American-Eurasian J. Agric. & Environ. Sci., 2 (1): 33-38, 2007
ISSN 1818-6769

Response of Kalmegh to an Arbuscular Mycorrhizal Fungus and a Plant Growth Promoting Rhizomicroorganism at Two Levels of Phosphorus Fertilizer

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Abstract: A field investigation was conducted to know the influence of inoculation with the Arbuscular Mycorrhizal (AM) fungus *Glomus mosseae* and the Plant Growth Promoting Rhizomicroorganism (PGPR) *Trichoderma harzianum* singly and in combination on growth and yield of kalmegh (*Andrographis paniculata*) at two levels of P fertilizer application i.e. at the recommended level and 75% of the recommended level. The plant height, plant spread, number of branches per plant, number of leaves per plant, leaf area, plant dry matter, plant P content and andrographolide (alkaloid of pharmaceutical importance) concentration were significantly higher in plants inoculated with both the organisms, at both the levels of P as compared to uninoculated plants. Inoculation with both the organisms also reduced significantly days for 50% flowering. Higher root colonization and spore numbers in the root zone soil were also observed when both the organisms were inoculated, at both the levels of P. This clearly brings out that inoculation with *G. mosseae* + *T. harzianum* not only improved growth, biomass yield, P nutrition and andrographolide concentration of kalmegh but also helped in saving 25% of P fertilizer application.

Key words: *Andrographis paniculata* · Arbuscular mycorrhiza · *Glomus mosseae* · kalmegh · *Trichoderma harzianum*

INTRODUCTION

Kalmegh (*Andrographis paniculata* Nees.) belonging to the family Acanthaceae is one of the medicinal plants that is recommended for cultivation in India, as there is a great demand for the plant by the pharmaceutical industries mainly for export. It is a source of several diterpenoids of which andrographolide (alkaloid) is important. The drug is used for treating general debility, dyspepsia, chronic malaria, jaundice and dysentery. Some scientists have observed that andrographolide has the potential to be included in the cocktail vaccine against AIDS by virtue of its antagonistic property with HIV II virus [1]. It has been already used for treating cancer as it promotes cell differentiation in tumour cells [2]. Though leaves contain maximum andrographolide, the entire plant is used for extracting the active ingredient.

Currently, the emphasis is on sustainable agriculture, which uses less of chemical inputs like fertilizers and pesticides having adverse effect on soil health, fertility and environment. Thus microbial inoculants play an important role in sustainable agriculture.

Arbuscular Mycorrhizal (AM) fungi are considered important in growth and development of plants. This is brought about by enhanced uptake of phosphorus [3, 4] and also other diffusion-limited elements like Zn, Cu etc. [5] by increasing the nutritional status of the host. AM fungi are also involved in increasing the uptake of water [6, 7] and in protecting the plants from phytopathogenic fungi, bacteria and parasitic nematodes invading the roots [8].

AM fungi enhancing the activity of beneficial soil organisms, like nitrogen fixers and phosphate solubilizers with consequential beneficial effect on plant growth has been reported [9]. *Trichoderma* spp. are known to induce plant growth by producing growth regulating factors [10] and suppressing the activity of pathogenic organisms [11]. They are also known to solubilize unavailable form of P to a form available for plant growth [12]. Recently it was found to enhance the activity of AM fungus and hence was designated as

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Mycorrhiza Helper Organism (MHO) [13]. But the information available on the interaction between AM fungi and *Trichoderma* and their consequential effect on plant growth is meager. Recently, it was reported that dual inoculation with *Glomus mosseae and Trichoderma viride* increased the yield and forskolin content in *Coleus forskohlii* [14]. The present study was undertaken to understand the response of kalmegh to the AM fungus *Glomus mosseae* and the Plant Growth Promoting Rhizomicroorganism (PGPR)/MHO, *Trichoderma harzianum,* at the recommended level of P and 75% of the recommended P, under field condition.

MATERIALS AND METHODS

A microplot investigation was conducted to examine the response of kalmegh *Andrographis paniculata* Nees. to inoculation with the AM fungus *G. mosseae* and the Plant Growth Promoting Rhizomicroorganism (PGPR)/MHO *T. harzianum* at two levels of P fertilizer application. The experimental plot was prepared and brought to fine tilth. The soil was an alfisol of a fine kaolinitic, isohyperthermic typic kanhaplustafs type with a pH of 5.93, organic carbon of 0.56 g per 100 g solution and available phosphorus of 25 kg ha^{-1} (Brays method). The net plot size was 1×1 m with proper irrigation channels. Well decomposed farm yard manure at the rate of 2.5 kg per plot was applied and mixed thoroughly. Similarly the recommended dose of N (75 kg ha^{-1}) and K (41 kg ha^{-1}) were applied in the form of urea and muriate of potash respectively, to all plots. P was added in the form of single super phosphate according to the treatment level, the recommended level being 22 kg ha^{-1}. Half the dose of N along with full dose of P and K were applied at the time of planting, while the remaining half of N was applied four weeks later. The AM fungus *Glomus mosseae* was maintained in pot culture with sterilized sand: soil 1:1 by vol. as the substrate and Rhodes grass as the host. The inoculum containing substrate with extramatrical hyphae, spores and infected root bits of Rhodes grass was used at the proportion of 10 g per planting point, as per the treatment. The inoculum contained 106 infective propagules per gram of substrate as estimated by 4-fold dilution method [15]. *Trichoderma harzianum* culture was grown in potato dextrose broth for 8 days. The culture was then filtered through Whatman no. 1 filter paper and the mycelial mat was macerated using a waring blender for 1 min and suspended in 0.1 M $MgSO_4.7H_2O$. Ten-ml inoculum containing 5×10^4 c.f.u. ml^{-1} was added to the planting point as per the treatment. Seedlings of kalmegh were first raised in nursery by sowing seeds in polythene bags containing a mixture of soil: sand: FYM in ratio of 1:1:1 by vol. Fifty-day-old seedlings were transplanted to the field as per the following treatments:

T1: Uninoculated control with 100% recommended P (22 kg ha^{-1})
T2: Inoculated with *G. mosseae* at 100% recommended P
T3: Inoculated with *T. harzianum* at 100% recommended P
T4: Inoculated with *G. mosseae* + *T. harzianum* at 100% recommended P
T5: Uninoculated control with 75% recommended P (16.5 kg ha^{-1})
T6: Inoculated with *G. mosseae* at 75% recommended P
T7: Inoculated with *T. harzianum* at 75% recommended P
T8: Inoculated with *G. mosseae* + *T. harzianum* at 75% recommended P

Each treatment had 3 replications. The experiment was laid out as a Randomized Complete Block Design (RCBD). The seedlings were planted with a spacing of 30 cm between rows and 15 cm between plants, with a total of 18 plants/plot. Plots were irrigated twice a week for the first 4 weeks and subsequently at weekly intervals to maintain enough moisture and the plants were raised for 90 days after transplanting.

Plant growth parameters like plant height, plant spread, number of branches/plant, leaf area and days for 50% flowering were recorded at 30 days interval commencing from the Day after Transplanting (DAT). Three such observations were made during entire growth period of the crop. But the data on 90 DAT (i.e. at harvest) alone is presented in this paper. Plant spread was measured along north-south and east-west direction, leaf area was measured using a leaf area meter. After harvest, the plants were dried at 60°C to attain a constant weight to get the plant dry matter (herbage yield)/plot. Phosphorus content in shoot and root was estimated colorimetrically by the vanadomolybdate yellow colour method [16]. The andrographolide concentration in the whole plant was estimated by spectrophotometric method [17].

Fresh root samples were stained using 0.05 g per 100 g solution trypan blue [18] and the percent root colonization was estimated by adapting the gridline intersect method [19]. Extramatrical spores in the root zone soil were enumerated using stereozoom microscope after

wet sieving and decanting the soil samples [20]. Data thus generated were subjected to statistical analysis of variance by RCBD and the treatment means were separated by Duncan's Multiple Range Test (DMRT) [21].

RESULTS AND DISCUSSION

Arbuscular mycorrhizal fungi are well known to enhance the nutritional status of several plants and thereby aid in increased growth and yield. The present investigation was carried out in order to evaluate the role of an AM fungus and a plant growth promoting rhizomicro organism (PGPR)/Mycorrhiza Helper Organism (MHO) on the growth and P nutrition of kalmegh and the possibility of reducing application of phosphate fertilizer through microbial inoculation. Inoculation of kalmegh with *G. mosseae* significantly increased plant height compared to uninoculated plants at both the levels of P (Table 1). Similar results were obtained in the medicinal plant palmarosa [22]. Inoculation with *T. harzianum* also increased plant height compared to uninoculated plants at both the levels of P. Inoculation with *G. mosseae* + *T. harzianum* resulted in higher plant height compared to *T. harzianum* alone, but on par with *G. mosseae* alone. Inoculation with *G. mosseae* + *T. harzianum,* at the two levels of P, significantly increased plant height compared to uninoculated plants. The spread of plants was higher when inoculated with both the organisms, followed by *G. mosseae* alone and *T. harzianum* alone at both the levels of P as compared to uninoculated plants (Table 1).

Number of branches per plant was also significantly larger in inoculated treatments as compared to uninoculated treatments, highest being in the treatment *G. mosseae*+ *T. harzianum,* at both the levels of P (Table 1). Similar trend was observed in the number of leaves per plant and leaf area per plant. Inoculated plants took significantly less number of days for 50 percent flowering as compared to the uninoculated treatments at both the levels of P. At 75% P level, the number of days taken for flowering was significantly less in the treatment *G. mosseae* + *T. harzianum* as compared to treatments with *G. mosseae* or *T. harzianum* alone, both being on par with each other.

Plant dry matter in plants inoculated with both the organisms and plants inoculated with *G. mosseae* alone and with *T. harzianum* alone, at both levels of P, had significantly higher biomass as compared to uninoculated plants (Table 1). The plant dry matter increased by 84.8% when inoculated with both organisms at 100% P followed by plants inoculated with *G. mosseae* alone by 48.4% as compared to the uninoculated plants. When inoculated with both the organisms at 75% P the increase in plant dry matter was by 49.8% followed by *G. mosseae* alone by 28.8% as compared to uninoculated control. Increase in plant biomass because of AM fungal inoculation has been reported in other medicinal plants like Palmarosa [22] and *Coleus forskohlii* [14].

The inoculated treatments with *G. mosseae* increased significantly the P content in both shoot and root as compared to uninoculated plants, at both levels of P (Table 2). Mycorrhizal association is known to increase the availability of diffusion limited nutrients, phosphorus being the most important of these nutrients [23, 24]. Various mechanisms have been suggested for increased P uptake by mycorrhizal plants. The external hyphae of AM fungi allow the root system to exploit greater volume of soil phosphorus (i) by extending away from roots and translocating P from some distance to the root zone [25] and (ii) by exploiting smaller soil pores not reached by root hairs and by adding surface area to the adsorptive system [26]. However, maximum shoot and root P content was observed in plants when *T. harzianum* was inoculated along with *G. mosseae,* at both levels of P,

Table 1: Influence of *Glomus mosseae* (Gm) and *Trichoderma harzianum* (Th) on the plant growth parameters of kalmegh at 90 DAT grown at 100% and 75% recommended level of P fertilizer

Treatments	Plant height (cm)	Plant spread (cm^2)	Branches (No./plant)	Leaves (No./plant)	Leaf area (cm^2/plant)	50% flowering (No. of days)	Dry matter (g/plant)
Uninoculated + 100% P	26.2^{cd}	344.3^{d}	20.6^{e}	43.3^{d}	133.8^{e}	126.6^{a}	154.6^{e}
Inoculated with *G. mosseae* (Gm) at 100% P	33.9^{a}	370.0^{b}	38.0^{a}	56.3^{b}	151.2^{b}	117.0^{cd}	229.5^{b}
Inoculated with *T. harzianum* (Th) at 100% P	26.5^{cd}	355.0^{c}	29.4^{d}	46.0^{c}	145.3^{c}	115.6^{d}	180.4^{d}
Inoculated with Gm + Th at 100% P	32.0^{ab}	375.0^{a}	40.7^{a}	61.0^{a}	155.4^{a}	115.6^{d}	285.7^{a}
Uninoculated + 75% P	24.1^{d}	330.6^{f}	16.5^{f}	34.6^{f}	125.2^{g}	123.3^{b}	142.5^{g}
Gm inoculated + 75% P	31.6^{ab}	347.6^{d}	30.7^{cd}	40.0^{e}	130.4^{f}	118.0^{c}	183.5^{d}
Th inoculated + 75% P	29.0^{bc}	335.0^{e}	28.4^{d}	38.0^{e}	128.4^{g}	119.0^{c}	150.1^{f}
Gm-Th inoculated + 75% P	31.9^{ab}	357.3^{c}	33.4^{bc}	44.6^{cd}	138.3^{d}	114.3^{e}	213.4^{c}

Means followed by the same letter within a column do not differ significantly at $p \leq 0.05$ by Duncan's Multiple Range Test, DAT= Days after transplanting

Table 2: Influence of *Glomus mosseae* (Gm) and *Trichoderma harzianum* (Th) on the P content and andrographolide concentration of kalmegh at 90 DAT, grown at 100% and 75% recommended level of P fertilizer

	P content (mg/plant)		
Treatments	Shoot	Root	Andrographolide concentration (%)
Uninoculated + 100% P	4.4^{e}	2.4^{e}	1.3^{c}
Inoculated with *G. mosseae* (Gm) at 100% P	7.0^{c}	4.1^{c}	1.9^{ab}
Inoculated with *T. harzianum* (Th) at 100% P	5.5^{d}	2.7^{e}	1.4^{b}
Inoculated with Gm + Th at 100% P	21.2^{a}	9.0^{a}	2.1^{a}
Uninoculated + 75% P	4.0^{e}	2.5^{e}	1.1^{c}
Gm inoculated + 75% P	5.5^{d}	3.7^{d}	1.5^{bc}
Th inoculated + 75% P	4.3^{e}	2.9^{e}	1.4^{bc}
Gm-Th inoculated + 75% P	15.3^{b}	8.1^{b}	2.1^{a}

Means followed by the same letter within a column do not differ significantly at P≤0.05 by Duncan's Multiple Range Test, DAT = Days after transplanting

Table 3: Influence of *Glomus mosseae* (Gm) and *Trichoderma harzianum* (Th) on mycorrhizal root colonization, spore numbers and population of *T. harzianum* in the root zone soil of kalmegh at 90 DAT grown at 100% and 75% recommended level of P fertilizer

Treatments	Colonization (%)	Spore number/50g of soil	*T. harzianum* (Xx10^{5} c.f.u /g soil)
Uninoculated + 100% P	25.3^{e}	48.3^{c}	ND
Inoculated with *G. mosseae* (Gm) at 100% P	45.3^{c}	105.0^{a}	ND
Inoculated with *T. harzianum* (Th) at 100% P	22.3^{f}	56.0^{c}	1.1
Inoculated with Gm + Th at 100% P	52.0^{a}	123.0^{a}	6.6
Uninoculated + 75% P	22.0^{f}	42.3^{c}	ND
Gm inoculated + 75% P	40.0^{d}	92.3^{b}	ND
Th inoculated + 75% P	25.0^{e}	55.6^{c}	1
Gm-Th inoculated + 75% P	48.0^{b}	112.3^{a}	5.6

ND = Not Determined, Means followed by the same letter within a column do not differ significantly at P≤0.05 by Duncan's Multiple Range Test, c.f.u = colony forming units, DAT = Days after Transplanting

which was as compared to plants inoculated with *G. mosseae* alone (Table 2). The least uptake was observed in uninoculated plants. *T. harzianum* is known to solubilize unavailable forms of P and convert them to an available form [12] thus enabling better P uptake by AM fungi [27, 28].

Both the organisms when inoculated singly increased the andrographolide concentration of the plant as compared to uninoculated plants. At the recommended level of P, dual inoculation increased slightly the andrographolide concentration as compared to plants inoculated with *G. mosseae* alone but statistically they were on par with each other. But at 75% recommended P level, the andrographolide concentration in plants inoculated with *G. mosseae* + *T. harzianum* was significantly larger as compared to plants treated singly with *G. mosseae or T. harzianum* (Table 2). Higher curcumin content in *Curcuma longa* because of inoculation with *Azotobacter* and *Azospirillum* [29] and larger forskolin concentration in *Coleus forskohlii* plants inoculated with *Glomus mosseae* + *Trichoderma viride* [14] were reported earlier under field conditions.

All the inoculated treatments except *T. harzianum* at 75% P level, increased significantly the percentage mycorrhizal root colonization as compared to uninoculated plants (Table 3). Among the single inoculated treatments, highest mycorrhizal colonization was observed in plants inoculated with the AM fungus *G. mosseae,* thus supporting the well-documented fact that inoculation with effective AM fungi enhances mycorrhizal root colonization [30]. Highest mycorrhizal root colonization in kalmegh was observed when *G. mosseae* was co-inoculated with *T. harzianum* at both levels of P. The least mycorrhizal root colonization was observed in uninoculated plants. A similar trend was observed in the mycorrhizal spore numbers in the root zone soil (Table 3). This may be because of synergistic interaction between AM fungus and PGPR/MHO supporting earlier reports [13, 14]. The stimulation was attributed to the volatile compounds produced by *Trichoderma* spp. [7]. Enhanced mycorrhizal root colonization and sporulation by *G. mosseae* because of co-inoculation with *T. harzianum* upholds this fungus in the category of MHO as reported earlier [27].

Population of *T. harzianum* was found to increase in plants inoculated with both the organisms. The colony-forming-units of *T. harzianum* were higher when both organisms were inoculated together and least when inoculated alone (Table 3). Increased population of *T. harzianum* was observed when micropropagated ficus and sugarcane were co-inoculated with *G. mosseae* as compared to single inoculation with *T. harzianum* [27].

The results of the present study clearly brought out the beneficial effect of inoculation with *Glomus mosseae* plus *Trichoderma harzianum* on the growth parameters like plant height, number of branches, plant spread, number of days taken for 50% flowering, P nutrition, yield and andrographolide concentration of an important medicinal plant, cultivated commercially. Further, through this *G. mosseae-T. harzianum* co-inoculation, application of phosphatic fertilizer can be reduced by 25 percent.

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