 per cent P. Plants inoculated with the VAM fungus at 50 per cent recommended P had the lowest root biomass.

Table 5 : Effect of soil inoculation with VAM fungus at different P levels on plant biomass of Chrysanthemum.

Treatment	Biomass (g/plant)		
	Shoot	Root	Total
U P 100	99.85b	36.03ab	135.88b
I P 100	100.85b	31.96b	132.81b
I P 75	118.70a	44.59a	163.29a
I P 50	76.85c	29.20b	106.05c

Means followed by the same superscript within each column do not differ significantly at P = 0.05

U P 100 = Uninoculated, 100 per cent recommended P.

I P 100 = Inoculated, 100 per cent recommended P.

I P 75 = Inoculated, 75 per cent recommended P.

I P 50 = Inoculated, 50 per cent recommended P.

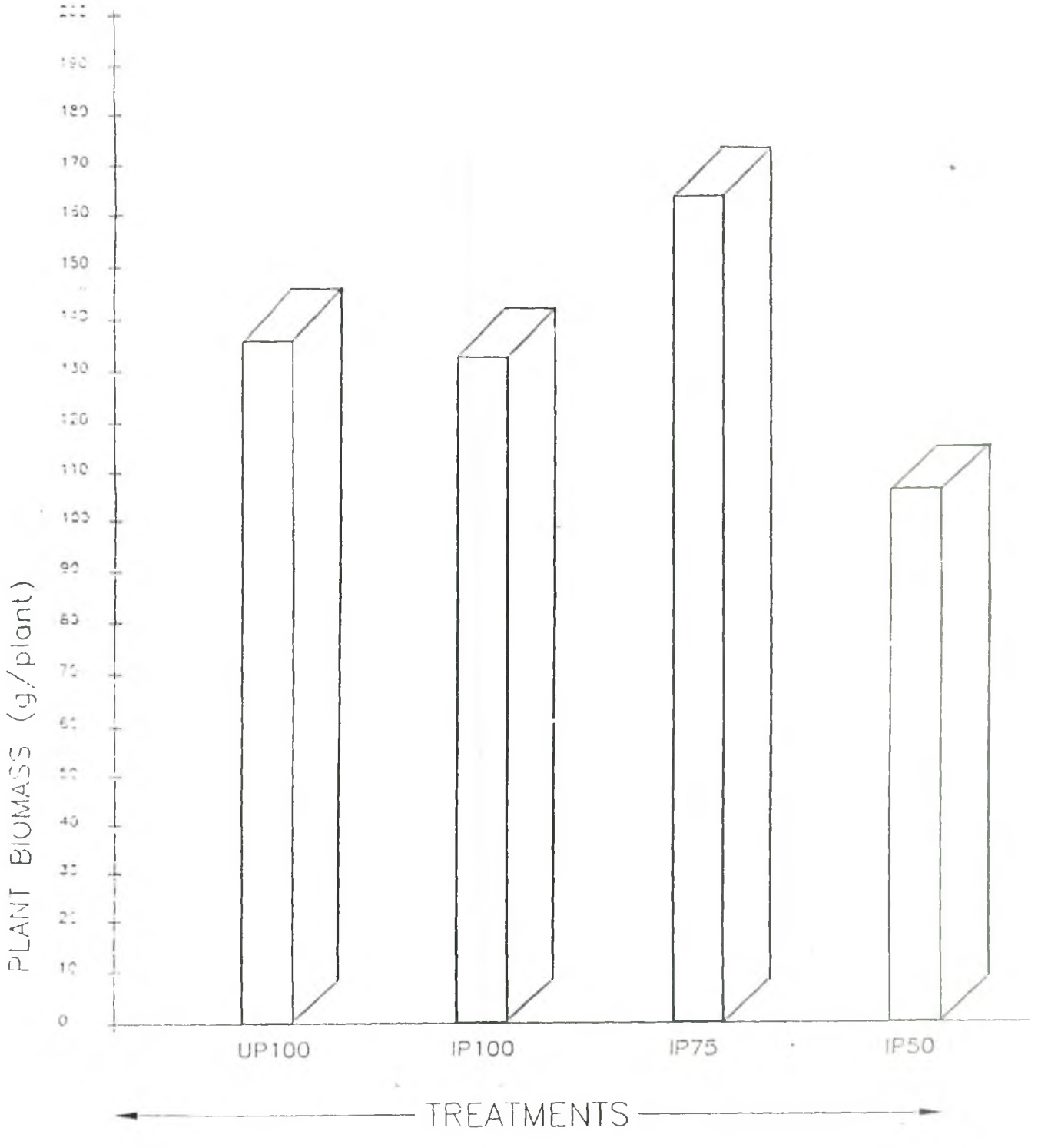

FIG 3 : Effect of soil inoculation with VAM fungus at different P levels on plant biomass of Chrysanthemum.

4.1.5.c. Total Plant Biomass :

The total plant biomass varied significantly among the treatments.

Maximum total plant biomass was observed in plants inoculated with the VAM fungus at 75 per cent recommended P (163.29 g/plant) which was significantly higher than that of other treatments. The total biomass of the plants inoculated with the VAM fungus at 100 per cent P and that of uninoculated plants at 100 per cent P was on par with each other but significantly higher than the total biomass of plants inoculated with VAM fungus at 50 per cent P (106.05 g/plant).

4.1.6 Effect of VAM at Different P Levels on Shoot P Concentration and Content of Chrysanthemum :

Shoot P concentration was maximum in plants inoculated with the VAM fungus at 75 per cent P and it was significantly greater than that of other treatments. Shoot P concentration was minimum in uninoculated plants (Table 6).

There was significant effect of VAM fungus at different P levels on shoot P content of plants (Table 6). Shoot P content was maximum (379.84 mg/plant) in plants inoculated with VAM fungus at 75 per cent P which was significantly higher than that of other treatments. Minimum shoot P content (119.82 mg/plant) was observed in uninoculated plants which were given 100 per cent P (Figure 4).

4.1.7. Effect of VAM Fungus at Different P Levels on Root P Concentration and Content of Chrysanthemum :

VAM fungus did not have significant effect on root P concentration (Table 6).

Table 6 : Effect of soil inoculation with VAM fungus at different P levels on shoot and roo
concentration and content of Chrysanthemum.

Treatment	Shoot		Root	
	P concentration (%)	P content (mg/plant)	P concentration (%)	P content (mg/plant
U P 100	0.12b	119.82d	0.26b	93.67b
I P 100	0.16b	161.36b	0.27b	86.29c
IP 75	0.32a	379.84a	0.33a	147.14a
I P 50	0.16b	122.19c	0.26b	75.92d

Means followed by the same superscript within each column do not differ significantly at P = 0.05

U P 100 = Uninoculated, 100 per cent recommended P.

I P 100 = Inoculated, 100 per cent recommended P.

I P 75 = Inoculated, 75 per cent recommended P.

I P 50 = Inoculated, 50 per cent recommended P.

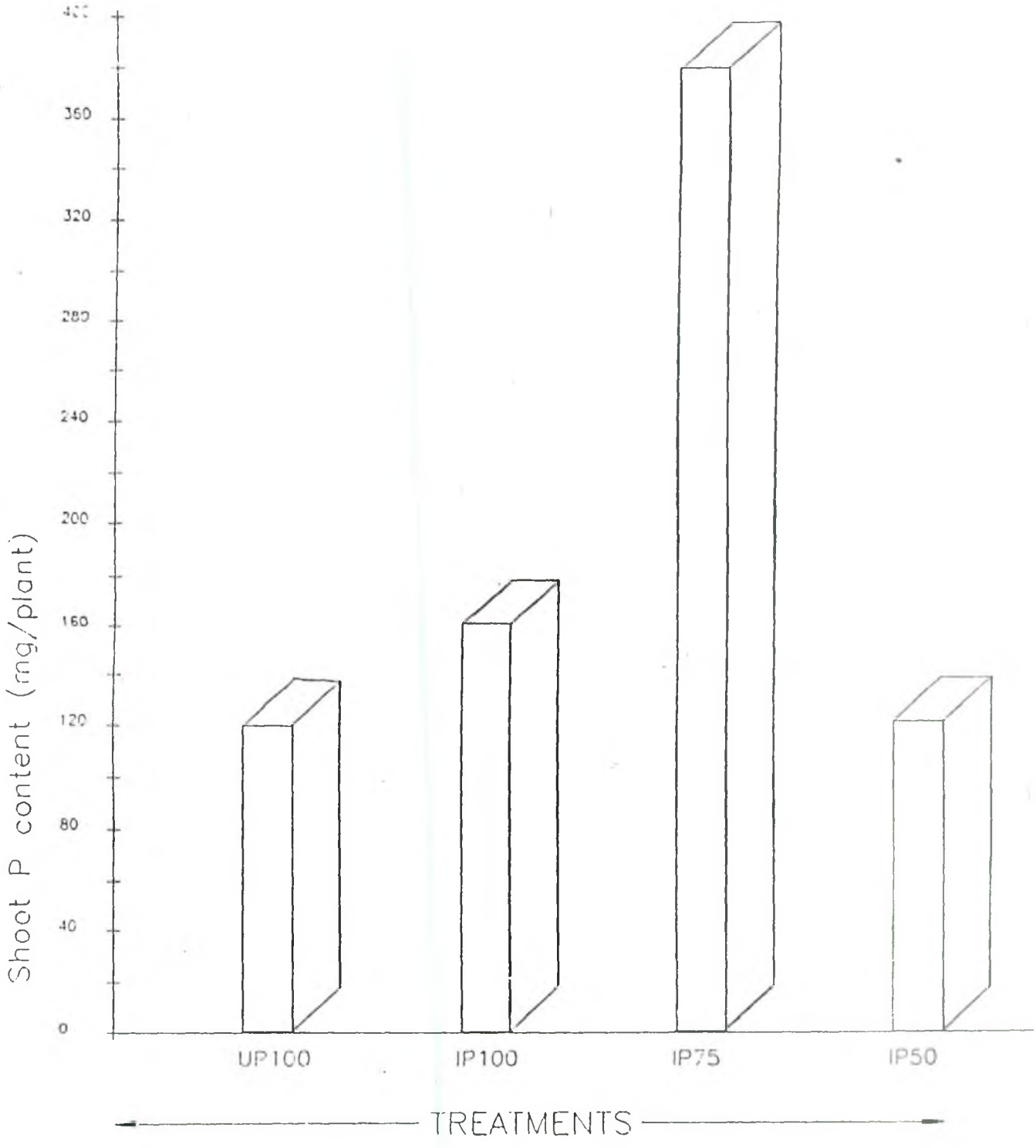

FIG 4 : Effect of soil inoculation with VAM fungus at different P levels on shoot P content of Chrysanthemum.

However, maximum root P concentration was observed in plants inoculated with VAM fungus at 75 per cent P (0.33). The minimum root P concentration was recorded in uninoculated plants and in the inoculated plants at 50 per cent P which was statistically on par with inoculated plants at 100 per cent P.

VAM fungal inoculation had significant effect on root P content (Table 6). Maximum root P content was observed in plants inoculated with the VAM fungus and given 75 per cent of the recommended P which was significantly higher than all the other treatments. Minimum root P content was seen in inoculated plants given 50 per cent P.

4.1.8. Mycorrhizal Spore Number in Root Zone Soil :

The mycorrhizal spore number in root zone soil as influenced by mycorrhizal inoculation at different P levels is presented in Table 7. Treatment differences were found to be statistically significant.

Spore count was maximum in plants inoculated with the VAM fungus at 75 per cent P followed by plants inoculated with VAM at 50 per cent P and plants inoculated with VAM at 100 per cent P. Spore count in the uninoculated control was least.

4.1.9. Per cent VAM Root Colonization :

The data on per cent VAM root colonization is presented in Table 7. There was significant effect of VAM on per cent root colonization at different P levels. It was maximum in inoculated plants given 75 per cent recommended P which was significantly higher than the other treatments. The root colonization in inoculated plants at 50 per cent P (85.02 per cent) was significantly more than that of

Table 7 : Effect of soil inoculation with VAM fungus at different P levels on mycorrhizal spore number in root zone soil and per cent mycorrhizal root colonization in Chrysanthemum.

Treatments	Spore number per 50 ml soil	Root colonization (%)
U P 100	182.92d	46.36d
I P 100	227.70c	78.19c
I P 75	325.54a	94.70a
I P 50	266.00d	85.02b

Means followed by the same superscript within each column do not differ significantly at P = 0.05

U P 100 = Uninoculated, 100 per cent recommended P.

I P 100 = Inoculated, 100 per cent recommended P.

I P 75 = Inoculated, 75 per cent recommended P.

I P 50 = Inoculated, 50 per cent recommended P.

inoculated plants given 100 per cent P. The uninoculated plants had least root colonization of 46.32 per cent.

4.2. Effect of VAM Inoculation at Different P Levels on :

4.2.1. Growth Parameters of China aster :

4.2.1.a. Plant Height :

The data on plant height as influenced by soil inoculation with the VAM fungus at different P levels is presented in Table 8.

In general, the inoculated plants had greater plant height compared to uninoculated plants at 45, 60, 90 days after planting (DAP). At all the stages, maximum plant height was observed in plants inoculated with the VAM fungus and given 75 per cent recommended P but it was statistically on par with that of plants inoculated with VAM at 100 and 50 per cent recommended P. The uninoculated plants which were given 100 per cent recommended P showed the least plant height.

4.2.1.b. Number of Branches :

The data on the number of branches observed at 60 and 90 DAP is presented in Table 8.

Number of branches did not differ significantly due to VAM inoculation at different P levels at 60 DAP. However, maximum number of branches were observed in plants inoculated with VAM fungus at 75 per cent and 50 per cent P. Least number of branches was seen in uninoculated control plants given 100 per cent P.

At 90 DAP, the number of branches did not differ significantly in plants

Table 8 : Effect of soil inoculation with VAM fungus at different P levels on plant height, number of branches and leaves of China aster .

Treatment	Plant height (cm)			Number of branches			Number of leaves			
	Days after planting									
	30	45	60	30	60	90	30	45	60	90
U P100	6.00	10.80b	18.30ab	24.70b	1.8a	2.7bc	9.3a	14.3b	22a	25.5a
I P 100	6.00	14.20a	20.60a	30.40a	2.2a	3.1abc	10.3a	18.4ab	22.8a	26.7a
I P 75	5.80	14.70a	23.30a	33.60a	2.6a	4.3a	10.1a	19.8a	22.6a	26.9a
I P 50	5.80	14.80a	23.40a	33.40a	2.6a	3.8ab	10.3a	16.6ab	24.8a	27.2a

Means followed by the same superscript within each column do not differ significantly at P = 0.05

U P 100 = Uninoculated, 100 per cent recommended P.

I P 100 = Inoculated, 100 per cent recommended P.

I P 75 = Inoculated, 75 per cent recommended P.

I P 50 = Inoculated, 50 per cent recommended P.

inoculated with the VAM fungus at different P levels. However, the number of branches in inoculated plants at 75 per cent P was significantly higher than that of the uninoculated plants.

4.2.1.c. Number of Leaves :

There was no significant difference on the number of leaves as a result of VAM inoculation at different P levels at different stages of growth (Table 8). At 30 DAP, maximum number of leaves was observed in inoculated plants given 50 per cent P and 100 per cent P. Least number of leaves were observed in uninoculated plants.

At 45 DAP maximum number of leaves was observed in inoculated plants given 75 per cent P followed by inoculated plants given 100 per cent P and inoculated plants at 50 per cent P. The least number of leaves was found in uninoculated plants.

At harvest (90 DAP), maximum number of leaves was observed in plants inoculated with the VAM fungus at 50 per cent P and minimum number of leaves in uninoculated plants.

4.2.2. Effect of VAM at Different P Levels on The Time Taken for Flower Bud Initiation and 50 Per cent Flowering of China aster :

The data on the number of days taken for flower bud initiation and 50 per cent flowering is presented in Table 9 and Figure 5.

Inoculation with the VAM fungus gave significant results with respect to the number of days taken for flower bud initiation. Flower bud initiation was earlier in inoculated plants as compared to uninoculated plants. The uninoculated plants

took maximum number of days for bud initiation (68.90).

Plants inoculated with the VAM fungus at 75 per cent recommended P took significantly less number of days for bud initiation (63) as compared to the other treatments. Flower bud initiation was earlier in inoculated plants at 100 per cent P compared to inoculated plants with 50 per cent P.

Plants inoculated with the VAM fungus flowered earlier than the uninoculated plants. The uninoculated plants took maximum number of days for flowering (85) followed by inoculated plants at 50 per cent P (82.8 days) and 100 per cent P (81.60 days). Inoculated plants at 75 per cent P took minimum number of days for flowering (80.4). The number of days taken for 50 per cent flowering by inoculated plants was significantly different from that of the uninoculated plants.

4.2.3. Effect of VAM Fungus at Different P Levels on Flower Number and Flower Yield :

The data on flower number and flower yield as influenced by inoculation with the VAM fungus at different P levels is presented in Table 10.

Maximum number of flowers per plant was observed in inoculated plants with 75 per cent P which differed significantly from the other treatments. The number of flowers in inoculated plants at 100 per cent P and 50 per cent were statistically on par with each other but significantly more than the uninoculated control plants which had the least number of flowers (7.7).

Flower yield was maximum in inoculated plants at 75 per cent P (45.20) which was significantly greater than the other treatments. Plants inoculated with the VAM fungus at 50 per cent P gave slightly higher yield than inoculated plants at 100 per cent P though they were statistically on par with each other.

PLATE 3: Response of China aster to VA mycorrhizal inoculation.

A = Uninoculated, recommended P.
C = Inoculated, 75 percent P.

PLATE 3

Table 9 : Effect of soil inoculation with VAM fungus at different levels of P on time taken for flower bud initation, 50 per cent flowering, flower number and flower yield of China aster.

Treatment	Days taken for flower bud initiation	Days taken for 50 per cent flowering	Number of flowers	Flower yield per plant (g)
U P 100	68.90a	85.00a	7.7c	32.0c
I P 100	65.50b	81.60bc	10.9b	34.7bc
I P 75	63.00c	80.40c	13.7a	45.2a
I P 50	68.30a	82.80b	10.2b	39.04b

Means followed by the same superscript within each column do not differ significantly at P = 0.

U P 100 = Uninoculated, 100 per cent recommended P.

I P 100 = Inoculated, 100 per cent recommended P.

I P 75 = Inoculated, 75 per cent recommended P.

I P 50 = Inoculated, 50 per cent recommended P.

UP100 – Uninoculated, recommended P

IP100 – Inoculated, recommended P

IP75 – Inoculated, 75 percent P

IP50 – Inoculated, 50 percent P

☒ No. of days taken for flower bud initiation.

☐ No. of days taken for 50 percent flowering

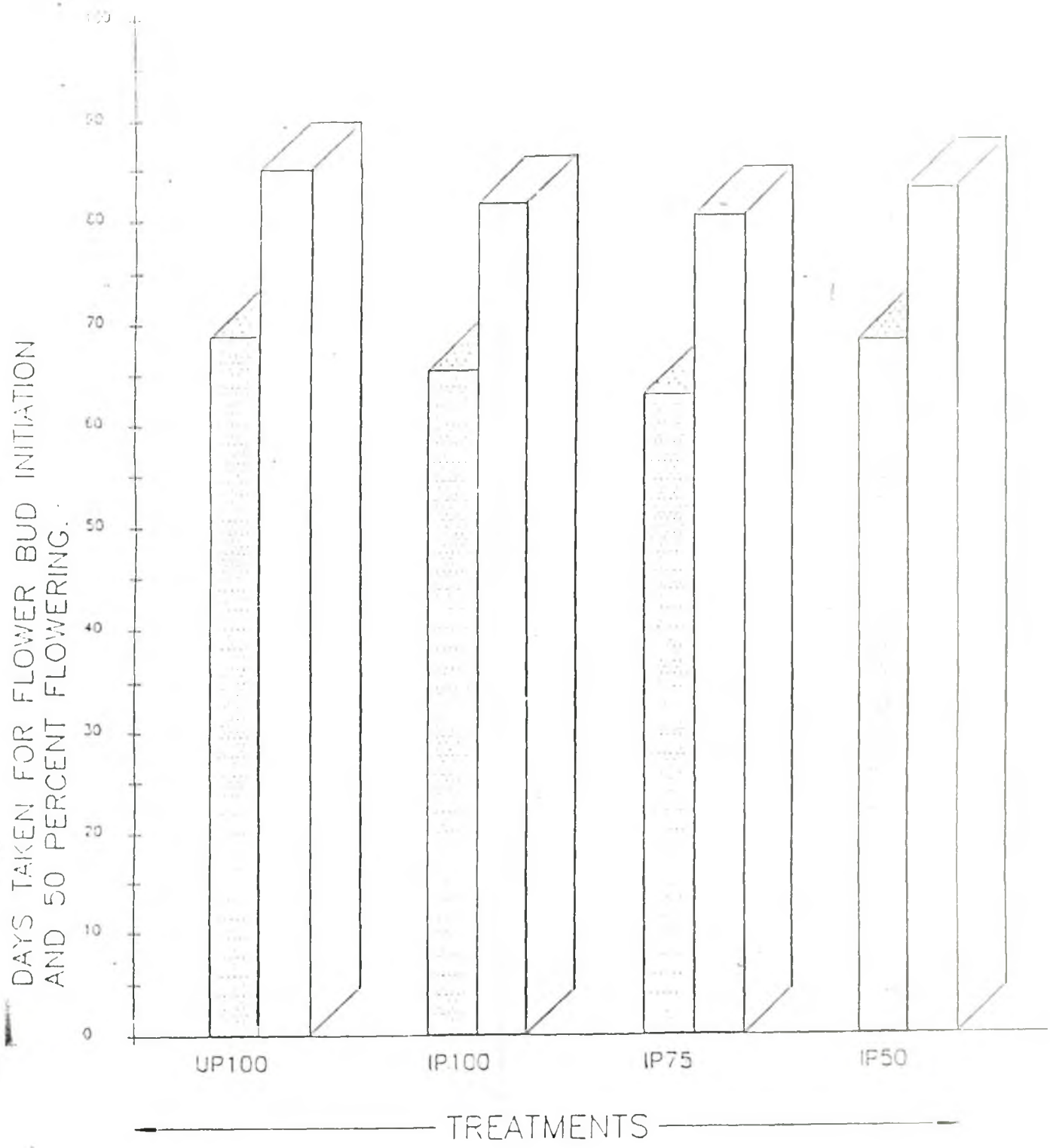

FIG 5 : Effect of soil inoculation with VAM fungus at different P levels on time taken for flower bud initation and 50 per cent flowering of China aster.

UP100 – Uninoculated, recommended P

IP100 – Inoculated, recommended P

IP75 – Inoculated, 75 percent P

IP50 – Inoculated, 50 percent P

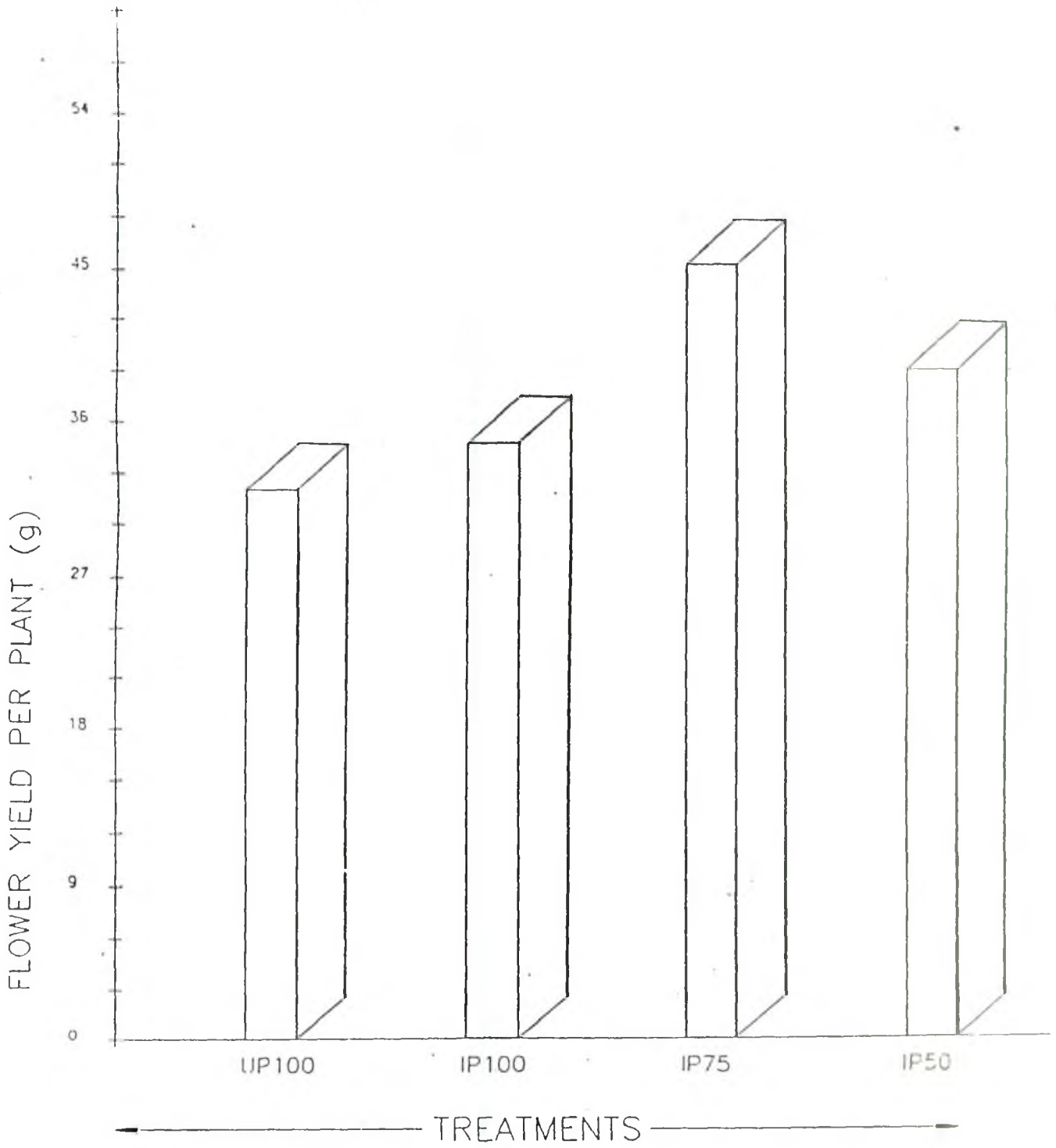

FIG 6 : Effect of soil inoculation with VAM fungus at different P levels on flower yield per plant of China aster.

Uninoculated plants had the lowest yield of 32 g / plant and was statistically on par with the yield of plants inoculated with VAM at 100 per cent P (Figure 6).

4.2.4. Effect of VAM at Different P Levels on Flower Characteristics of China aster :

4.2.4.a. Flower Diameter :

There was significant effect of VAM fungus on flower diameter. In general, the inoculated plants at different P levels had higher flower diameter compared to uninoculated control plants given 100 per cent recommended P (Table 10).

Maximum flower diameter was recorded in plants inoculated with the VAM fungus at 75 per cent recommend P (6.18 cm) which was significantly greater than that of other treatments. The flower diameter of inoculated plants given 50 per cent P was on par with inoculated plants given 100 per cent P and they were significantly greater than that of uninoculated control plants given 100 per cent P which had recorded the lowest flower diameter (5.58 cm).

4.2.4.b. Stalk Length :

The stalk length was maximum in inoculated plants given 75 per cent P (24.6 cm) and it was significantly higher than that of the other treatments. The lowest stalk length was recorded in inoculated plants at 50 per cent recommended P (22.60 cm) which was statistically on par with that of inoculated plants given 100 per cent P.

Table 10 : Effect of soil inoculation with VAM fungus at different P levels on flower characteristics of China aster.

Treatment	Flower diameter (cm)	Stalk length (cm)
U P 100	5.58c	23.95b
I P 100	5.96b	22.80c
I P 75	6.18a	24.60a
I P 50	5.97b	22.60c

Means followed by the same superscript within each column do not differ significantly at P = 0.05

U P 100 = Uninoculated, 100 per cent recommended P.

I P 100 = Inoculated, 100 per cent recommended P.

I P 75 = Inoculated, 75 per cent recommended P.

I P 50 = Inoculated, 50 per cent recommended P.

4.2.5. Effect of VAM Fungus at Different P Levels on Plant Biomass of China aster :

The data on plant biomass as influenced by soil inoculation with VAM fungus as different P levels is presented in Table 11 and Figure 7.

4.2.5.a. Shoot :

Maximum shoot biomass was recorded in inoculated plants given 75 per cent of the recommended P which was significantly higher than the other treatments. The lowest shoot biomass was observed in uninoculated plants given 100 per cent P which was statistically on par with that of inoculated plants given 100 and 50 per cent recommended P.

4.2.5.b. Root :

Plants inoculated with VAM fungus at 75 per cent P had maximum root biomass but it was statistically on par with that of inoculated plants given 50 per cent P. Uninoculated plants given 100 per cent P showed minimum root biomass which was on par with that of inoculated plants given 100 per cent P.

4.2.5.c. Total Biomass :

There was significant difference among the treatments with respect to total plant biomass.

Total biomass was maximum in inoculated plants given 75 per cent recommended P which was significantly higher than other treatments. The biomass of inoculated plants at 50 per cent P was significantly higher than that of

Table 11: Effect of soil inoculation with VAM fungus at different P levels on plant biomass of China aster.

Treatment	Biomass (g/plant)		
	Shoot	**Root**	**Total**
U P 100	13.52b	10.55c	24.07c
I P 100	13.73b	10.77bc	24.50c
I P 75	18.42a	11.88a	30.30a
I P 50	14.48b	11.13ab	25.61b

Means followed by the same superscript within each column do not differ significantly at P = 0.05

U P 100 = Uninoculated, 100 per cent recommended P.

I P 100 = Inoculated, 100 per cent recommended P.

I P 75 = Inoculated, 75 per cent recommended P.

I P 50 = Inoculated, 50 per cent recommended P.

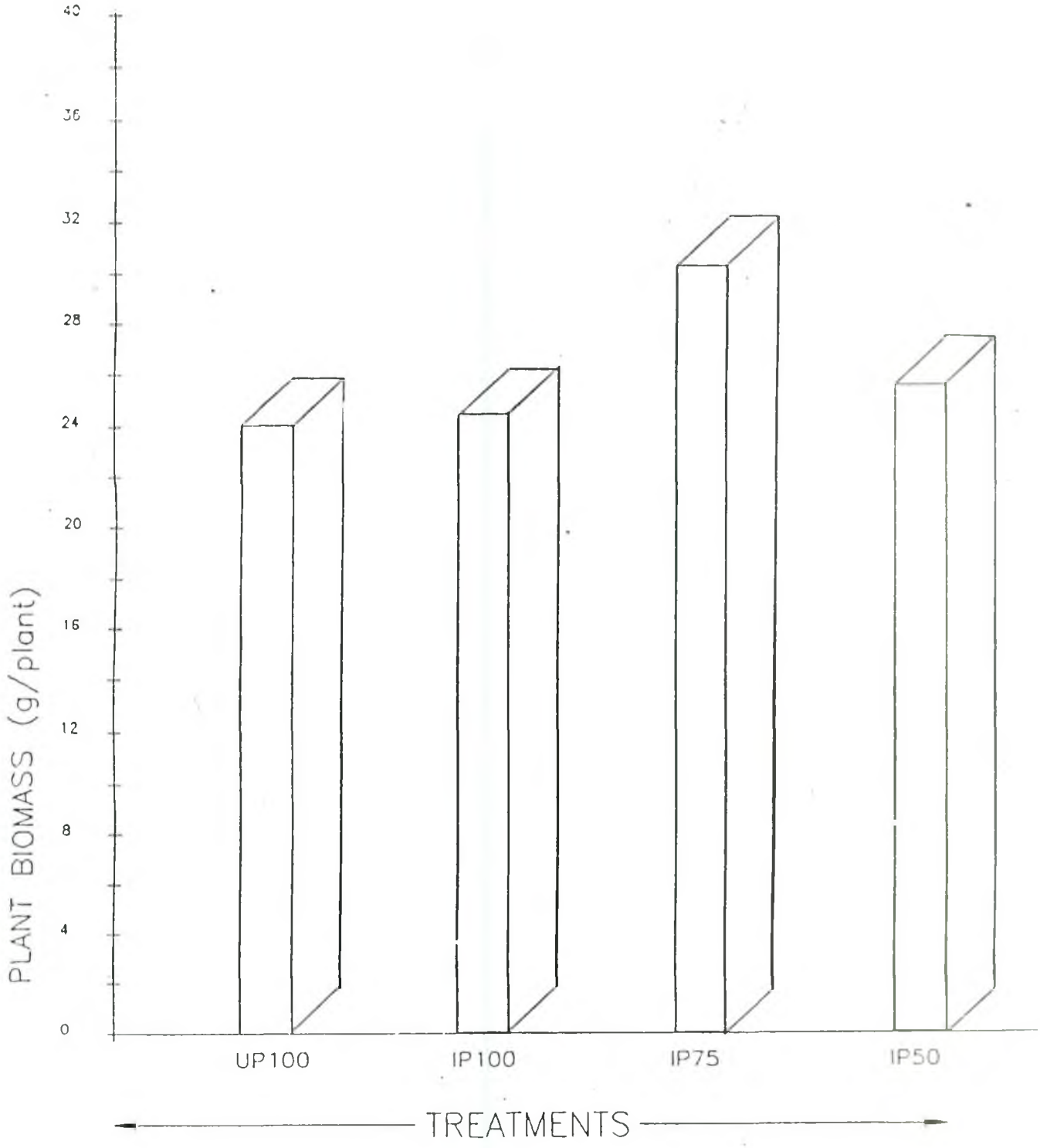

FIG 7 : Effect of soil inoculation with VAM fungus at different P levels on plant biomass of China aster.

cent P, the latter two being statistically on par with each other.

4.2.6. Effect of VAM fungus at Different P Levels on Shoot P Concentration and Content of China aster :

The data on the effect of VAM fungus at different P levels on shoot P concentration and content is given in Table 12.

There was no significant difference among the inoculated plants at different P levels with regard to shoot P concentration but shoot P concentration in inoculated plants was significantly higher than that of uninoculated plants. However, the highest shoot P concentration was recorded in inoculated plants at 75 per cent P.

There was significant effect of VAM fungus at different P levels on shoot P content of plants. Shoot P content was maximum in inoculated plants at 75 per cent P which was significantly higher than that of other treatments. This was followed by plants inoculated with VAM at 50 per cent P which had significantly higher shoot P content compared to uninoculated control plants and inoculated plants given 100 per cent P. The uninoculated plants given 100 per cent P had least P content (Figure 8).

4.2.7. Effect of VAM Fungus at Different P Levels on Root P Concentration and Content of China aster :

Maximum root P concentration was recorded in inoculated plants at 50 per cent P which was significantly greater than that of the other treatments. Minimum root P concentration was seen in uninoculated plants given 100 per cent P but it was statistically on par with inoculated plants given 100 per cent P and 75 per cent P.

Table 12 : Effect of soil inoculation with VAM fungus at different P levels on shoot and root P concentration and content of China aster.

Treatment	Shoot		Root	
	P concentration (%)	P content (mg/plant)	P concentration (%)	P content (mg/plant)
U P 100	0.22b	30.20d	0.128b	13.50c
I P 100	0.31a	41.91c	0.250b	26.92b
IP 75	0.35a	57.47a	0.140b	15.23c
I P 50	0.34a	49.23b	0.374a	41.62a

Means followed by the same superscript within each column do not differ significantly at P = 0.05

U P 100 = Uninoculated, 100 per cent recommended P.

I P 100 = Inoculated, 100 per cent recommended P.

I P 75 = Inoculated, 75 per cent recommended P.

I P 50 = Inoculated, 50 per cent recommended P.

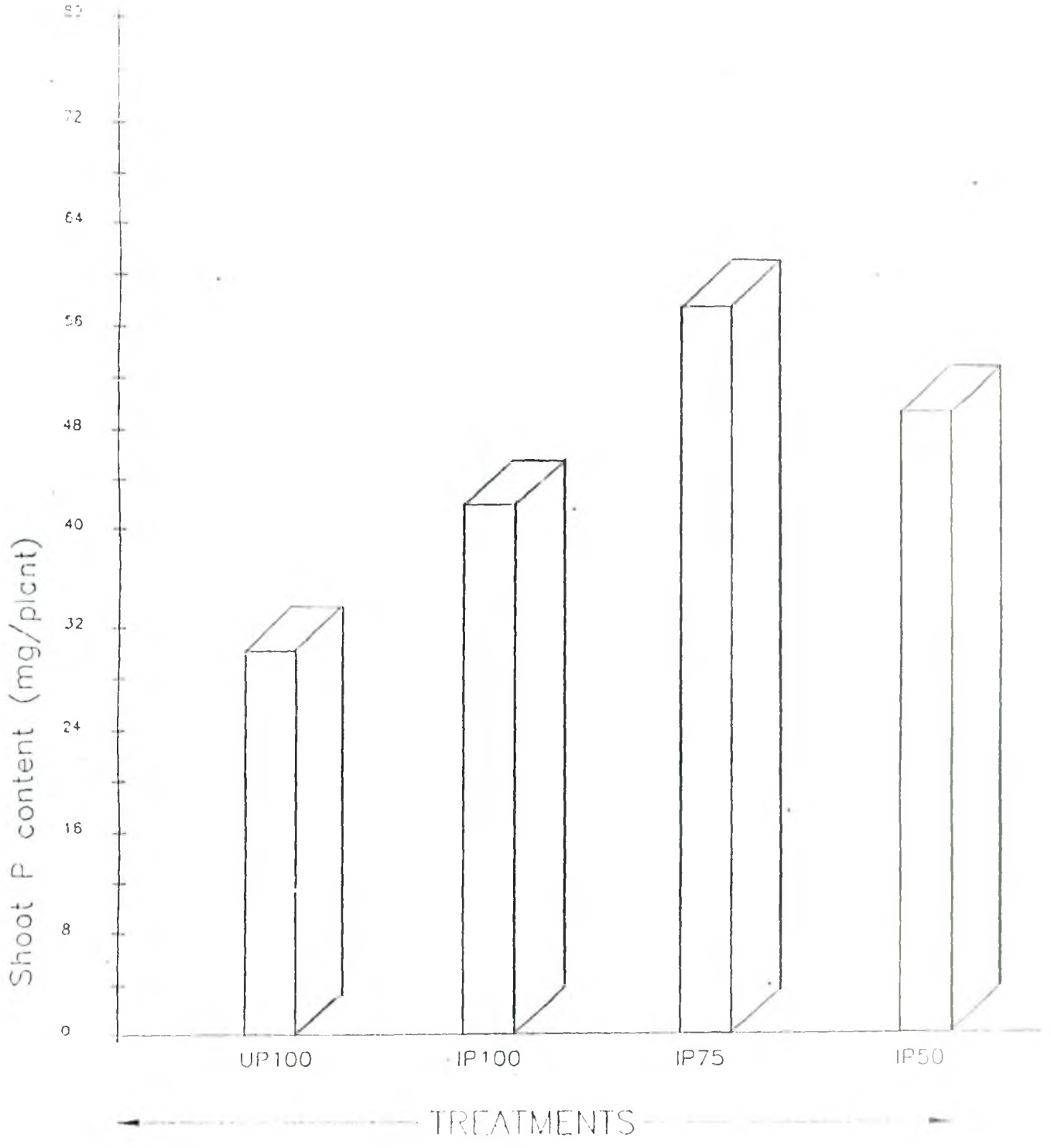

FIG 8 : Effect of soil inoculation with VAM fungus at different P levels on shoot P content of China aster.

VAM fungus had significant effect on the root p content (Table 12). Maximum root P content was observed in inoculated plants at 50 per cent recommended P (41.62 mg/plant) which was significantly higher than that of the other treatments. The lowest P content (13.50 mg/plant) was recorded in uninoculated plants given 100 per cent P but it was statistically on par with that of plants inoculated with VAM at 75 per cent P.

4.2.8. Mycorrhizal Spore Number in Root Zone Soil of China aster :

The mycorrhizal spore number in root zone soil as influenced by mycorrhizal inoculation at different P levels is presented in Table 13. Treatment differences were found to be statistically significant.

Spore count per 50 ml soil was maximum in plants inoculated with VAM fungus at 50 per cent recommended P followed by inoculated plants at 75 per cent P. Spore count in uninoculated plants was the least.

4.2.9. Per cent VAM Root Colonization of China aster :

There was significant effect of VAM on root colonization at different P levels. Root colonization was maximum in inoculated plants given 50 per cent P but it was statistically on par with that of inoculated plants given 75 per cent P. Root colonization in plants inoculated with VAM at 100 per cent P was significantly higher than that of uninoculated control plants. The uninoculated plants had the least mycorrhizal root colonization.

Table 13: Effect of soil inoculation with VAM fungus at different P levels on mycorrhizal spore number in root zone soil and per cent mycorrhizal root colonization in China aster

Treatment	Spore number per 50 ml soil	Root colonization (%)
U P 100	186.80d	42.10c
I P 100	368.40c	56.63b
I P 75	468.80b	71.40a
I P 50	513.70a	71.91a

Means followed by the same superscript within each column do not differ significantly at P = 0.05

U P 100 = Uninoculated, 100 per cent recommended P.

I P 100 = Inoculated, 100 per cent recommended P.

I P 75 = Inoculated, 75 per cent recommended P.

I P 50 = Inoculated, 50 per cent recommended P.

4.3. Effect of VAM Inoculation at Different P Levels On :

4.3.1. Growth Parameters of Marigold :

The data on growth parameters as influenced by inoculation with VAM at different P levels is presented in Table 14.

4.3.1.a. Plant Height :

At 30 DAP, maximum plant height was observed in inoculated plants given 100 per cent P. The lowest plant height was observed in inoculated plants given 50 per cent P. At 45 DAP, there was no significant difference among the treatments except in inoculation treatment given 50 per cent P which had the least plant height. Similar trend was seen at 60 DAP.

4.3.1.b. Number of Leaves :

At 30 DAP, maximum number of leaves was observed in inoculated plants with 100 per cent P which was statistically on par that of plants inoculated with VAM at 50 per cent P and uninoculated control plants given 100 per cent P. Minimum number of leaves was seen in inoculated plants at 75 per cent P.

At 45 DAP, maximum number of leaves was seen in inoculated plants given 75 per cent P which was significantly higher than the other treatments.

At 60 DAP, maximum number of leaves was seen in inoculated plants given 75 per cent P which was statistically on par with that of uninoculated plants given 100 per cent P. Plants inoculated with VAM fungus at 100 and 50 per cent recommended P showed significantly less number of leaves.

Table 14 : **Effect of soil inoculation with VAM fungus at different P levels on plant height, number of branches and leaves of Marigold.**

Treatment	Plant height (cm)			Number of leaves			Number of branches		
	Days after planting								
	30	45	60	30	45	60	30	45	60
U P100	35.00ab	40.17a	41.08a	12.33ab	27.83b	40.17a	4.50a	6.08a	7.17a
I P 100	38.25a	41.42a	43.33a	13.25a	27.17b	29.83b	4.00a	5.67a	7.58a
I P 75	37.67ab	41.92a	43.42a	11.67b	31.08a	41.92a	4.58a	5.83a	7.58a
I P 50	34.17b	36.67b	38.08b	12.67ab	27.83b	30.00b	4.17a	5.92a	7.00a

Means followed by the same superscript within each column do not differ significantly at P = 0.05

U P 100 = Uninoculated, 100 per cent recommended P.

I P 100 = Inoculated, 100 per cent recommended P

I P 75 = Inoculated, 75 per cent recommended P

I P 50 = Inoculated, 50 per cent recommended P

4.3.1.c. Number of Branches :

The effect of VAM fungus at different P levels on number of branches was not significant at different stages of plant growth. However, maximum number of branches was observed in inoculated plants at 75 per cent P.

4.3.2. Effect of VAM at Different Levels of P on The Time Taken for Bud Initiation and 50 Per cent Flowering of Marigold :

The effect of VAM fungus on the number of days taken for flower bud initiation was significant (Table 15) at different levels of P. The uninoculated plants took maximum number of days for bud initiation (32.25) which was significantly higher than the number of days taken for bud initiation in the other treatments. Next higher number of days was taken by plants inoculated with VAM at 100 per cent P which was statistically higher than the number of days taken for bud initiation in inoculated plants given 75 and 50 per cent of the recommended P. Inoculated plants at 50 per cent recommended P took the least number of days for bud initiation which was significantly different from the other treatments

Significant effect of the VAM fungus on 50 per cent flowering was seen (Table 15). Inoculated plants at 50 per cent recommended P flowered earlier than the other plants. Maximum number of days for 50 per cent flowering was taken by the uninoculated plants which was significantly more than that of other treatments (Figure).

4.3.3. Effect of VAM Fungus at Different P Levels on Flower Number and Flower Yield of Marigold :

The data on the effect of VAM fungus on flower number and flower yield

PLATE 4: Response of Marigold to VA mycorrhizal inoculation

A = Uninoculated, recommended P.
D = Inoculated, 50 percent P.

PLATE 4

Table 15 : Effect of soil inoculation with VAM fungus at different levels of P on time taken for flower bud initation, 50 per cent flowering, flower number and flower yield of Marigold.

Treatment	Days taken for flower bud initiation	Days taken for 50 per cent flowering	Number of flowers	Flower yield per plant (g)
U P 100	33.25a	35.42a	7.25b	28.92b
I P 100	29.92b	33.92b	7.42b	31.10b
I P 75	26.33c	28.92c	8.75a	44.05a
I P 50	23.33d	25.17d	8.07a	36.02a

Means followed by the same superscript within each column do not differ significantly at P = 0.05

U P 100 = Uninoculated, 100 per cent recommended P.

I P 100 = Inoculated, 100 per cent recommended P.

I P 75 = Inoculated, 75 per cent recommended P.

I P 50 = Inoculated, 50 per cent recommended P.

UP100 – Uninoculated, recommended P

IP100 – Inoculated, recommended P.

IP75 – Inoculated, 75 percent P

IP50 – Inoculated, 50 percent P

▨ No. of days taken for flower bud initiation.

☐ No. of days taken for 50 percent flowering

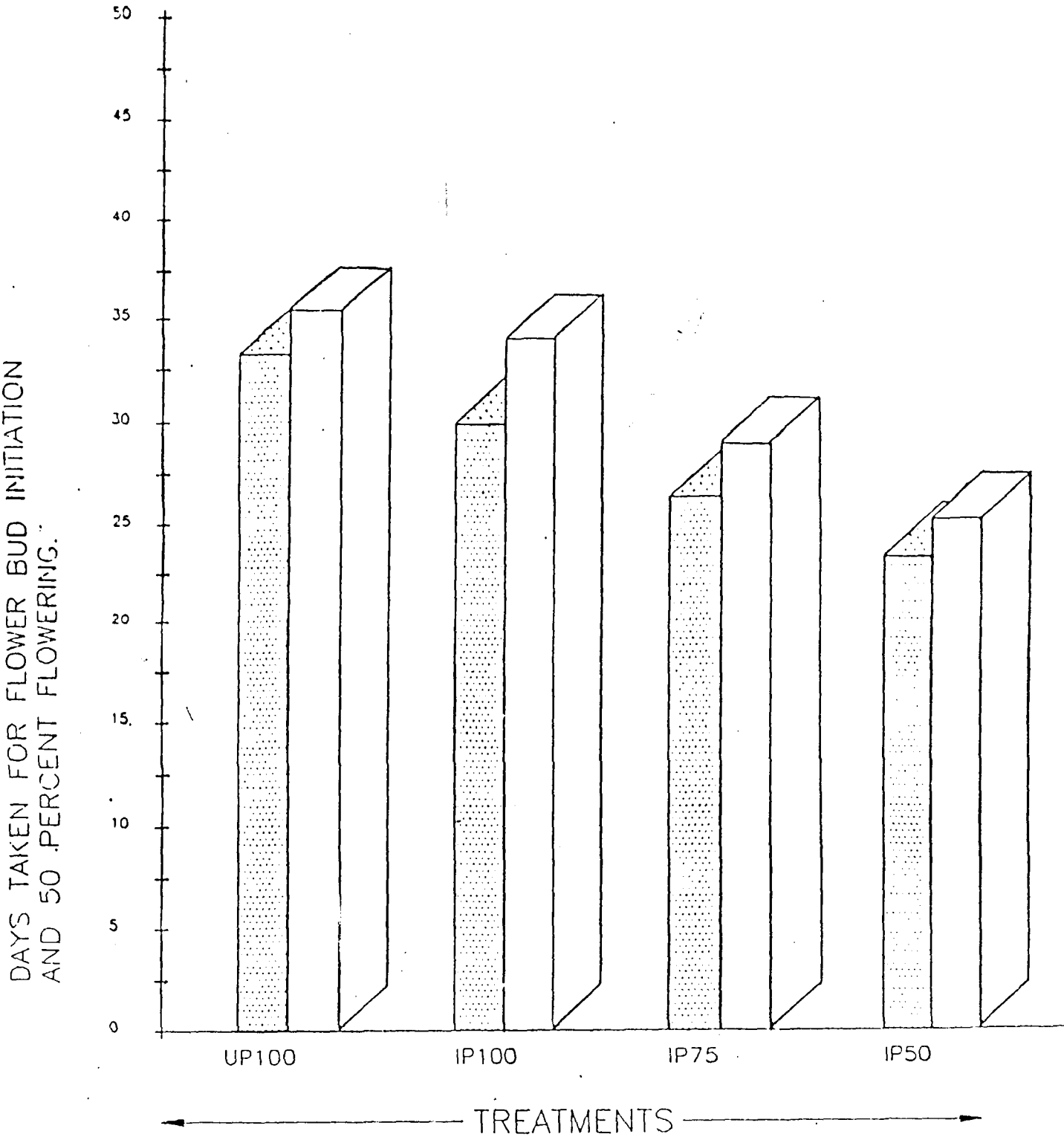

FIG 9 : Effect of soil inoculation with VAM fungus at different P levels on time taken for flower bud initation and 50 per cent flowering of Marigold.

UP100 – Uninoculated, recommended P

IP100 – Inoculated, recommended P

IP75 – Inoculated, 75 percent P

IP50 – Inoculated, 50 percent P

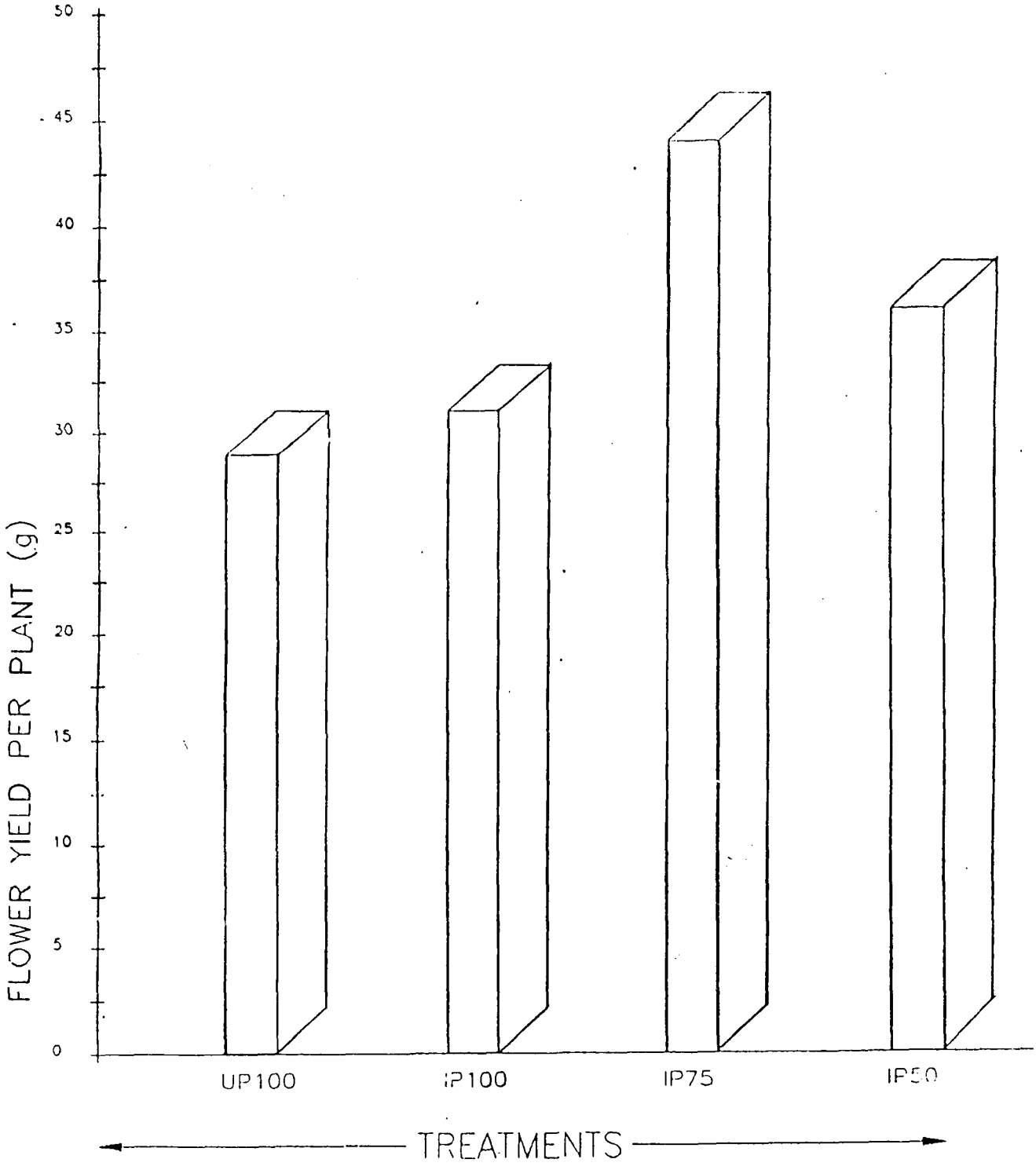

FIG 10 : Effect of soil inoculation with VAM fungus at different P levels on flower yield per plant of Marigold

per plant is presented in Table 15.

Maximum number of flowers were harvested in the inoculated plants at 75 per cent P which was statistically on par with that of inoculated plants given 50 per cent P. Least number of flowers occurred in uninoculated plants given 100 per cent P but it was on par with that of inoculated plants given 100 per cent P.

Flower yield per plant was maximum in inoculated plants given 75 per cent recommended P but was on par with that of inoculated plants given 50 per cent P. Flower yield per plant was least in uninoculated plants given 100 per cent P but was on par with that of uninoculated plants given 100 per cent P (Figure 10).

4.3.4. Effect of VAM Fungus at Different P Levels on Flower Characteristics of Marigold :

4.3.4.a. Flower Diameter :

Flower diameter was maximum in the inoculated plants given 50 per cent P but it was statistically on par with that of inoculated plants given 75 per cent P. Flower diameter was lowest in uninoculated plants but was on par with inoculated plants given 100 per cent P. (Table 16).

4.3.4.b. Stalk Length :

There was no significant difference among different treatments with respect to stalk length. However, stalk length was maximum in the inoculated plants which were given 50 per cent of the recommended P and it was least in the uninoculated plants. (Table 16).

Table 16 : Effect of soil inoculation with VAM fungus at different P levels on flower characteristics of Marigold.

Treatment	Flower diameter (cm)	Stalk length (cm)	Dry weight (g)
U P 100	5.58b	5.86b	4.06a
I P 100	6.00b	6.00b	3.82a
I P 75	6.25a	6.85a	3.44a
I P 50	6.75ab	7.00a	4.34a

Means followed by the same superscript within each column do not differ significantly at P = 0.05

U P 100 = Uninoculated, 100 per cent recommended P.

I P 100 = Inoculated, 100 per cent recommended P.

I P 75 = Inoculated, 75 per cent recommended P.

I P 50 = Inoculated, 50 per cent recommended P.

4.3.4.c. Flower Dry weight :

There was no significant effect of VAM fungus at different P levels on flower dry weight (Table 16). However, maximum dry weight (4.34 g/plant) was recorded in the inoculated plants given 50 per cent recommended P The minimum dry weight was seen in the inoculated plants given 50 per cent P

4.3.5. Effect of VAM at Different P Levels on Plant Biomass of Marigold :

The data on the effect of VAM at different P levels on plant biomass is presented in Table 17 and Figure 11.

4.3.5.a. Shoot Biomass :

There was no significant difference in shoot biomass among the inoculated plant at different P levels but the shoot biomass was significantly greater in inoculated plants when compared to the uninoculated plants. Among the inoculated plants, maximum shoot biomass was seen in plants supplied with 50 per cent recommended P.

4.3.5.b. Root Biomass :

Trend in the root biomass was similar to that of shoot biomass.

4.3.5.c. Total Biomass of Plants :

The effect of VAM fungus on total biomass showed significant difference among the treatments. Maximum plant biomass was observed in plants inoculated with VAM fungus at 50 per cent recommended P (5.12) which was significantly higher than that of other treatments. The biomass of inoculated plants

Table 17 : Effect of soil inoculation with VAM fungus at different P levels on plant biomass of Marigold.

Treatment	Biomass (g/plant)		
	Shoot	Root	Total
U P 100	2.07b	0.71b	3.41c
I P 100	3.26a	0.88a	4.14b
I P 75	3.86a	1.26a	5.12a
I P 50	3.48a	0.97a	4.45b

Means followed by the same superscript within each column do not differ significantly at P = 0.05

U P 100 = Uninoculated, 100 per cent recommended P.

I P 100 = Inoculated, 100 per cent recommended P.

I P 75 = Inoculated, 75 per cent recommended P.

I P 50 = Inoculated, 50 per cent recommended P.

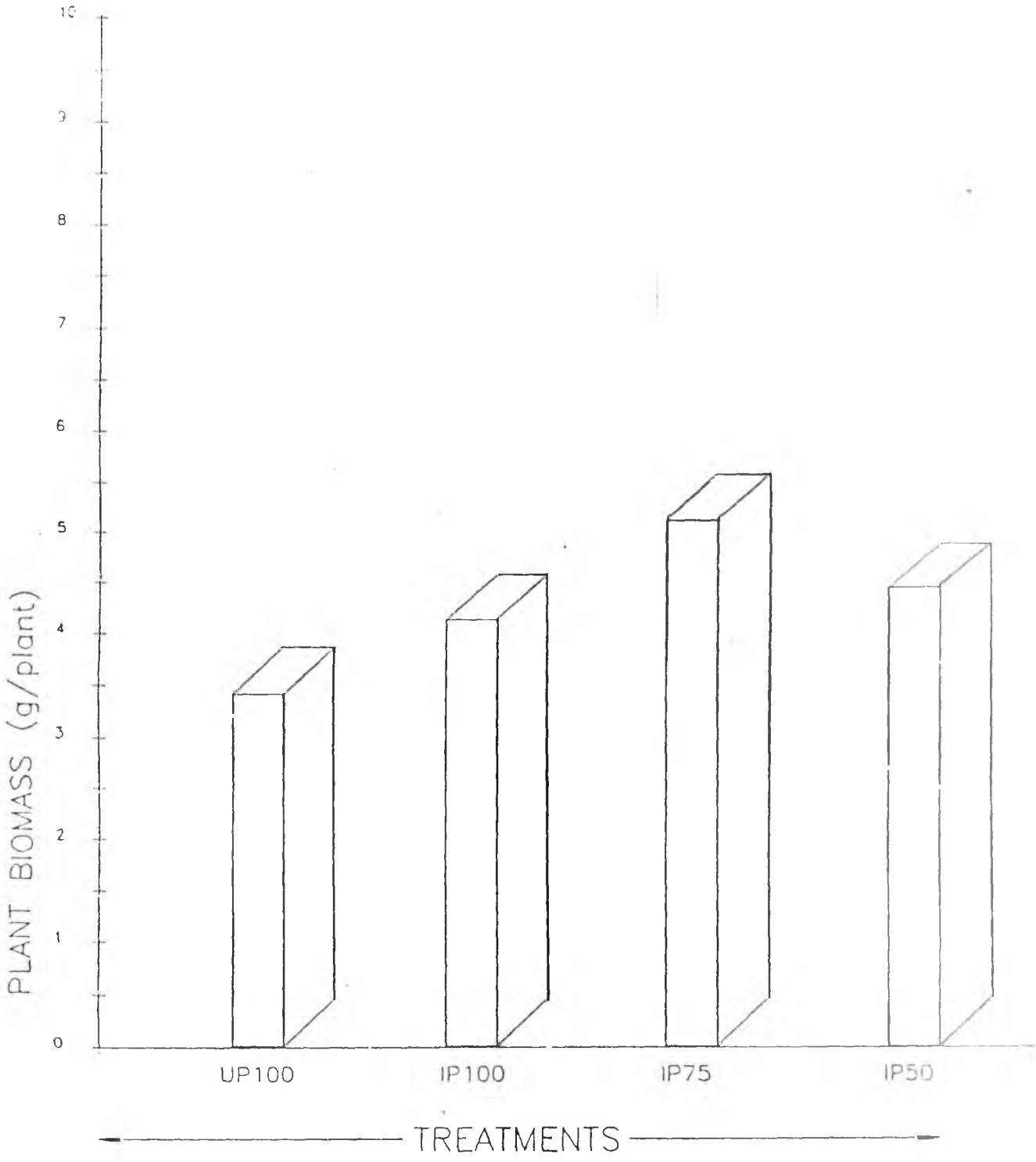

FIG 11 : Effect of soil inoculation with VAM fungus at different P levels on plant biomass of Marigold.

at 75 and 100 per cent P was on par with each other but significantly higher than that of uninoculated plants.

4.3.6. Effect of VAM Fungus at Different P Levels on Shoot P Concentration and Content of Marigold :

Maximum shoot P concentration was observed in inoculated plants at 75 per cent recommended P but it was on par with the shoot P concentration of plants at 50 per cent P. Both were significantly higher than the other two treatments. The least shoot P concentration was observed in uninoculated plants given 100 per cent P which was on par with that of inoculated plants at 100 per cent P. (Table 18).

Maximum shoot P content was seen in inoculated plants at 75 per cent P which was on par with the shoot P content of inoculated plants at 50 per cent recommended P. Both differed significantly from that of inoculated plants at 100 per cent P and uninoculated plants given 100 per cent P. Least shoot P content was seen in the uninoculated plants given 100 per cent P (Figure 12).

4.3.7. Effect of VAM Fungus at Different P Levels on Root P Concentration and Content of Marigold :

Root P concentration was maximum in uninoculated plants but was on par with that of inoculated plants given 100 and 50 per cent P. The root P concentration was lowest in plants inoculated with VAM at 75 per cent of the recommended P which was significantly lower than the other treatments.

Maximum root P content was seen in plants inoculated with VAM fungus at 50 per cent P which was significantly greater than that of other treatments. The P

Table 18 : Effect of soil inoculation with VAM fungus at different P levels on shoot and root concentration and content of Marigold.

Treatment	Shoot		Root	
	P concentration (%)	P content (mg/plant)	P concentration (%)	P content (mg/plant)
U P 100	0.142b	8.71c	0.19a	1.66b
I P 100	0.145b	10.26b	0.189a	1.83b
IP 75	0.180a	14.23a	0.079b	1.56c
I P 50	0.176a	13.76a	0.189a	2.39a

Means followed by the same superscript within each column do not differ significantly at P = 0.05

U P 100 = Uninoculated, 100 per cent recommended P.

I P 100 = Inoculated, 100 per cent recommended P.

I P 75 = Inoculated, 75 per cent recommended P.

I P 50 = Inoculated, 50 per cent recommended P.

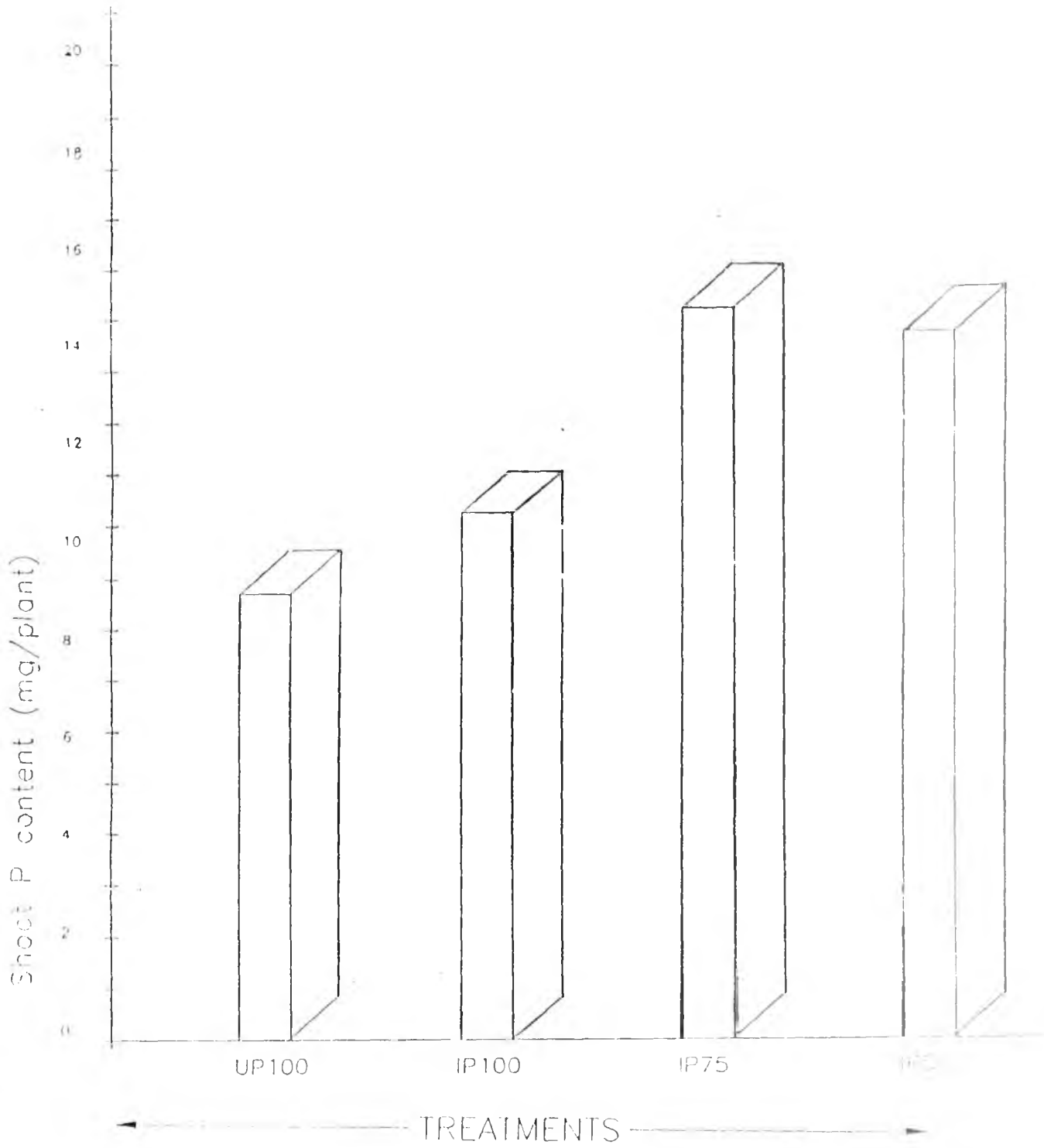

FIG 12 : Effect of soil inoculation with VAM fungus at different P levels on shoot P content of Marigold.

content of uninoculated plants was on par with that of inoculated plants given 100 per cent P. (Table 18).

4.3.8. Mycorrhizal Spore Number in Root Zone Soil :

Treatment differences were found to be statistically significant (Table 19). Spore count per 50 ml soil was maximum (355.7) in plants inoculated with VAM fungus at 50 per cent P followed by plants inoculated with VAM at 75 per cent and 100 per cent P. Spore count in the uninoculated control plants was the least (149.7).

4.3.9. Per cent VAM Root Colonization :

There was significant effect of VAM on per cent root colonization at different P levels (Table 19). Mycorrhizal colonization was maximum in plants inoculated with the VAM fungus at 75 per cent P which was significantly higher than that of the other treatments. Lowest mycorrhizal colonization was observed in uninoculated plants given 100 per cent P.

Table 19 : Effect of soil inoculation with VAM fungus at different P levels on mycorrhizal spore number in root zone soil and per cent mycorrhizal root colonization in Marigold.

Treatments	Spore number per 50 ml soil	Root colonization (%)
U P 100	149.67d	54.08c
I P 100	289.58c	63.92b
I P 75	308.50b	78.83a
I P 50	355.75a	69.83b

Means followed by the same superscript within each column do not differ significantly at P = 0.05

U P 100 = Uninoculated, 100 per cent recommended P.

I P 100 = Inoculated, 100 per cent recommended P.

I P 75 = Inoculated, 75 per cent recommended P.

I P 50 = Inoculated, 50 per cent recommended P.

DISCUSSION

V DISCUSSION

The beneficial effects of VAM fungi on crop plants is well documented. The benefits of mycorrhizal association include improved plant nutrition, growth, stress tolerance and resistance to root pathogens. There is great potential for the use of VAM fungi in ornamental plants. Use of VAM fungi is especially beneficial in nursery production. (Gonzalez Chavez and Ferrera, 1994). In the present investigation, the symbiotic response of the VAM fungus Glomus mosseae in three commercially important ornamental crops viz. Chrysanthemum (Chrysanthemum morifolium), China aster (Callistephus chinensis) and Marigold (Tagetus erecta) at three levels of phosphorous i.e., 100, 75 and 50 per cent of the recommended P was studied. It was compared with the uninoculated plants given 100 per cent P. This was thought to provide information on the possibility of reducing the application of phosphatic fertilizer through VAM inoculation in these plants.

5.1. Effect of VAM at Different P Levels on the Growth Parameters :

Inoculation with the VAM fungus significantly influenced the morphological characters like plant height, number of branches per plant, number of leaves and plant spread. In chrysanthemum, plant height, number of branches and plant spread observed in the inoculated plants given 75 per cent of the recommended P was on par with uninoculated plants given 100 per cent P. From this, it is clear that it is possible to reduce P application by 25 per cent by inoculating with VAM which does not affect the growth parameters adversely.

In China aster, plant height, number of branches and number of leaves

were significantly more in the inoculated plants as compared to the uninoculated plants which were given 100 per cent P. However, maximum effect of VAM fungus on these parameters was observed when soil was inoculated at 75 per cent recommended P. This suggested that growth of China aster is not affected by reducing P fertilizer application by 25 per cent when inoculated with the VAM fungus.

In Marigold, inoculation of soil with the VAM fungus at different P levels did not cause significant increase of growth parameters like plant height, number of leaves and number of branches. However, they were more in the inoculated plants as compared to the uninoculated plants which again suggests the possibility of reducing the application of P fertilizer in Marigold by inoculating the soil with the VAM fungus.

Bagyaraj and Powell (1985) in Marigold, Kale et al. (1987) in Aster and Salvias and Cazares and Smith (1996) in Tagetus erecta and Zinnia elegans reported increase in growth parameters through inoculation with VAM fungi. This influence of VAM fungi on morphological characters may be due to increased P uptake which might have caused cell elongation and multiplication (Black, 1965).

5.2. Effect of VAM at Different P Levels on Flower Characters, Flower Yield and Yield Components :

Inoculation with the VAM fungus influenced flower bud initiation, flowering and flower yield of Chrysanthemum, China aster and Marigold. In all the three crops, early flower bud initiation and early flowering was observed in plants inoculated with the VAM fungus compared to the uninoculated plants. In Chrysanthemum and China aster, early flower bud initiation and early flowering

was observed in the inoculated plants given 75 per cent of the recommended P where as in Marigold early flower bud initiation and flowering was observed in plants inoculated with the VAM fungus at 50 per cent recommended P.

Phosphorous is the major element limiting the growth and yield of flowers. In many ornamental plants P deficiency retards flowering. In most of the ornamental plants, the number of flower buds and florets increase in proportion to the application of increased P levels (Joiner, 1967).

Early flowering in ornamental plants due to inoculation with VAM fungus has been reported by several workers. Wen (1991) reported that Gerbera plants inoculated with Glomus etunicatum flowered 16 days earlier than the non-mycorrhizal control. Cheng (1989) reported that Zinnia plants inoculated with Glomus mosseae flowered 24 days earlier than the uninoculated plants. Daft and Okasanyas (1973) reported early flowering in Petunias due to VAM inoculation and Backhans (1982) reported early flowering in Chrysanthemum frutescens inoculated with VAM fungus.

In the present study, Chrysanthemum morifolium plants flowered 3 days early compared to uninoculated plants, when the soil was inoculated with Glomus mosseae at 75 per cent of recommended P. China aster plants flowered 4 days earlier when inoculated with Glomus mosseae at 75 per cent recommended P. Marigold flowered 10 days earlier compared to uninoculated plants when the soil was inoculated with Glomus mosseae at 50 per cent recommended P. The early flowering might be due to increased P uptake by the inoculated plants and better translocation of P in the plants at the time of flowering.

There was significant effect of VAM fungus on the flower characteristics

like flower diameter and stalk length. Maximum flower diameter and stalk length of flowers was observed in plants inoculated with the VAM fungus at 75 per cent recommended P in Chrysanthemum; the increasing being 5.4 per cent and 6.73 per cent with respect to flower diameter and stalk length.

In China aster, there was an increase in flower diameter by 10.75 per cent and 4.65 per cent increase in stalk length in plants inoculated with VAM fungus and given 75 per cent P compared to the uninoculated plants given 100 per cent P.

In Marigold, there was 20.96 per cent increase in flower diameter in plants inoculated with the VAM fungus at 50 per cent recommended P compared to the uninoculated plants which were given 100 per cent P. There was 19.45 per cent increase in stalk length of inoculated plants given 50 per cent P.

Flower yield is the manifestation of yield contributing characters like number of flowers per plant and flower size. In chrysanthemum, maximum number of flowers were harvested from plants inoculated with the VAM fungus at 75 per cent recommended P level; an increase of about 17 per cent over the uninoculated plants given 100 per cent P. In China aster, about 39 per cent increase in flower number was recorded in plants inoculated with the VAM fungus at 75 per cent P over the uninoculated control plants given 100 per cent P. In Marigold, of 20.69 per cent increase in flower number was seen when the plants were inoculated with VAM fungus at 75 per cent of the recommended P compared to uninoculated plants given 100 per cent P.

The increase in flower yield of Chrysanthemum was about 23.32 per cent in the inoculated plants given 75 per cent P compared to uninoculated plants supplied with 100 per cent P. In China aster, the increase in flower yield due to

inoculation at 75 per cent P was about 29 per cent over uninoculated plants given 100 per cent P. In Marigold, the flower yield was almost double in the inoculated plants given 75 per cent and 50 per cent P compared to the uninoculated plants given 100 per cent P. There was 24.55 per cent increase in yield in the inoculated plants given 50 per cent P. Also the flower characters namely stalk length and flower diameter were significantly superior in the inoculated plants at 50 per cent recommended P in case of Marigold. Since the flower yield per plant was statistically on par in the inoculated plants at 50 per cent and 75 per cent recommended P, there is a possibility of reducing P application by 50 per cent in case of Marigold.

Ronkodori and Pokorny (1982) in Juniperus chinensis and Backhans (1982) in Chrysanthemum frutescens reported increase in flower number and flower yield per plant when inoculated with VAM fungus as compared to the uninoculated plants. The differences in the yield components could be attributed to the physiological characters, both in vegetative and reproductive stages of crop growth. Differences in dry matter production and its distribution into different plant parts like leaf, stem and flower with the inoculation of VAM fungus might be mainly responsible for the increase in flower yield, flower number and flower size.

Regarding plant biomass, in case of chrysanthemum and china aster, maximum total plant biomass was observed in plants inoculated with th VAM fungus at 75 per cent recommended P. Maximum plant biomass in Marigold was observed when the plants were treated with the VAM fungus at 50 per cent of the recommended P. These results uphold the observation made by Vandel Ploeg (1974) in Lilium regale; and Johnson et al. (1982) in Chrysanthemum morifolium.

Inoculation with the VAM fungus is known to increase the uptake of micronutrients like Cu, Mn, Fe and Zn in addition to P uptake. (Cooper and Tinker, 1978; Krishna and Bagyaraj, 1984). VAM fungi enhancing water uptake in plants has also been reported (Allen et al., 1980). These beneficial effects of VAM fungus might have contributed for maximum dry matter production in the inoculated plants.

Regarding the flower dry weight, an increase of 5.38 per cent was recorded in Chrysanthemum when the plants were grown in soil inoculated with VAM fungus at 75 per cent of the recommended P when compared to uninoculated plants given 100 per cent P. In China aster, an increase of about 19.5 per cent was observed over the uninoculated plants given 100 per cent P when the plants were inoculated with VAM at 75 per cent of the recommended P. In Marigold 6.9 per cent increase in flower dry weight was observed over the control when the plants were grown in soil inoculated with the VAM fungus at 50 per cent P.

This increase in the dry matter accumulation of flowers may be attributed to more number of flowers and better translocation of carbohydrates from other plant parts to the flower. The dry matter production and its accumulation in flowers depends upon the photosynthetic capacity of the plant during flower development period. The photosynthetic capacity of the plants inturn depend upon dry matter accumulation in leaves, leaf area and leaf area index. Biermann and Lindermann (1983) reported greater leaf area and leaf area index in china aster plants which were inoculated with the VAM fungus.

Maximum shoot and root dry weights were observed in Chrysanthemum, China aster and Marigold when they were grown in soils inoculated with the VAM fungus at 75 per cent of the recommended P. A similar trend was reported by Wang et al. (1993) in Gerbera and Syngonium, and Bagyaraj and Powell (1985) in Marigold.

Chrysanthemum flowers from inoculated plants at 75 per cent recommended P lasted 4 days more in vase in 2 per cent sucrose solution as compared to flowers obtained from uninoculated plants, given 100 per cent P. Wen (1991) reported that Gerbera plants inoculated with Glomus mosseae produced flowers which lasted three days longer in vase than the non-mycorrhizal plants. The increased vase life of flowers from mycorrhizal plants may be due to greater development of water conducting tissues in mycorrhizal plants than in non-mycorrhizal plants. (Chang, 1992; Wen, 1991).

Inoculation with VAM fungus causes greater uptake of P by their expanded network of hyphae (Bolon, 1991 and Srinivasa, 1992). In the present investigation mycorrhizal plants at 75 and 50 per cent recommended P had maximum shoot P concentration and shoot P content in all the three crops as compared to other treatments. This clearly explains the role of VA mycorrhizal fungi in translocation of less mobile element P. The increased P content could be attributed to increased P uptake. Various mechanism have been suggested for increase in P uptake by the mycorrhizal plants. These include exploration of large volume of soil and faster movement of P into the mycorrhizal hyphae. (Rhodes and Gerdemann, 1975).

Inoculation with Glomus mosseae markedly increased the level of VAM colonization in roots of all the three plant species at all the three P levels as compared to the uninoculated plants. Per cent root colonization was significantly higher in mycorrhizal plants at 75 per cent P in Chrysanthemum and China aster but in Marigold, it was more in inoculated plants given 50 per cent P. The extramatricular chlamydospore number in the root zone soil also followed a similar trend. This suggests that the number of infective propagules in the soil are low and the infectivity of native VAM fungi is lower than that of the inoculant fungus. Such increases in root colonization levels of plant species grown in unsterile soil inoculated with VAM fungus have been observed earlier (Bagyaraj and Manjunath, 1980; Bagyaraj and Sreeramulu, 1982; Bagyaraj, et al., 1988). Further, there was decrease in VAM colonization level at 100 per cent P. Such a decrease in VAM colonization level at higher soil P levels has also been observed (Hoepfner et al., 1983; Lim and Cole, 1984). Menge et al. (1978) attributed this reduction to the decrease in permeability of plant cell membrane at high P levels by which VAM fungus was deprived of photosynthates for its development.

The results of the present study thus clearly indicate that Chrysanthemum, China aster and Marigold respond well to inoculation with Glomus mosseae. By giving importance to early flowering, flower characteristics and flower yield, it can be stated that soil inoculation of Glomus mosseae at 75 per cent recommended P gave most beneficial results in Chrysanthemum and China aster, and inoculation with Glomus mosseae at 50 per cent recommended P gave most beneficial results in Marigold. It can be concluded that through mycorrhizal inoculation, P fertilizer application can be reduced by 25 per cent in case of Chrysanthemum and China aster and by 50 per cent in case of Marigold.

SUMMARY

VI SUMMARY

A green house investigation was conducted to study the effect of VA mycorrhizal inoculation on the growth and yield of Chrysanthemum, China aster and Marigold at different P levels namely 100 per cent, 75 per cent and 50 per cent of the recommended P as compared to the uninoculated plants given 100 per cent recommended P. All the plants received recommended N and K fertilizer and the plants were grown in unsterile soil in pots. Glomus mosseae was the VAM fungus used for inoculating the soil.

In general, plants inoculated with Glomus mosseae and given 75 per cent of recommended P had more plant height, number of leaves, branches and plant spread compared to that of uninoculated plants given 100 per cent recommended P. In Chrysanthemum and China aster, maximum plant biomass was observed in the inoculated plants given 75 per cent P, while in Marigold, maximum plant biomass was observed in the inoculated plants given 50 per cent P.

Inoculation with G. mosseae markedly increased the VAM colonization in the roots of all the three plant species at all the three P levels as compared to the uninoculated plants. Mycorrhizal root colonization was significantly higher in the inoculated plants at 75 per cent P in Chrysanthemum and China aster, and in Marigold it was more in the inoculated plants given 50 per cent recommended P. The extra matricular chlamydospore numbers in the root zone soil followed a similar trend.

Mycorrhizal inoculation also induced early flower bud initiation and early flowering. Inoculation with the fungus enhanced the flower number and flower yield per plant in all the three plant species. Chrysanthemum and China aster

plants inoculated with the VAM fungus and given 75 per cent P flowered 3 and days earlier respectively as compared to the uninoculated plants given 100 p cent P. Marigold plants inoculated with the VAM fungus and given 50 per cent flowered 10 days early as compared to the uninoculated plants given 100 per ce P. There was 23.32 per cent increase in the flower yield per plant Chrysanthemum and 29 per cent increase in flower yield per plant in case of Chi aster when the plants were inoculated with the VAM fungus and given 75 per ce P compared to uninoculated plants given 100 per cent P. In Marigold there w an increase of 24.55 per cent in flower yield per plant when plants we inoculated with the VAM fungus at 50 per cent P compared to uninoculated plan given 100 per cent P.

Giving more importance to early flower bud initiation and flowering, flow number, size and yield per plant, it can be concluded that the best flowering c be obtained in the mycorrhizal plants given 75 per cent recommended P in ca of Chrysanthemum and China aster and at 50 per cent recommended P in case Marigold. It can thus be concluded that through mycorrhizal inoculation fertilizer application can be reduced by 25 per cent in Chrysanthemum and Chi aster and by 50 per cent in Marigold.

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APPENDICES

APPENDIX I

Composition of Ruakara plant nutrient solution

1. **Major Elements**		g/4.5 litres
Solution A :	$Mg(NO_3)_2\ 6H_20$	22.25
	$Ca(NO_3)_2\ 4H_20$	75.50
	NH_4NO_3	38.40
	KNO_3	10.25

2. **Minor Elements**		g/500 ml	
a) Boric acid	(H_3BO_3)	6.3831	Dissolved in 500 ml water
b) Manganese	($MnCl_2\ 4H_2O$)	20.9647	Dissolved in 20.20 ml of 0.1 N HCl, make up to 500 ml H_2O
c) Zinc	($ZnCl_2$)	5.684	Dissolved in 117.3 ml of 0.1 N HCl, make up to 500 ml H_2O
d) Copper	($CuCl_2\ 2H_2O$)	1.7023	Dissolved in 12.07 ml of 0.1 N HCl, make up to 500 ml H_2O
e) Molybdenum	($(NH_4)_6MO_2\ 4H_2O$)	0.2070	Dissolved in 500 ml water
f) Cobalt	($COCl_2\ H_2O$)	0.4088	Dissolved in 500 ml water

Five ml of each mineral element nutrient solution was mixed and diluted to 2.5 litres with water.

3. Iron

Ferric citrate (Fe $C_6H_3O_7$ $5H_2O$) (13.5 g) was dissolved in 119 ml of 1 N HCl and diluted to 2.5 litres with water.

The nutrient solution which was added to the growth medium was prepared by mixing 300 ml of solution A, 300 ml of solution B, 150 ml of solution containing minor elements (diluted), 22.5 ml of ferric citrate solution and diluted to 4.5 litres of water.

For mycorrhizal plants - omit K_2HPO_4 and KH_2PO_4

APPENDIX II

Vanadomolybdate reagent

Solution A is prepared by dissolving 25 g of ammonium molybdate in 400 ml of distilled water. Solution B is prepared by dissolving 1.25 g ammonium metavanadate in 300 ml of boiling water. Solution B is cooled and then 200 ml of concentrated HNO_3 is added and the solution is again cooled to room temperature. Finally solution A is poured into solution B and the mixture is diluted to one litre.