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The effect of AM fungi and phosphorous level on the biomass yield and ajmalicine production in *Catharanthus roseus*

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Abstract
A field study was conducted to evaluate the effectiveness of arbuscular mycorrhizal fungi (AMF) and phosphorus levels (100, 150 and 200 kg) for increasing biomass yield and ajmalicine content in a medicinal plant (*Catharanthus roseus*). The plants treated with 150 and 200 kg P_2O_5/ha along with AMF had the maximum plant height, number of leaves, root biomass, phosphorus content, root colonization, spore count and ajmalicine content 120 days after planting when compared with the control plants. The results suggested that these treatments could be recommended for enhancing biomass and alkaloid content in *C. roseus*.

Keywords: Ajamlicine, AM fungi, *Catharanthus roseus*, phosphorus.

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INTRODUCTION

Catharanthus roseus (L) G. Don (Madgaskar periwinkle) of the Apocynaceace family is widely grown in tropical and subtropical regions of the world. The plant produces several pharmaceutically important alkaloids (Jaleel and Panneerselvam 2007). Among these valuable compounds are the powerful antineoplasmic agents vinblastine and vincristine. The monomeric alkaloids ajmalcine and serpentine are used in the treatment of circulatory diseases (Heijden et al. 2004, Jaleel et al. 2006, 2007a-g).

The occurrence of Arbuscular Mycorrhizal Fungi (AMF) in the roots of several medicinal plants was noticed by Govinda Rao et al. (1989), Lakshman and Raghavendra (1990) and these help the plants acquire mineral nutrients from the soil. These fungi increase the surface area of roots and thus help in absorbing some diffusion-limited nutrients (P, Zn, Cu etc.). They also help in water uptake, thereby protecting the plants under mild drought stress and also help to deter the activity of root pathogens. They produce growth-promoting substances such as Indole acetic acid (IAA), cytokinins and gibberellin like substances. AMF enhances the plant growth as a result of the improved phosphate nutrition of the host plant. This has been confirmed by the use of isotropic traces (Bolan 1991).

The inoculation of AM and other beneficial soil microorganisms significantly increased the biomass of different medicinal plants (Sena and Das 1998, Kothari et al. 1999). Hence, in the present study we analyzed the effect of

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AM fungi of *Glomus mosseae* along with phosphorous levels on the growth and alkaloid content of *C. roseus.*

MATERIAL AND METHODS

Glomus mosseae (AM fungi) was obtained from the Department of Microbiology, Faculty of Agriculture, Annamalai University to evaluate the inoculation effect of *G. mossae* and phosphorous levels on the growth and alkaloid content of *C. roseus*. A field trail was conducted in the department pot culture yard from Feb 2006 till June 2006. One hundred (100g) grams of soil based *G. mossae* inoculum (50 spores per 100 g) was added to the planting hole in the field. Three replicates were conducted in each treatment.

C. roseus plants from each treatment were randomly selected after 90 and 120 DAP and their per cent root colonization, spore count, growth parameters like plant height, number of leaves, root length, root dry weight and ajmalicine alkaloid content were recorded. The Tryphan Blue technique was adapted to detect and evaluate the AM fungi root colonization (Philips and Hayman 1970). Wet sieving and decanting techniques were adopted to access the AM fungal spores (Gerdemann and Nicolson 1963). The Phosphorus content was estimated by the Vandomolybdate yellow colour method (Jackson 1973).

Ajmalicine extraction from the roots was carried out by following the standard extraction method (Zhao et al. 2000) with small modification as explained previously (Jaleel et al. 2006).

Statistical analysis was performed using the one-way analysis of variance (ANOVA) followed by a post hoc test. The data is expressed as bars. Bar values that are not sharing a common superscript (a, b, c, d, e, f) differ significantly at $P \leq 0.05$ as determined by the post hoc test.

RESULTS

The maximum plant height was recorded in the combined treatment of 200 kg P_2O_5/ha + AMF (41.6 cm, 47.66 cm/plant) on 90 DAP, 120 DAP followed by 150 kg P_2O_5/ha + AMF, 100 kg P_2O_5/ha + AMF. The minimum plant height was recorded in the control treatment. The combined treatment of 200 kg P2O5/ha + AMF and 150 kg P_2O_5/ha + AMF were on par, but the other treatments were significantly different than the control plants (Fig. 1).

The number of leaves of *C. roseus* that were recorded were more in 200 kg P_2O_5/ha + AMF (29.6, 35.1/plant) on both days of sampling the period followed by 150 kg P_2O_5 P_2O_5/ha + AMF (22, 27.6/plant). The number of leaves were recorded less in the control (Fig. 2).

The root length and root dry weight of *C. roseus* measured on the influence of AM fungi and phosphorous levels. The maximum root length and root dry wt. were observed in combined treatment (200 kg P_2O_5/ha + AMF) on both days of sampling period (26.50, 29.50 cm/plant, 14.20, 15.26 g/plant) followed by 100 kg P_2O_5/ha + AMF. The minimum root length and root dry wt was also observed in the control treatment (3.50, 5.70 cm/plant, 5.14, 6.50 g/plant). The combined treatment of 200 kg P_2O_5/ha + AMF and 150 kg P_2O_5/ha + AMF were on par, but the other treatments are significantly different with the control plants (Figs. 3, 4).

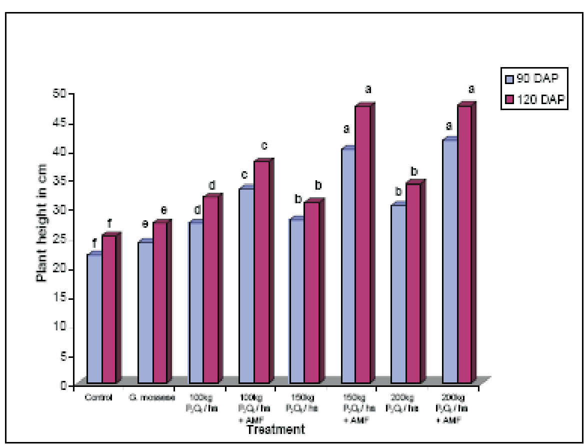

Fig. 1. Effect of *Glomus mosseae* inoculation and phosphorous concentration on plant height (cm) of *C. roseus*.

Bar values that are not sharing a common superscript (a, b, c, d, e, f) differ significantly at $p \leq 0.05$ as determined by a posthoc test.

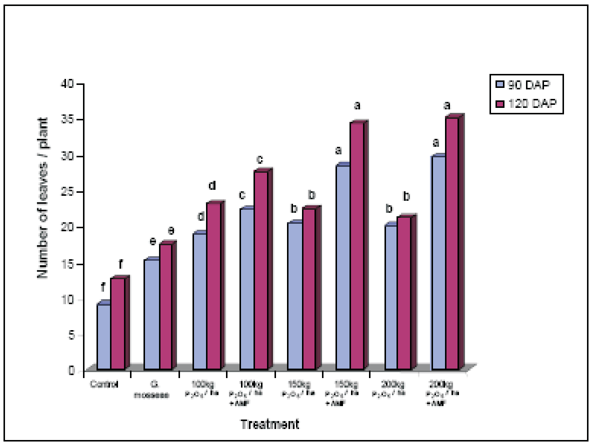

Fig. 2. Effect of *Glomus mosseae* inoculation and phosphorous concentration on the no of leaves/ plant of *C. roseus*.

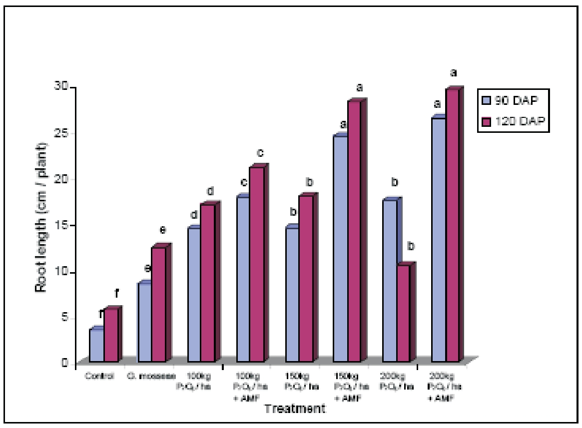

Fig. 3. Effect of *Glomus mosseae* inoculation and phosphorous concentration on the root length (cm/plant) of *C. roseus*.

The phosphorus content of *C. roseus* was recorded as more in the combined treatment of 200 kg P_2O_5/ha + AMF (27.16, 32.44 mg/g/plant) on both days of the sampling periods followed by 100 kg P_2O_5/ha + AMF. A low phosphorous content was recorded in the control plants (14.42, 17.32 mg/g/plant). The combined treatment of 200 kg P_2O_5/ha + AMF and 150 kg P_2O_5/ha + AMF were on par, but the other treatments were significantly different with the control plants (Fig. 5).

The root colonization of *C. roseus* during different treatments of AM fungi and phosphorous levels were recorded. The maximum root percent colonization were recorded in the combined treatment of 200 kg P_2O_5/ha + AMF (77.45, 82.14 per cent/plant) on both days of the sampling period followed

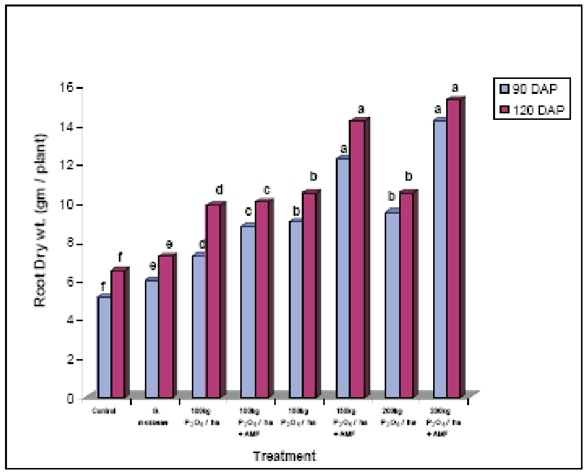

Fig. 4. Effect of *Glomus mosseae* inoculation and phosphorous concentration on the root dry wt (g/ plant) of *C. roseus*.

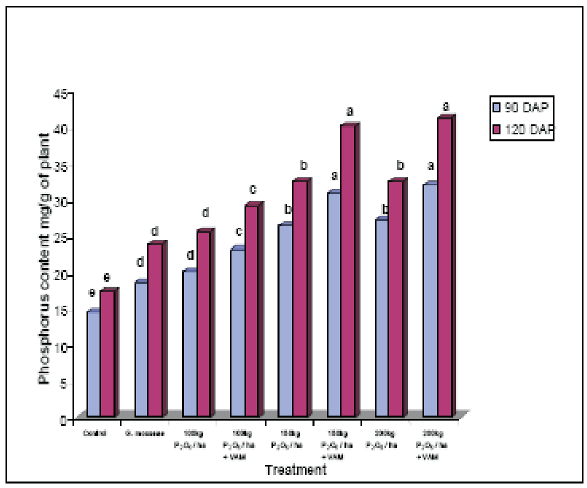

Fig. 5. Effect of *Glomus mosseae* inoculation and phosphorous concentration on the nutrient content (P) of *C. roseus*.

by 150 kg P_2O_5/ha + AMF. The minimum percent of root colonization was observed in the control treatment (20.46, 25.46 percent/plant) on both days of the sampling period. The treatment of 200 kg P_2O_5/ha + AMF and 150 kg P_2O_5/ha + AMF were on par, but the other treatments were significantly different with the control plants (Fig. 6).

The maximum AM spore count was recorded in the combined treatment of 200 kg P_2O_5/ha + AMF (77.00 and 88.00 spores/100 gm of soil) followed by 150 kg P_2O_5/ha + AMF on both days of the sampling periods. The minimum AMF spore count was recorded in the control treatment (15 and 22 spores/100 gm of soil). The combined treatment 200 kg P_2O_5/ha + AMF and 150 kg P_2O_5 per/ha + AMF were on par, but the other treatments were significantly different with the control plants (Fig. 7).

The maximum ajmalicine content was recorded in the combined treatment of AMF and P_2O_5 (200 kg P_2O_5/ha + AMF) (1.22 ± 0.66, 1.68 ± 0.44 mg/g/plant) followed by 150 kg P_2O_5/ha + AMF on both days of the sampling periods (0.80 ± 0.66, 110 ± 0.42 mg/g plant). The minimum ajmalicine content was recorded in the control treatment on both days of sampling period (0.30 ± 0.18, 0.45 ± 0.80 mg/g of plant) (Fig. 8). The combined treatment of 200 kg P_2O_5/ha + AMF and 150 P_2O_5/ha + AMF were on par, but the other treatments were significantly different with the control plants.

DISCUSSION

AMF are ubiquitous and their occurrences are well studied in different crop plants (Tholkappian et al. 2000). The present study was undertaken to study the effect of different phosphorus levels and AMF in *C. roseus.*

The study revealed an increased growth in phosphorous levels. The plant height, number of leaves, root length, phosphorous content, root colonization, AMF spore count and ajmalicine content recorded highest in the combination treatment of 200 kg P_2O_5 per ha with AMF. The values will also recorded for 100, 150 and 200 kg P_2O_5 per ha with AMF. Bolan (1991) reported the role of mycorrhizal plants in the uptake of phosphorous by the plants. The results indicated that the plant height, number of leaves, root length, root dry weight, phosphorous content, per cent root colonization and AMF spore count and ajmalicine content were significantly increased in the combined treatment of 200 kg P_2O_5/ha + AMF followed by 150 kg P_2O_5/ha + AMF on both days of sampling period (90,120 DAP). This shows the efficiency of AM fungus and *G. mossea* on

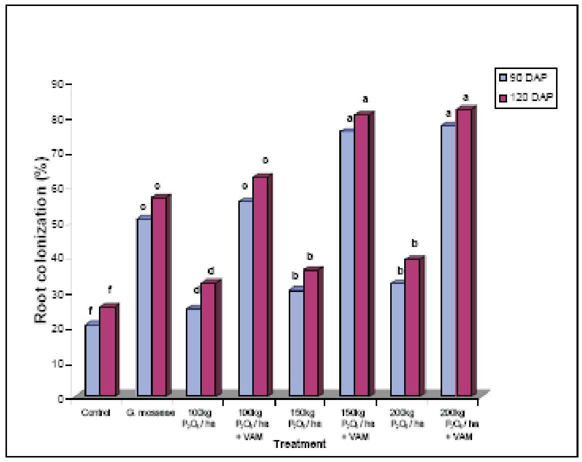

Fig. 6. Effect of *Glomus mosseae* inoculation and phosphorous concentration on the root colonization (%) of *C. roseus.*

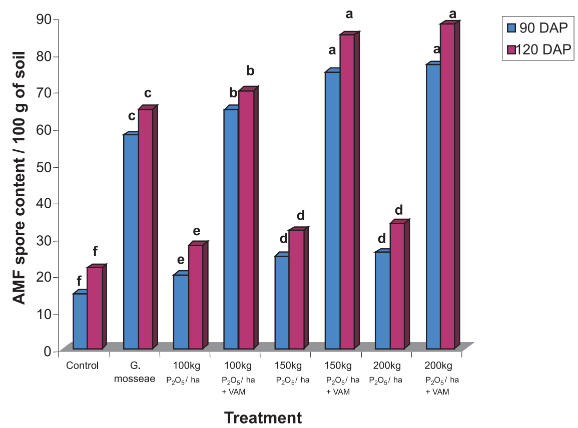

Fig. 7. Effect of *Glomus mosseae* inoculation and phosphorous concentration on the AMF spore count of *C. roseus.*

the growth of *C. roseus.* Sena and Das (1998) reported similar results for other medicinal plants. Similar results were reported by Earanna et al. (2001) in *Coleus aromaticus. Coleus forskholii* showed an increase in plant height, number of branches, biomass, 'P' content and forskolin content when it was inoculated with *Glomus bagyarajii* (Sailo and Bagyaraj 2005).

Root biomass was increased in *C. roseus* when inoculated with *Glomus mosseae*. The increase in fresh root weight and root dry weight in 200 kg P_2O_5/ha + AMF on both days of the sampling period followed by 150 kg P_2O_5/ha + AMF. The increase in fresh root weight in inoculated plants could be correlated with increased mycorrhizal

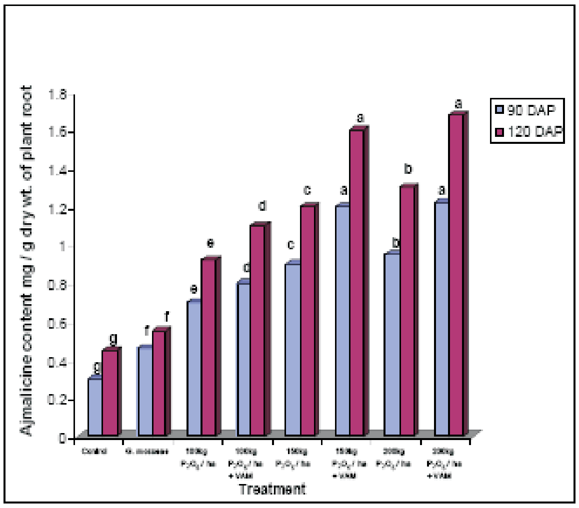

Fig. 8. Effect of *Glomus mosseae* inoculation and phosphorous concentration on the ajmalicine content (mg/g dry wt of *C. roseus.*

colonization. The reason may also be due to the formation of external mycelium around the roots by AMF fungi. These results are in agreement with the findings of earlier Investigators (Peterson et al. 1991, Gupta and Janardhanam 1991) the observed improved biomass production in different plants due to AM fungi inoculation.

The phosphorous content of the plant was increased in the combined treatment 200 kg P_2O_5/ha+AMF on both days of the sampling period (32.00, 41.22 mg/g of plant). The role of mycorrhizae on plant growth has often been related to the increase in the uptake of immobile nutrients especially phosphorous. Inoculation with arbuscular-mycorrhizal fungi improved the phosphorous uptake in *Coleus aromaticus* (Earanna et al. 2001).

In the present study, the percent of mycorrhizal colonization and AMF spore number was the maximum in the combined treatment of 200 kg P_2O_5/ha+AMF on both days of the sampling periods (77.45 and 82.14, 77 and 88 spores/100 gm of soil) followed by other treatments. Similar findings were observed on the root colonization studies done by Griffee and Metha (2000) who reported the pot culture studies on *Catharanthus roseus* showed a positive response to *Glomus mosseae* and *Glomus aggregatum* inoculation in respect to per cent colonization and spore count as Earanna et al. (2001) reported on *Coleus aromaticus.*

The ajmalicine content was increased in the combined treatment of 200 kg P_2O_5/ ha+AMF when compared to the control plants. Similar findings were obtained by Tholkappian et al. (2000) and Sailo and Bagyaraj (2005). The biosynthesis of total indole alkaloids can be stimulated by the addition of exogenous elicitors such as fungal preparations. Many studies reveal that fungal elicitors profoundly affect the regulation of indole alkaloid biosynthesis (Sayed and Verpoorte 2007). Biotic and abiotic elicitors have been used to increase the production the de novo synthesis of secondary metabolites in cell cultures (Aerts et al. 1996).

CONCLUSIONS

The present study revealed the positive effect of *G. mosseae* and 'P' levels on growth, biomass yield and ajmalicine content of *C. roseus.* On the basis of these findings, it may be concluded that the application of 150 kg P_2O_5/ha+AMF was equally as good as the 200 kg P_2O_5/ha in respect to productivity, root colonization, AMF spore count, 'P' levels and alkaloid content.

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AM Mantar ve Fosfor Iceriginin *Catharanthus roseus*'ta Biyokutle Miktarina ve Ajmalisin Uretimine Etkisi

Ozet

Arbuskuler mikorizal mantar (AMM) ve fosfor seviyelerinin (100, 150 ve 200 kg), tibbi bir bitkide (*Catharanthus roseus*) biyokutle miktarini ve ajmalisin icerigini artirmadaki etkisini olcmek amaciyla bir alan caliṣmasi gerceklestirildi. 150 ve 200 kg P_2O_5/ha ve AMM ile muamele edilen bitkiler, ekimden 120 gun sonra kontrol bitkileri ile kiyaslandiginda, maksimum bitki yuksekligi, yaprak sayisi, kok biyokutlesi, fosfor icerigi, kok kolonizasyonu, spor sayisi ve ajmalisin icerigine sahipti. Sonuclar, bu muamelelerin *C. roseus*'ta biyokutle ve alkaloit icerigini artirmak icin kullanilmasinin tavsiye edilebilecegini gostermektedir.

Anahtar Kelimeler: Ajmalisin, arbuskuler mikorizal mantar (AMM), *Catharanthus roseus*, fosfor.

