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Effect of Inoculation with Vam Fungi at Different Phosphorus Levels on Shoot, Vegetative Parts, Flower P Concentration and Uptake of Nitrogen and Phosphorus at Different Growth Stages of *Tagetes erecta* L.

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A B S T R A C T

In this experiment the VAM fungi viz., *Glomus fasciculatum* (Thaxter) Gerd. And Trappe, *Glomus mossea* (Nicol. and Gerd.) Gerd and Trappe, *Glomus intraradices* Schenck and Smith. With an un-inoculated control was maintained and three P levels viz., 60, 90, 120 kg ha^{-1} were tried. The results brought out that Shoot P concentration, P from vegetative parts and P from flower, P-uptake and N-uptake were significantly maximum in the plants inoculated with *G. fasciculatum* and given P at 90 kg/ha (0.23%, 0.43%, 0.26%, 24.64 kg/ ha and 43.54 kg/ ha, respectively).

Introduction

Marigold (*Tagetes erecta* L.) belongs to the family Asteraceae and genus *Tagetes*. The two main popularly grown species in marigold are *Tagetes erecta* L. and *Tagetes patula* L. which have their origin in Mexico and South Africa, respectively. *Tagetes erecta* L. is popularly known as "African marigold" while *Tagetes patula* L. as "French marigold".

Marigold is grown for cut flowers, making garlands, decoration during pooja and several religious functions, besides its use in landscape gardening. Apart from its significance in ornamental horticulture, it has been valued for other purposes too. The aromatic oil extracted from marigold, is called as "tagetes oil". It is used in preparation of high grade perfumes and also as an insect fly repellent.

Marigold is a heavy feeder of nutrients, at present these nutrients are supplied through chemical fertilizers. The indiscriminate and continuous use of chemical fertilizers in intensive cropping system has led to an imbalance of nutrients in soil which has an adverse effect on soil health. The balanced

use of chemical fertilizers improves the physico-chemical properties of soil besides increasing the efficiency of applied fertilizers.

Mycorrhiza literally means 'fungus root'. The fungus obtains photosynthesis from plant, while the plant is able to utilize the network of fungal hyphae, (which effectively act as an extended root system). The uptake of inorganic nutrients by plants is influenced by microorganisms in the rhizosphere. Symbiotic endophytes such as mycorrhizae are examples of microorganisms that are involved in the uptake of vital plant nutrient element, phosphorus.

Phosphorus is an important plant macronutrient, making up about 0.2 % of a plant's dry weight. Mycorrhizae are important for plant P acquisition, since fungal hyphae greatly increase the volume of soil that plant roots explore (Smith and Read, 1997). In certain plant species, root clusters (proteoid roots) are formed in response to P limitations. These specialized roots exude high amounts of organic acids (up to 23 % of net photosynthesis), which acidify the soil and chelate metal ions around the roots, resulting in the mobilization of P and some micronutrients (Marschner, 1995).

Considering its importance as commercial flower crop, the study on effect of VAM fungi on marigold at different phosphorus levels was initiated.

Materials and Methods

A factorial experiment was laid out in Randomised Block Design. There were 12 treatment combinations each three replications. In the present experiment VAM fungi (*Glomus fasciculatum, G. mosseae, G. intraradices* with an uninoculated control) and three levels of phosphorus (60, 90, 120 kg ha^{-1}) were tried in all possible combinations.

Treatment details are as follows,

Factor I = Mycorrhizal species

M_1- *Glomus fasciculatum* (Thaxter) Gerd. and Trappe.

M_2- *Glomus mossea* (Nicol. and Gerd.) Gerd. and Trappe.

M_3- *Glomus intraradices* Schenck and Smith.

M_o- Uninoculated control

Factor II = Phosphorus levels: 3 (225kg N + 60kg K_2O as constant)

P_1- 60 kg P_2O_5 ha^{-1}
P_2- 90 kg P_2O_5 ha^{-1}
P_3- 120 kg P_2O_5 ha^{-1}

Chemical analysis of plants

Plant samples were collected from each treatment at two stages of crop growth (60 and 90 days after transplanting). The oven dried plant material of dry matter production was used for the purpose. The dried samples were made into powder, mixed well and stored for further analysis.

Nitrogen estimation

The estimation of nitrogen was done by Kjeldhal method as outlined by Jackson (1967). One gram of powdered plant sample was taken and transferred to Kjeldahl digestion flask carefully. Added 2-3 gram of digestion mixture and about 10-15 ml of concentrated H_2SO_4. Digested the content in a digestion chamber over a low temperature and then over a high temperature till a light bluish green residue is obtained. Then cooled the content and make up the volume to 100 ml with distilled water. Distillation was done by pipette out of 10 ml of the digested sample in

to a microkjeldhal flask and added 10 ml of distilled water. 25-30 ml of 4 per cent boric acid mixed indicator added in a receiving flask and connected that to receiving tube. Added 10 ml of 40 per cent NaOH solution to the distillation flask and immediately closed it and distilled the content till wine red colour turned to green colour as an end point. After complete distillation disconnected the receiving flask and transferred to titration unit. Titration was done against the standard sulphuric acid (0.5 N) till the colour changed from green to wine red colour. From the titrated value calculated the per cent nitrogen by using the formula and expressed in per cent.

Total phosphorus (kg/ha)

Digested plant sample with triacid mixture were used for estimation of phosphorus, and is expressed in kilogram per hectare. It was estimated by Vanidomolybdate method as given by Jackson (1967) and the intensity of colour developed was read in spectrophotometer at 460nm.

Shoot phosphorous concentration

Shoot P concentration (%) was estimated by the procedure given by Jackson (1967). The plant sample was dried to obtain consistent dry weight.

It was then powdered and 0.5 g of this sample was taken in 100 ml conical flasks and digested with tri acid mixture (concentrated nitric acid, 60 per cent perchloric acid and concentrated sulphuric acid 10: 4: 1) at 50 ^{0}C, till they turned to white colour.

To each flask, a small quantity of distilled water was added, then the contents were transferred to 100 ml volumetric flask and volume was made up to 100 ml by adding distilled water. From this 10 ml was transferred to 50 ml volumetric flasks, to which 10 ml of vanadomolybdate reagent (Appendix- III) was then added and volume was made up to 50 ml by adding distilled water. The flasks were kept undisturbed for 20 minutes.

Then the optical density of each sample was measured at 420 nm using spectrophotometer (a blank was maintained in a similar manner, without any plant sample). The percentage of P in the sample was computed by the following formula,

$$\% P = \frac{x \times 50}{10} \times \frac{100}{0.5} \times \frac{100}{10^{6}}$$

Where, 10 is the aliquot used for colour development 0.5 g is the weight of sample used for digestion and X = graph ppm obtained from standard curve prepared as follows.

For obtaining the standard curve, 0.439 g of pure KH_2PO_4 was dissolved in distilled water and made up to 1000 ml in volumetric flask to get 1000 ppm P solution.

Aliquot of 1, 2, 3, 4, 5..... 10 ml transferred to 50 ml volumetric flask and 10 ml KNO_3 vanadomolybdate reagent was added to each flask including a distilled water blank.

Half an hour later readings were taken in a spectrophotometer at 420 nm. One to ten ml transfers mentioned above corresponds to 2 to 20 ppm in the volume made up. By plotting concentration on X- axis and corresponding absorbance along the Y- axis, the standard curve was obtained.

Uptake of phosphorous by plant

Total phosphorous uptake was calculated for each treatment separately using the formula.

$$\text{P uptake (kg/ ha)} = \frac{\text{Per cent of nutrient concentration} \times \text{Biomass (kg/ ha)}}{100}$$

And it was expressed in kg per hectare.

Results and Discussion

The data on shoot P concentration, P concentration from vegetative parts and flower parts as influenced by inoculation of *Glomus* fungi at different level of P recorded at 60 and 90 DAT are presented in table 1 and the data on nitrogen uptake and phosphorus uptake as influenced by inoculation of *Glomus* fungi at different levels of P recorded at 60 and 90 DAT are presented in table 2.

Shoot P concentration (%)

The interaction effect of inoculation of *Glomus* fungi and P-fertilization was significant at both stages.

The plants inoculated with *G. fasciculatum* and given P at 90 kg/ ha recorded significantly maximum shoot P concentration (0.28 and 0.23 %, respectively) as compared to the other species of *Glomus* fungi and uninoculated control. And least was observed in uninoculated control plants with given P at 60kg/ ha (0.12 and 0.09 %, respectively) at 60 and 90 DAT, respectively.

Phosphorus from vegetative parts (%)

The interaction effect of inoculation of *Glomus* fungi and P-fertilization was significant on vegetative parts P concentration at both stages. The plants inoculated with *G. fasciculatum* and given P at 90 kg/ ha recorded significantly maximum P concentration from vegetative parts (0.53 and 0.43 %, respectively) as compared to the other species of *Glomus* fungi and uninoculated control. And least was observed in uninoculated control plants with given P at 60kg/ ha (0.23 and 0.16 %, respectively) at 60 and 90 DAT, respectively.

Phosphorus from flower (%)

The interaction effect of inoculation of *Glomus* fungi and P-fertilization was significant on flower P concentration at both stages. The plants inoculated with *G. fasciculatum* and given P at 90 kg/ ha recorded significantly maximum flower P concentration (0.26 %) as compared to the other species of *Glomus* fungi and uninoculated control. And least was observed in uninoculated control plants with given P at 60kg/ ha (0.09 %).

Nitrogen uptake (kg/ ha)

The interaction effect of inoculation of *Glomus* fungi and P-fertilization was significant on uptake of N.

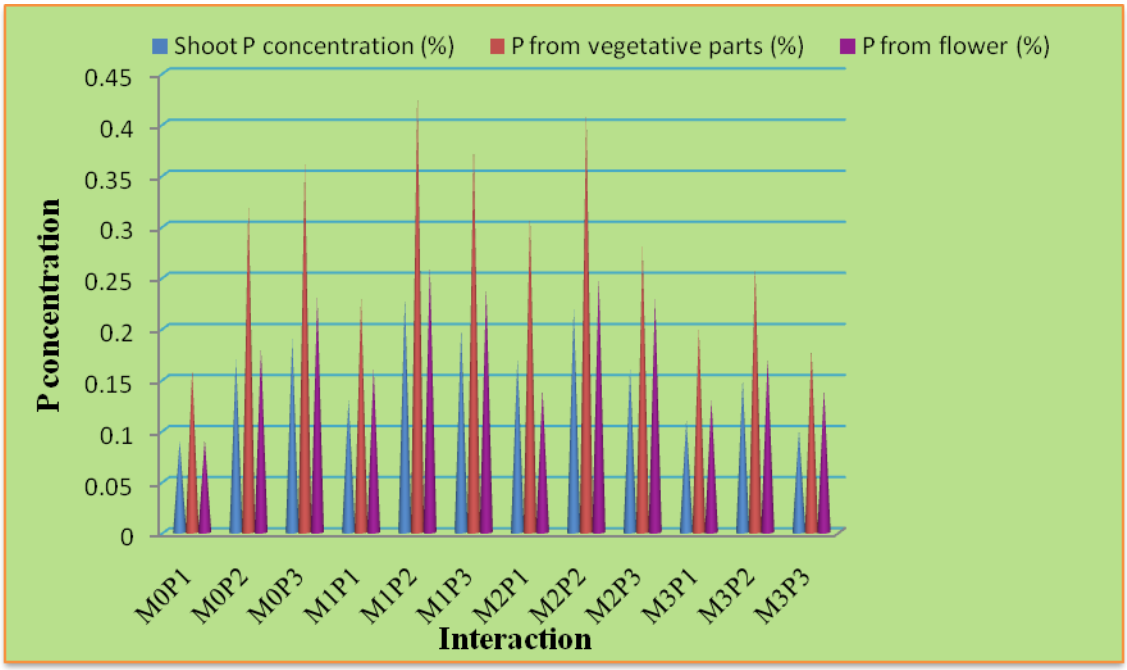
The plants inoculated with *G. fasciculatum* and given P at 90 kg/ ha recorded significantly highest uptake of N in marigold (54.78 and 43.54 kg/ ha, respectively) as compared to other species of *Glomus* fungi and uninoculated control plants applied with P at 120 kg/ ha and least was observed in uninoculated control plants with given P at 60kg/ ha (24.63 and 20.72 kg/ ha, respectively) at 60 and 90 DAT, respectively.

Phosphorus uptake (kg/ ha)

The interaction effect of inoculation of *Glomus* fungi and P-fertilization was significant on uptake of P. The plants inoculated with *G. fasciculatum* and given P at 90 kg/ ha recorded significantly highest uptake of P in marigold (24.02 and 24.64 kg/ ha, respectively) as compared to other species of *Glomus* fungi and uninoculated control

plants applied with P at 120 kg/ ha and least was observed in uninoculated control plants with given P at 60kg/ ha (7.59 and 6.14 kg/ ha, respectively) at 60 and 90 DAT, respectively.

Fig.1 Effect of inoculation with VAM fungi at different P levels on shoot, vegetative parts, Flower P concentration of *Tagetes erecta* L. at final stage of growth



M_0 - Uninoculated control
M_1 - *Glomus fasciculatum*
M_2 - *Glomus mossae*
M_3 - *Glomus intraradices*

P_1 – 60 kg P_2O_5/ ha
P_2 – 90 kg P_2O_5/ ha
P_3 – 120 kg P_2O_5/ ha

Treatment Combination

Treatment No.	Treatment	Combination
T_1	M_0P_1	Uninoculation + 60 kg P_2O_5 ha $^{-1}$
T_2	M_0P_2	Uninoculation + 90 kg P_2O_5 ha $^{-1}$
T_3	M_0P_3	Uninoculation + 120 kg P_2O_5 ha $^{-1}$
T_4	M_1P_1	*G. fasciculatum* + 60 kg P_2O_5 ha $^{-1}$
T_5	M_1P_2	*G. fasciculatum* + 90 kg P_2O_5 ha $^{-1}$
T_6	M_1P_3	*G. fasciculatum* + 120 kg P_2O_5 ha $^{-1}$
T_7	M_2P_1	*G. mosseae* + 60 kg P_2O_5 ha $^{-1}$
T_8	M_2P_2	*G. mosseae* + 90 kg P_2O_5 ha $^{-1}$
T_9	M_2P_3	*G. mosseae* + 120 kg P_2O_5 ha $^{-1}$
T_{10}	M_3P_1	*G. intraradices*+ 60 kg P_2O_5 ha $^{-1}$
T_{11}	M_3P_2	*G. intraradices* + 90 kg P_2O_5 ha $^{-1}$
T_{12}	M_3P_3	*G. intraradices* + 120 kg P_2O_5 ha $^{-1}$

Table.1 Effect of inoculation with VAM fungi at different P levels on shoot, vegetative parts, Flower P concentration of *Tagetes erecta* L.

Treatment	Shoot P concentration (%)		P from vegetative parts (%)		P from flower (%)
	60 DAT	90 DAT	60 DAT	90 DAT	
Mycorrhiza					
M_0 - Uninoculated control	0.19	0.15	0.36	0.28	0.17
M_1 - *Glomus fasciculatum*	0.23	0.19	0.43	0.35	0.22
M_2 - *Glomus mosseae*	0.22	0.18	0.41	0.33	0.21
M_3 - *Glomus intraradices*	0.16	0.12	0.29	0.21	0.14
S.Em ±	0.0002	0.0002	0.0002	0.0002	0.002
C.D. (P=0.05)	0.0005	0.0005	0.0005	0.0005	0.006
Phosphorus levels (kg/ha)					
P_1 – 60	0.12	0.09	0.23	0.17	0.10
P_2 – 90	0.18	0.14	0.33	0.27	0.16
P_3 – 120	0.15	0.12	0.28	0.23	0.15
S.Em ±	0.0001	0.0001	0.0001	0.0001	0.002
C.D. (P=0.05)	0.0004	0.0004	0.0004	0.0004	0.004
Interaction (MXP)					
M_0P_1 - Uninoculated control + P @ 60	0.12	0.09	0.23	0.16	0.09
M_0P_2 - Uninoculated control + P @ 90	0.21	0.17	0.41	0.32	0.18
M_0P_3 - Uninoculated control + P @ 120	0.23	0.19	0.44	0.36	0.23
M_1P_1 - *Glomus fasciculatum* + P @ 60	0.17	0.13	0.32	0.23	0.16
M_1P_2 - *Glomus fasciculatum* + P @ *90*	0.28	0.23	0.53	0.43	0.26
M_1P_3 - *Glomus fasciculatum* + P @ 120	0.24	0.20	0.46	0.38	0.24
M_2P_1 - *Glomus mosseae* + P @ 60	0.20	0.17	0.38	0.31	0.14
M_2P_2 - *Glomus mosseae* + P @ 90	0.26	0.22	0.50	0.41	0.25
M_2P_3 - *Glomus mosseae* + P @ 120	0.19	0.16	0.36	0.28	0.23
M_3P_1 - *Glomus intraradices* + P @ 60	0.15	0.11	0.29	0.20	0.13
M_3P_2 - *Glomus intraradices* + P @ 90	0.19	0.15	0.35	0.26	0.17
M_3P_3 - *Glomus intraradices* + P @ 120	0.13	0.10	0.25	0.18	0.14
S.Em ±	0.0006	0.0006	0.0006	0.0006	0.006
C.D. (P=0.05)	0.002	0.002	0.002	0.002	0.018

Table.2 Effect of inoculation with VAM fungi at different P levels on uptake of N and P at Different growth stages of *Tagetes erecta* L.

Treatment	N uptake (kg/ ha)		P uptake (kg/ ha)	
	60 DAT	90 DAT	60 DAT	90 DAT
Mycorrhiza				
M_0 - Uninoculated control	33.57	30.63	13.92	13.65
M_1 - *Glomus fasciculatum*	42.25	36.01	18.40	18.51
M_2 - *Glomus mosseae*	39.29	33.67	16.61	16.96
M_3 - *Glomus intraradices*	28.64	24.42	10.48	9.38
S.Em ±	0.32	0.02	0.02	0.03
C.D. (P=0.05)	0.95	0.06	0.07	0.09
Phosphorus levels (kg/ha)				
P_1 - 60	21.91	18.95	8.20	7.52
P_2 - 90	32.66	27.54	14.11	14.19
P_3 - 120	26.29	23.69	11.11	11.19
S.Em ±	0.24	0.02	0.02	0.02
C.D. (P=0.05)	0.71	0.04	0.05	0.07
Interaction (MxP)				
M_0P_1 - Uninoculated control + P @ 60	24.63	20.72	7.59	6.14
M_0P_2 - Uninoculated control + P @ 90	36.98	34.63	16.19	16.07
M_0P_3 - Uninoculated control + P @ 120	39.09	36.55	17.98	18.73
M_1P_1 - *Glomus fasciculatum* + P @ 60	29.90	25.67	11.57	10.37
M_1P_2 - *Glomus fasciculatum* + P @ *90*	54.78	43.54	24.02	24.64
M_1P_3 - *Glomus fasciculatum* + P @ 120	42.06	38.84	19.61	20.51
M_2P_1 - *Glomus mosseae* + P @ 60	34.24	30.71	14.35	14.89
M_2P_2 - *Glomus mosseae* + P @ 90	50.32	41.25	22.10	22.87
M_2P_3 - *Glomus mosseae* + P @ 120	33.29	29.06	13.36	13.14
M_3P_1 - *Glomus intraradices* + P @ 60	28.09	23.96	10.22	8.73
M_3P_2 - *Glomus intraradices* + P @ 90	32.09	27.44	12.96	12.10
M_3P_3 - *Glomus intraradices* + P @ 120	25.74	21.87	8.27	7.30
S.Em ±	0.97	0.06	0.07	0.09
C.D. (P=0.05)	2.84	0.18	0.21	0.27

Inoculation of VAM fungi causes the greater uptake of P by their expanded network of hyphae (Kale *et al.,* 1987 and Bolan, 1991), recorded greater shoot P content of China aster and salvia with the inoculation of VAM fungi in the worm cast amended soil.

Similarly Sreenivasa, (1992) and Sreenivasa *et al.,* (1993) also recorded greater shoot P concentration of chilli with the inoculation of *G. macrocarpum* and applied with recommended level of P than applied with P alone.

In the present investigation also the plants inoculated with *G. fasciculatum* and given P at 90 kg/ ha recorded significantly maximum shoot P (0.23 %), P concentration in vegetative parts and flower (0.43 and 0.26 % respectively), and the same fungus along with the application of P at 90 kg/ ha recorded significantly maximum P-uptake and N uptake (24.64 and 43.54 kg/ha, respectively) (Figure 1). It was superior over all other *Glomus* fungi and it was comparable with uninoculated control plants

(18.73 kg/ ha) applied with P at 120 kg/ ha. Shoot P concentration at 90 DAT was reduced as compared to 60 DAT this might be due to the translocation of P towards flower development and cell expansion (Black, 1965). In the present investigation, increased P content could be attributed to the increased P-uptake.

Various mechanisms have been suggested for increase in the P-uptake by mycorrhizal plants. These includes: exploration of large soil volume, faster movement of P into mycorrhizal hyphae and solubilisation of soil P. Exploration of large soil volume by mycorrhizal plants is achieved by decreasing the distance, that P ion must diffuse to plant roots and by increasing the surface area of absorption. Faster movement of P into mycorrhizal hyphae is achieved by increasing the affinity for P ions and by decreasing the threshold concentration required, for absorption and solubilisation of soil P is achieved by the release of organic acids and phosphatase enzyme (Bolan, 1991). These results are supported by Csima *et al.*, (2012).

In conclusion, Shoot P concentration, P from vegetative parts, P from flower, P-uptake and N-uptake were significantly maximum in the inoculated plants and given P at 90 kg/ ha (0.23%, 0.43%, 0.26%, 24.64 kg/ ha and 43.54 kg/ ha, respectively). This indicates the possibility of reducing P fertilizer application by 25 per cent of the recommended dose to marigold by inoculation with a suitable strain of VAM fungi, *i. e., G. fasciculatum* and *G. mosseae*.

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