



RESEARCH ARTICLE

Influence of P Fertilizer and AM Fungal inoculation on Biomass yield and Nutrients Uptake in Two Medicinal plants

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Abstract

Inoculation of *Glomus macrocarpum* (AMF) with additional, super phosphate and rock phosphate treatment showed varied results on *Solanum nigrum* and *Solanum indica*. At 30 days interval experimental plants have not exhibited any better growth or nutrient uptake in mycorrhizal plants nor non-mycorrhization plants. However, both the plants significantly showed higher growth, biomass production nutrients uptake in both shoot and root of mycorrhizal plants over non-mycorrhizal plants. When the plants of *Solanum nigrum* treated with 3.0mg rock phosphate/kg soil, with mycorrhizal inoculation, and *Solanum indica* showed plants treated with 1. 0mg super phosphate /kg soil with mycorrhizal inoculation, on contrast to this per cent of AM fungal colonization and spore number decreased with the increased dosage of Super phosphate.

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INTRODUCTION

Proper fertilizer application is an essential method to increase plant, production that can be used on infertile soils. Most of the Indian tropical soils lack required phosphorus to many types of plants (Nambiar, 2002). It has been well documented that Arbuscular mycorrhizal fungi (AMF) are the members of phycmycetes associated with most of the terrestrial plants, play vital role in uptake and translocation of diffusion limited nutrients mainly 'P' there by promote plant growth (Koide and Mosse, 2004; Lakshman, 2009). The beneficial effect of AM fungi had a special importance, for those plants having coarse and poorly branched root systems. Since AM fungi external hyphae can extend as much as 5-8 cm away from the roots, absorb nutrients from a much larger soil volume than the absorption zone surrounding the non-mycorrhizal root system (Lakshman, 1996; Gai *et al.*, 2006). And thus absorption of phosphate ions could possible through some beneficial micro symbionts that are associated with rhizospheric zones of numerous plants. It is Arbuscular mycorrhizal fungi have associated symbolically almost all types of plants including medicinal plants. In many instances, when nutrients are exhausted from the soil, a balanced fertilizer will be necessity in order to maintain nutrient balance in mycorrhizal fungi especially important for the absorption of nutrients of low mobility in soil solution such as P, K, M, Zn, Fe and Cu etc. It was suggested that P is usually absorbed in the form of orthophosphate and transported actively through the much-branched hyphae as polyphosphate (Nye and Kirk, 1987; Bagyaraj, 2006). The major transfer of 'P' from the AM fungi to the plants occurred in those roots colonized with Arbuscular mycorrhizal main components called arbuscules. And thus P transfer from the fungus to the host plants takes place in a process of interchange with carbon compound metabolites. It has been observed that, 80-85% of total applied P is not available to plants because of their inaccessibility, fixation and immobilization. In this context, many experiments have been proved that, AM fungal inoculation increase the recovery of phosphate fertilization from soil.

Researches on AM fungi dependence of many plants have clearly demonstrated that tropical crops will not grow well in low P soils without an effective mycorrhizal association (Redhead, 1971; Rhodes and Gerdeman, 1978; Jøner and Jakobsen, 1995). Since mycotrophic plants depend on AM fungal colonization when they grown under low external P conditions, their yield can be enhanced by increasing efficiency of Arbuscular mycorrhizal fungi, either by inoculation of more efficient fungal strains or by fungi through the use of agronomic practices. But, less report are available on phosphate fertilizer application on medicinal plants to know their effect along with mycorrhizal inoculation.

The present study investigated the effects of the different levels of super phosphate and rock phosphate on two medicinal plants with and without inoculation of AM fungus (*Glomus macrocarpum*) was investigated.

Materials and methods:

The seeds of experimental plants (*Solanum nigrum* and *Solanum indica*) were sown in 15x20 cm diameter earthen pots containing four kg of sterilized phosphorus deficient soil. The soil was mixed in 1:1 proportion (1 part of garden soil and 1 part of pure sand). The Physico-chemical characteristic of this soil was analyzed given in (Table. 1). The random black designed triplicate sets of pots were maintained with proper controls. Finally, three different levels of super phosphate and rock phosphate at the rate of 1.0, 1.5 and 3.0 mg/kg rock phosphate and super phosphate was added to each experimental pots, except control pots. 15 gm of AM fungal inoculum (*Glomus macrocarpum*) consists of freshly infected chopped root pieces, spores, sporocarps, mycelia and rhizospheric soil collected from the rhizospheric soil of *Sorghum sudanense* was placed 4 cm below the surface each experimental pots before sowing the seeds. To maintain the moisture content the experimental pots were watered on every alternate day. All the pots were maintained in green house. The following treatments were given for each experimental plant.

1. Non mycorrhizal plant (Control)
2. Three levels of Rock phosphate + AM fungus (*Glomus macrocarpum*)
3. Three levels of Super phosphate + AM fungus (*Glomus macrocarpum*).

Periodical data were recorded for three harvests in between thirty days intervals. The observations were recorded on the plant height, dry weight of shoot and root are recorded at every interval of 30 days, and AM fungal spores were isolated by the wet-sieving and decanting method following (Gerdeman and Nicholson, 1963). The per cent of AM fungal colonization of roots were estimated according to (Philips and Hayman, 1970). Phosphorus content in plant was determined calorimetrically by the vanadomolybdate/phosphoric-yellow colour method outlined by Jackson (1973). Total nitrogen content was determined by the Microkjeldahl method (Bremner, 1960). Potassium was estimated by Flame Photometer following the procedure of Chapman and Pratt (1961). Other Physico-chemical analysis of the Soil used for experimental pots like pH, Moisture content was estimated according to the procedures of Jackson (1973). Different levels of rock phosphate and super phosphate were mixed in distilled water and given to each of the experimental plants by using 1.0, 1.5 and 3.0mg / kg soil / pot.

Results:

The AM fungus (*Glomus macrocarpum*) inoculated plants grew much taller than the non-mycorrhizal ones with P fertilizer application in both of the experimental plants (Tables 2-3). The highest plant height (49.3 cm) was observed in *Solanum nigrum* when treated with 3.0g RP/kg soil, whereas, *Solanum indica* showed high growth (55.1 cm) when treated with 1.0 mg SP/kg soil with AM fungal inoculation. The plants showed increased plant height, shoot dry weight, per cent colonization and spore number with gradual increased application of rock phosphate dosages. But gradual increased per cent root colonization with increased spore number was not noticed in *Solanum indica* with increased Super phosphate fertilization. So, the relation between per cent colonization and spore number was seen only in *Solanum nigrum* treated with rock phosphate fertilization.

The super phosphate fertilization experiments were not similar to these of rock phosphate treated experiments; it was observed that the application SP level 1.0 mg SP/kg soil greatly influenced the plant height and biomass production in AM fungal inoculated experimental plants. These plants showed decreased percent colonization and spore number with increased SP levels in both *Solanum nigrum* and *Solanum indica*. Nutrient uptake (NPK) was determined in the shoots of mycorrhizal and non-mycorrhizal of both the experimental plants. The results revealed that the increased higher concentration of N, P, K was determined in all the experimental plants, only after the AM fungal (*Glomus macrocarpum*) inoculation (Figure 1-4). These significant results were obtained in both rock phosphate and super phosphate treatments (Table 2 and 3). Root-shoot ratio was drastically increased in all the non-inoculated plants over the mycorrhizal plants. The P fertilization on experiments indicated that mycorrhizal inoculation alone aids in the effective utilization of Rock phosphate than the super phosphate. This

is because of changing them into available form by AM fungi, which was later taken up by the plants for their better growth and development.

Discussion

Some soil microorganisms play vital role in solubilization of mineral compounds which later on mobilized by the production of organic acids, but many microorganisms can bring the insoluble inorganic compounds into solution form. In such cases, arbuscular mycorrhizal fungi clearly produce an increase in absorbing root surface area due to gross changes in morphology of the feeder roots (Hayman and Mosse, 1972; Manjunath and Bagyaraj, 1984; McGonigle *et al.*, 1990). The data obtained in the present study after positive indications that the mycorrhizal colonization of the host plants, these two medicinal plants were able to utilize quickly soluble phosphates. The super phosphate treatments were more efficient in *Solanum indica*. These two plants demonstrated greater growth and shoot dry weight and mineral uptake in their shoots. Although the decrease in number of chlamydospores associated with increased super phosphate fertilization was not statistically significant. The trend of lower per cent mycorrhizal colonization with increasing fertilizer (SP) agrees with the results of previous workers (Sylvia and Schenck, 1983; Sieverding and Howler, 1985). On the other hand, arbuscular mycorrhizal fungi are known to occur in soils with very high P contents (Davis *et al.*, 1984; Powel and Daniel, 1978) and thus among the species of mycorrhizal fungi there may be different response to low or high soil P conditions or to P fertilization treatments (Jones, 2000).

Studies on *Solanum nigrum* and *Solanum indica* treated with 3.0 mg rock phosphate/kg soil dosages had the beneficial response to mycorrhiza (*Glomus macrocarpum*). The plants grow much, greater than non-mycorrhizal plants. This could be attributed that low solubility of P source (rock phosphate) is slowly diffuse in to the soil and mycorrhizal hyphae slowly absorb available P more effective than the plants treated with super phosphate. Plant roots may increase the rate of dissolution of rock phosphate by lowering the concentration of P in the soil solution and in some instances by lowering the pH of the soil. All the three levels of rock phosphate with mycorrhizal inoculation showed increased plant height, total dry weight of shoot in *Solanum nigrum*. Thus, the treatment of AM fungus + (3.0mg/kg soil) rock phosphate was most effective in promoting plant growth. These studies indicated that mycorrhizal inoculation helps in the effective utilization of rock Phosphate by changing it into available form, which later is taken up by the plants for their better growth and development. These findings are in consistent with the earlier workers contributions (Jalali and Thereja, 1985 Pacovsky *et al.*, 1986). But, the super phosphate on *Solanum indica* revealed, that the mycorrhizal inoculation with 1.0 mg super phosphate/kg soil was most effective than the other two levels of fertilization as seen in *Solanum indica*.

Plants inoculated with AM fungus (*Glomus macrocarpum*) and treated with different levels of super phosphate and plants treated with phosphate grown in green house conditions showed enhanced nutrients concentration in shoots, compared to non-inoculated plants. These findings are supported by others who reported similar results (Ross, 1971 Powell and Daniel, 1978; Harley and Smith, 1983). In contrast to these results, higher concentration of Mg was detected in non-mycorrhizal plants than mycorrhizal ones. There were significantly increased root-shoot ratios in non-mycorrhizal plants over the mycorrhizal plants. These findings are par with the results obtained by early works of (Redhead, 1977; Kormanik *et al.*, 1977; Mosse, 1990). The fraction of P applied that is absorbed by the plant after mycorrhizal inoculation is considered to the density of roots in the soil (Smith, 1982). At a lower soil P buffer capacity, the effect of root uptake on the rate of dissolution should be greater. Many rhizospheric soils contain organic acids with low calcium, it could be desirable to practice AM fungal inoculation technique to young nursery plants with different levels of rock phosphate and indigenous mycorrhizal fungi. It may be concluded that mycorrhizal plants absorb 'P' only from the soluble 'P' pools in the soil and that they are unable to utilize source of 'P' that are unavailable to non-mycorrhizal roots. Results presented here for spore density and root colonization are in agreement with these found in the green house and the field showing that AM fungal species, host plant species and soil conditions have been reported to effect on mycorrhizal formation and sporulation in pot culture (Brundrett *et al.*, 1996). Phosphorus with mycorrhiza responses for the plant growth agreed with that reported for other plants grown in controlled environment (Youpensuk *et al.*, 2005), there by confirming mycorrhizal nutritional benefits that the strong interrelationship between P supply and mycorrhizal response under nutrient stressed conditions.

Table 1. Physico-chemical properties of soil used for pot experiments.

Characteristics	Values
Soil moisture (1%)	24.3
pH	6.8%
Organic matter (1%)	0.62%
E.C milli mhos	0.47%
Nitrogen	19.12%

Phosphorus	14.3%
Potassium	24.5%
Iron	29.2%
Zinc	7.51%
Copper	4.7%
Magnesium	3.58%

Each value is the mean of 12 samples

Table 2. Effect of different P levels with AM fungus (*Glomus macrocarpum*) inoculated *Solanum nigrum* L. for 30, 60, 90 days.

Duration Treatments	Plant height (cm)	Dry wt of shoot (g)	Dry wt of root (g)	Root/shoot ratio %	% VAM collonization	Spore na/50g soil	Shoot			root		
							N%	P%	K%	N%	P%	K%
For 30 days												
NM	11.0±1.0	0.91±10	0.71±0.0	0.78±0.0	-	-	1.32b	0.09e	1.15c	0.41d	0.05f	0.42d
M+RP1	12.3±2.0	1.65±1.0	0.81±0.0	0.49±0.0	49.6±2.1	50.7±21	1.42b	0.15e	2.13b	0.75 a	007e	0.61c
M+RP2	21.5±2.0	1.73±1.0	0.90±1.0	0.52±0.0	52.5±3.1	50.4±1.1	1.74 a	0.18d	2.30a	0.32e	0.11d	0.35c
M+RP3	29.8±2.1	2.11±1.0	1.20±0.0	0.57±0.0	54.5±1.0	60.2±3.1	2.11 a	0.22a	2.41b	0.91 a	0.12d	1.25 a
M+SP1	35.1±2.0	2.80±0.0	0.91±0.0	0.53±0.0	38.5±2.1	49.2±2.1	1.21b	0.22 a	2.54 a	0.32e	0.11d	0.95 a
M+SP2	29.1±2.0	2.10±0.0	0.80±0.0	0.73±0.0	36.3±2.2	42.5±3.1	0.83d	0.21b	2.41b	0.51c	0.04f	0.45c
M+SP3	28.5±1.0	2.62±0.0	0.83±0.0	0.32±0.0	35.2±1.0	47.2±1.1	0.96 b	0.23 a	2.52a	0.49c	0.07e	0.58b
For 60 days												
NM	13.1±1.0	1.02±0.0	0.73±0.5	0.78±0.0	-	-	1.32b	0.11e	1.18e	0.52d	0.08e	0.51c
M+RP1	18.3±2.1	1.70±1.0	1.85±0.0	0.50±0.0	51.4±2.1	53.4±21	1.61b	0.18d	2.17b	0.79b	0.10a	0.71c
M+RP2	35.1±3.1	1.91±1.0	0.94±1.0	0.45±0.0	54.5±3.1	57.3±11	1.83 b	0.21c	2.34 a	0.84a	0.10a	1.11b
M+RP3	40.2±2.2	3.30±0.0	1.23±0.0	0.39±0.0	55.4±2.0	62.5±2.1	2.21 a	0.23a	2.94 a	1.11a	0.15a	1.51a
M+SP1	42.1±1.1	3.15±0.0	0.37±0.0	0.37±0.0	36.3±1.1	47.4±1.1	1.42b	0.20c	2.61 a	0.75b	0.07d	1.32b
M+SP2	31.5±1.0	2.09±1.0	0.73±0.0	0.61±0.0	35.5±2.1	41.5±2.1	0.91c	0.22 b	1.58b	0.51d	0.05d	0.52d
M+SP3	38.2±2.0	3.11±1.0	0.90±0.0	0.30±0.0	35.4±1.0	45.3±3.5	1.11c	0.24 a	2.63a	0.63d	0.09b	0.67b
For 90 days												
NM	15.1±1.0	1.10±10	0.72±0.0	0.78±0.0	-	-	1.41b	0.11d	1.41d	0.56d	0.07d	0.58c
M+RP1	21.2±1.0	1.81±1.0	0.52±1.0	0.50±1.0	51.2±1.0	54.5±3.1	1.82b	0.31c	2.19c	0.09e	0.12a	0.95 a
M+RP2	40.2±2.1	2.10±0.0	1.00±0.0	0.48±0.0	55.4±3.0	58.3±2.1	1.93a	0.34 b	2.45b	0.95b	0.13a	1.41a
M+RP3	49.3±3.2	3.60±1.0	1.32±0.0	0.37±0.0	59.3±2.1	67.4±3.1	2.31 a	0.38 a	3.12 a	1.21a	0.19a	1.72a
M+SP1	42.5±2.1	3.15±1.0	1.40±0.0	0.29±1.0	35.5±1.0	45.3±2.1	1.62a	0.32a	2.71b	0.82b	0.11a	1.51a
M+SP2	32.0±1.0	1.80±0.0	0.90±0.0	0.50±0.0	33.4±0.0	41.2±1.1	1.11c	0.25 b	2.80 b	0.53d	0.09b	0.65b
M+SP3	46.5±1.1	3.40±3.44	1.25±0.0	0.36±1.0	31.7±1.0	43.1±2.1	1.31b	0.31 a	2.94 a	0.74c	0.07c	0.81b

NM=Non-Mycorrhizal. M+RP1= AMF + 1.0 mg Rock phosphate/kg of soil. M+RP2 = AMF+1.5mg RP/kg soil, M+RP3 = AMF+3.0mg RP/kg of soil, M+SP1 = AMF+1.0mg Super phosphate/kg soil, M+RP2= AMF+1.5mg SP/kg of soil, M+SP3= AMF+3.0mg SP/kg of soil.

Each value is the mean of four reading ie. N=4, and values represented as n=4, ± Standard duration. Means followed the same letter in each column do not differ significantly at P=0.05.

Table 3. Effect of different P levels with AM fungus (*Glomus macrocarpum*) inoculated *Solanum indica* L. for 30, 60, 90 days.

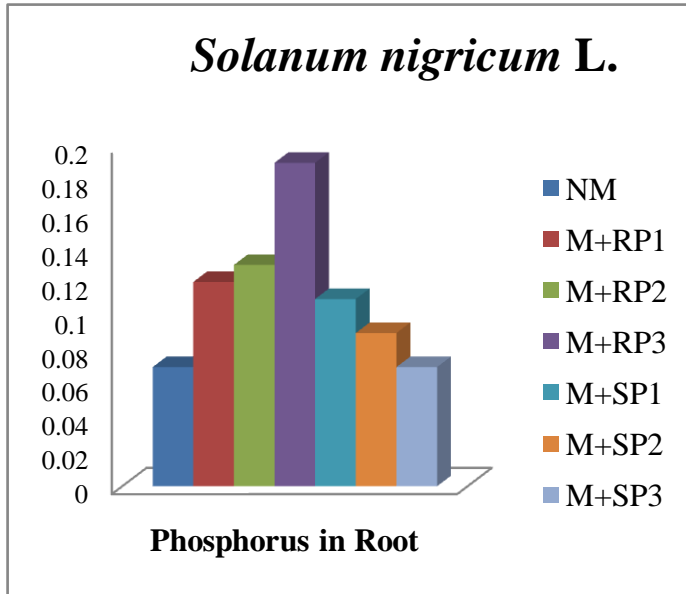
Duration Treatments	Plant height (cm)	Dry wt of shoot (g)	Dry wt of root (g)	Root/shoot ratio %	% VAM collonization	Spore No/50g soil	Shoot			root		
							N%	P%	K%	N%	P%	K%
For 30 days												
NM	13.1±0.0	0.95±0.0	0.72±0.0	0.75±0.0	-	-	1.32e	0.11e	2.24b	0.37e	0.05e	0.41d
M+RP1	16.2±2.0	1.70±1.0	0.85±0.	0.50±0.0	36.2±5.2	18.2±1.0	1.45d	0.12d	2.30b	0.38e	0.07d	0.45c
M+PP2	22.5±1.0	1.03±1.0	3.90±1.0	0.50±1.0	38.3±2.0	42.5±3.0	1.39d	0.29a	3.34a	0.44c	0.11c	0.52c
M+RP3	18.2±2.0	1.72±1.0	0.88±0.0	0.51±0.0	42.4±3.1	51.4±1.0	1.36d	0.14c	2.15c	0.42c	0.12c	0.46d
M+SP1	33.5±2.0	2.10±1.0	0.91±1.0	0.50±0.0	31.5±1.0	43.3±3.0	1.91a	0.28a	3.52a	0.52a	0.15a	1.51a
M+SP2	27.1±1.0	1.71±3.0	0.86±0.0	0.50±0.0	36.2±1.2	40.2±1.0	1.45c	0.21b	2.41d	0.45b	0.12c	0.96b
M+SP3	23.3±2.0	1.73±0.0	0.88±1.0	0.50±0.0	31.5±20	39.1±1.0	1.78b	0.25a	2.73c	0.48b	0.14b	0.99b
For 60 days												
NM	14.5±0.0	0.97±0.0	3.72±1.0	0.74±0.0	-	-	1.34c	0.23d	2.27c	0.38e	0.06e	0.41e
M+RP1	13.4±0.0	0.82±0.0	0.92±1.0	0.50±0.0	35.4±2.0	51.7±1.0	1.38c	0.24b	2.35d	0.42c	0.11d	0.46d
M+RP2	30.5±1.0	2.20±1.0	0.25±1.0	0.56±0.0	41.5±0.0	52.3±3.0	1.42b	0.25a	3.48a	0.46b	0.14c	0.57b
M+RP3	26.1±1.0	1.95±1.0	3.93±1.0	0.50±0.0	42.3±2.0	54.4±1.0	0.41e	0.19d	2.81c	0.42c	0.12c	1.48b
M+SP1	40.2±2.0	3.80±1.0	1.21±0.0	0.57±0.0	34.4±1.0	40.3±3.0	1.94a	0.31a	3.17a	0.54a	0.17a	2.53a
M+SP2	30.2±1.0	1.98±1.0	1.10±1.0	0.55±0.0	32.5±1.1	41.2±1.0	1.47b	0.22b	2.52d	0.46b	0.13c	0.47d
M+SP3	38.0±0.0	2.0±0.0	1.16±0.0	0.58±0.0	29.3±2.0	41.5±3.0	1.82a	0.22b	2.91c	0.52a	0.15b	0.42c
For 90 days												
NM	17.5±1.0	1.10±0.0	0.80±0.0	0.72±0.0	-	-	1.34e	0.23c	2.30e	0.38d	0.10e	1.41e
M+RP1	30.2±2.0	2.10±0.0	1.20±0.0	0.57±0.0	39.6±2.0	51.6±3.0	1.39d	0.18e	2.38d	0.44b	0.12d	1.48d
M+RP2	42.1±1.0	3.87±0.0	1.40±0.0	0.50±0.0	47.3±1.1	53.3±1.0	1.44b	0.23c	3.56a	0.48b	0.15c	1.59a
M+RP3	28.2±1.0	2.00±0.0	1.11±0.0	0.55±0.0	45.4±3.1	54.7±1.0	1.42c	0.21c	2.87b	0.43c	0.14d	1.45d
M+SP1	55.1±2.0	4.11±0.0	1.45±0.0	0.35±0.0	31.2±1.0	40.4±3.0	1.98a	0.35a	3.89a	0.57a	0.18a	1.55a
M+SP2	35.1±1.0	2.15±0.0	1.322±0.0	0.56±0.0	32.5±1.0	38.4±1.0	2.52a	0.24b	2.64c	0.49b	0.14d	1.49b
M+SP3	48.2±2.0	3.91±0.0	1.35±0.0	0.35±0.0	24.1±2.0	37.4±1.0	1.65b	0.32a	3.19a	0.54a	0.16b	1.48b

NM=Non-Mycorrhizal. M+RP1= AMF + 1.0 mg Rock phosphate/kg of soil. M+RP2 = AMF+1.5mg RP/kg soil, M+RP3 = AMF+3.0mg RP/kg of soil, M+SP1 = AMF+1.0mg Super phosphate/kg soil, M+RP2= AMF+1.5mg SP/kg of soil, M+SP3= AMF+3.0mg SP/kg of soil.

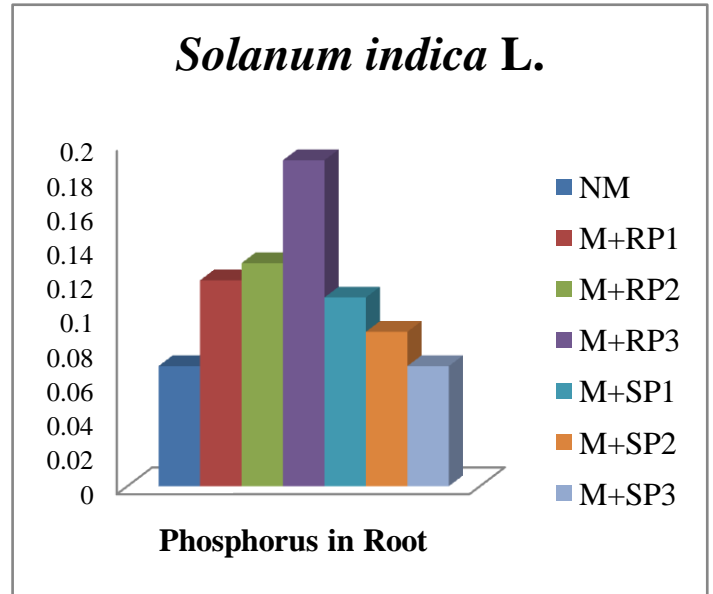
Each value is the mean of four reading ie. N=4, and values represented as n=4, ± Standard duration. Means followed the same letter in each column do not differ significantly at P=0.05.

Figure 1-4. Showing the effect of AM fungi, along with inoculation of Rock Phosphate and Super Phosphate on Phosphorus uptake in *Solanum nigrum* L. and *Solanum indicum* L. for 90 days.

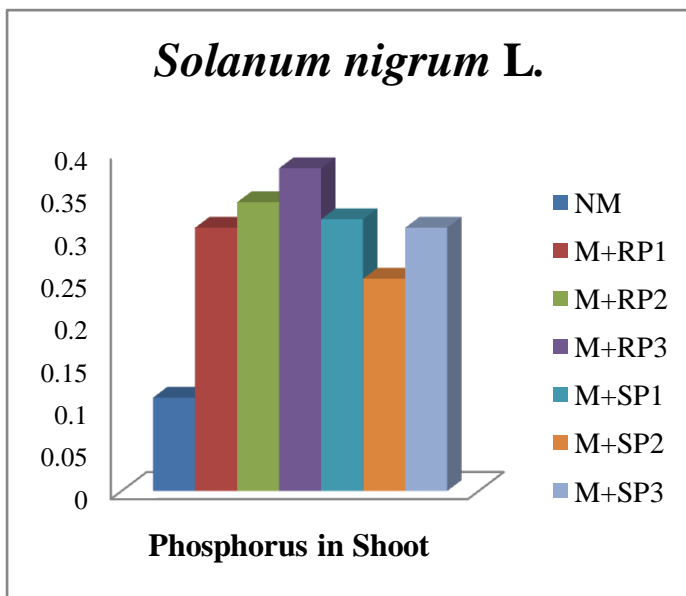
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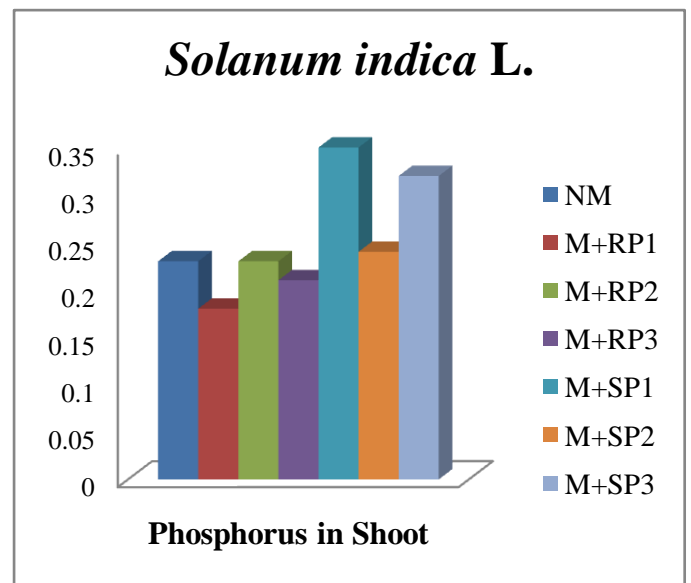
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