International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Int. J. of Life Sciences, 2020; 8 (4):719-725
ISSN:2320-7817(p) | 2320-964X(o)

Original Article **Open Access**

Influence of phosphorus fertilizer and mycorrhizal fungi on growth, protein content of *Psophocarpus tetragonolobus* (L.) DC.

Naqvi Nikhat

Department of Botany, SFS College, Seminary Hills, Nagpur
Email: naqvin@rediffmail.com

Manuscript details:

Received: 01.06.2020
Accepted: 10.11.2020
Published: 30.12.2020

Cite this article as:
Naqvi Nikhat (2020) Influence of phosphorus fertilizer and mycorrhizal fungi on growth, protein content of *Psophocarpus tetragonolobus* (L.) DC., *Int. J. of Life Sciences,* 2020; 8 (4): 719-725.

Available online on http://www.ijlsci.in
ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

ABSTRACT

The present growing concern about the negative effects of various chemicals which are used in plant production on environment is promoting the development of sustainable management practices. One such strategy is the better exploitation or management of microbes present in soil which contribute to soil fertility. The feasibility of using Arbuscular Mycorrhizal Fungi to reduce the need for fertilizer application, particularly phosphorus was examined using *Psophocarpus tetragonolobus* (L.) DC. (Winged bean). The plants were grown in fumigated plots. The effect of mycorrhizal inoculation as well as Superphosphate application on plants was studied. Mycorrhizal inoculation influenced plant growth responses and the application of Superphosphate stimulated the effects of the fungus. Mycorrhizal inoculation increased dry matter, yield, protein content. Maximum growth response was recorded when plants were inoculated with AM fungi and also given P amendment.

Keywords: *Psophocarpus tetragonolobus*, protein, phosphate fertilizer, mycorrhizal fungi, crop yield, sustainable agriculture.

INTRODUCTION

Nowadays increased emphasis is being given on sustainable management practices due to the negative effects of various chemicals used in plant production on environment. For achieving sustainable plant production, decrease in chemical inputs to economic but nonpolluting levels through the development of alternative strategies has been advocated. Sustainability in agriculture requires balanced functional microbial ecosystems. An important category of microbes includes Arbuscular mycorrhizal fungi. The association of plant roots with these fungi is a key factor in the below ground network essential to ecosystem function (Smith and Read, 2010). Arbuscular mycorrhizal fungi (AMF) belonging to phylum Glomeromycota symbiotically associate with the plant roots. (SchuBler and Walker, 2010). About 80% of the land plants are colonized with AMF.

This association is known to benefit plants under conditions of nutritional and water stress and pathogen challenge (Naqvi and Naqvi ,2004). The extraradical hyphae of mycorrhizal fungi play an important role in holding soil particles, maintaining soil structure ,increasing soil aggregation thereby increasing waterholding capacity of soil and reduced drought susceptibility(Yan et al. 2012) .Agriculture has a dominant role in economy of a developing country .These fungi are of increased interest due to their widespread distribution and their ability to colonize major agricultural crops thereby enhancing plant productivity and improving their mineral nutrition especially phosphorus (Marschner and Dell , 1994). Phosphorus is one of the important nutrients affecting plant growth. However, Phosphorus fertilizers is major expense to the farmers. Management of AM fungi to improve the efficiency of Phosphorus use is now-a-days a very important component of sustainable agriculture systems and IPM (Field et al.2020). The feasibility of using Arbuscular mycorrhizal fungi to reduce the need for fertilizer application, particularly phosphorus has been examined by many workers (Smith et al. 2008, Sun et al.2016, Zhang et al.2017). Phosphorus (P) supply is the most important feature mediated by AMF (Smith and Smith, 2011). Number of studies have demonstrated the beneficial role of AM fungi in plant growth and health. Growth and productivity of number of plant species have been shown to be enhanced by AMF including crops of agricultural importance such as soybeans (Stoffel et al.2020), corn (Guo et al., 2014), beans (Tajini et al. 2012), wheat (Ardakani et al. 2011) and Okra (Farhan and Khalifa, 2019).

*Psophocarpus tetragonolobus (L.) DC. (*winged bean) ranks first in importance among underutilized/ underexploited minor tropical legumes which is receiving considerable attention in recent years due to its high protein content and wide range of uses of all plant parts (multipurpose crop). It is grown for food, fodder, as a cover and fallow restorative crop or for green manure because of its exceptionally good nodulation.

This investigation was undertaken to determine the response of *Psophocarpus tetragonolobus* to colonization with AM fungi and to compare the AM inoculated plants to non-inoculated winged beans that received P fertilizer. This report provides information on growth, yield, protein content in various parts of mycorrhizal and non-mycorrhizal plants with P amendment.

MATERIALS AND METHODS

Study Area:
Four plots of 9×5 Sq metre area were selected for this study. The plots were sterilized by fumigating the soil with 0.1% Formalin(v/v). The plots were kept covered with polythene sheets for a week and then exposed for another week .This process was repeated twice. In plots given P amendment, pellets of Superphosphate were ground to a fine powder and dissolved in water. This was added to the soil before seed sowing.

Test Plant:
Seeds of *Psophocarpus tetragonolobus* (L.) DC. Var. AKWB1 was used.

Mycorrhizal Inoculation:
Spores of *Glomus macrocarpum* were used for inoculation. The inoculum consisted of mycorrhizal roots with extramatrical hyphae and soil containing spores. The AM inocula was placed at a depth of 3 cm in furrows before sowing the seeds.
Seeds were sown with following four treatments of soil.
Control
Mycorrhiza
Superphosphate amendment (50kg/ha)
Mycorrhiza+ Superphosphate (50 kg/ha)

Growth Studies
Various growth parameters like root length ,shoot length, number of nodules ,number of leaves ,root dry weight, shoot dry weight ,number of pods/plant ,number of seeds/pod, length of pod were recorded at different growth periods .Yield was estimated by taking dry weight of 20 seeds.

Mycorrhizal Status (a) AM Fungal spore isolation**: AM** Fungal spores were extracted using wet sieving and decanting technique (Gerdemann and Nicolson, 1963). Spores were counted under Stereozoom microscope. An average of 5 readings was taken.
(b)AM Fungi in roots**:** Percent mycorrhizal colonization in roots- The cleaned roots were cut into 1cm long pieces and stained with Trypan Blue (Phillips and Hayman ,1970). Minimum of 100 root segments were observed and colonization by AM fungi was calculated using the following formula: .

$$\text{Percent root colonization (\%)} = \frac{\text{Total No. of root segments colonized}}{\text{Total no. of root segments studied}} \text{X100}$$

Biochemical Analysis:

Protein content in Roots and Shoots- It was estimated by Bradford's method (1976).

Seed protein content- It was estimated by Microkjeldahl method.

RESULTS AND DISCUSSION

Plant growth responses:

Both P fertilizer (Superphosphate) as well as AM fungi substantially influenced the various growth parameters in *Psophocarpus tetragonolobus* (Table I). The increase with mycorrhizal fungi alone was much more as compared with Superphosphate alone. In treatments with Superphosphate alone or with combination of Superphosphate and AM fungi, there was early flowering and consequently fruiting. Number of pods per plant were more in mycorrhiza inoculated plants as compared to only Superphosphate treated plants. However, the combined treatment of AM fungi and superphosphate caused approximately five times increase as compared to control (Table 2). The yield was increased 1.5 times by combined treatment of AM fungi and Superphosphate as compared to control (Table 2). Thus, AMF application may allow considerable reduction in P rates applied to the soil. This reduction has a direct effect on soil quality, reducing the dependance on phosphate fertilizers, thereby reducing the expenses related to the purchase of fertilizers by the farmers.

Table 1: Effect of different treatments on various vegetative growth parameters of 60-day old plants of *Psophocarpus tetragonolobus* (Naqvi)

Serial no.	Growth parameters	Treatments	Mean	Observed 't' value
1	Root Length(cm)	Control	19.32 ± 2.53	-
		Mycorrhiza(M)	20.76 ±3.15	0.72
		Super Phosphate(SP)	21.16 ± 0.51	1.44
		SP+M	21.48 ± 2.64	1.19
2	Shoot Length(cm)	Control	97.16 ± 8.04	-
		Mycorrhiza(M)	122.08 ± 38.9	1.26
		Super Phosphate(SP)	124.88 ± 14.92	3.28*
		SP+M	142.42 ± 11.61	6.44***
3	Number of leaves	Control	21.4 ± 3.05	-
		Mycorrhiza(M)	26.6 ± 4.34	1.97
		Super Phosphate(SP)	24.8 ± 0.83	2.17
		SP+M	27.4 ± 1.82	3.39**
4	Number of Nodules	Control	1 ± 1	-
		Mycorrhiza(M)	4.4 ± 2.3	2.72*
		Super Phosphate(SP)	4.4 ± 1.14	4.47**
		SP+M	6 ± 2.35	3.94**
5	Root Dry Weight (g)	Control	0.507 ± 0.14	-
		Mycorrhiza(M)	1.268 ± 0.33	4.23**
		Super Phosphate(SP)	0.952 ± 0.13	4.94**
		SP+M	0.966 ± 0.05	5.74***
6	Shoot Dry Weight (g)	Control	4.133 ± 0.62	-
		Mycorrhiza(M)	7.636 ± 1.41	4.55**
		Super Phosphate(SP)	6.214 ± 2.46	1.65
		SP+M	7.099 ± 1.49	3.71**

Values are expressed as mean ± SD, where n=5

*Observed 't' value is significant at P ≤ 0.05

**Observed 't' value is significant at P ≤ 0.01

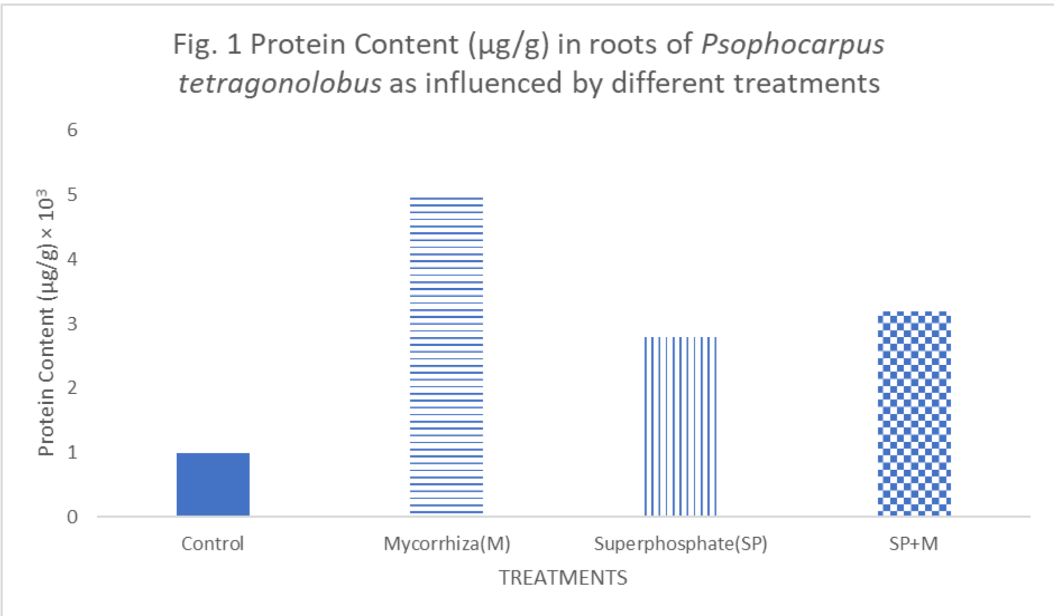
***Observed 't' value is significant at P ≤ 0.001

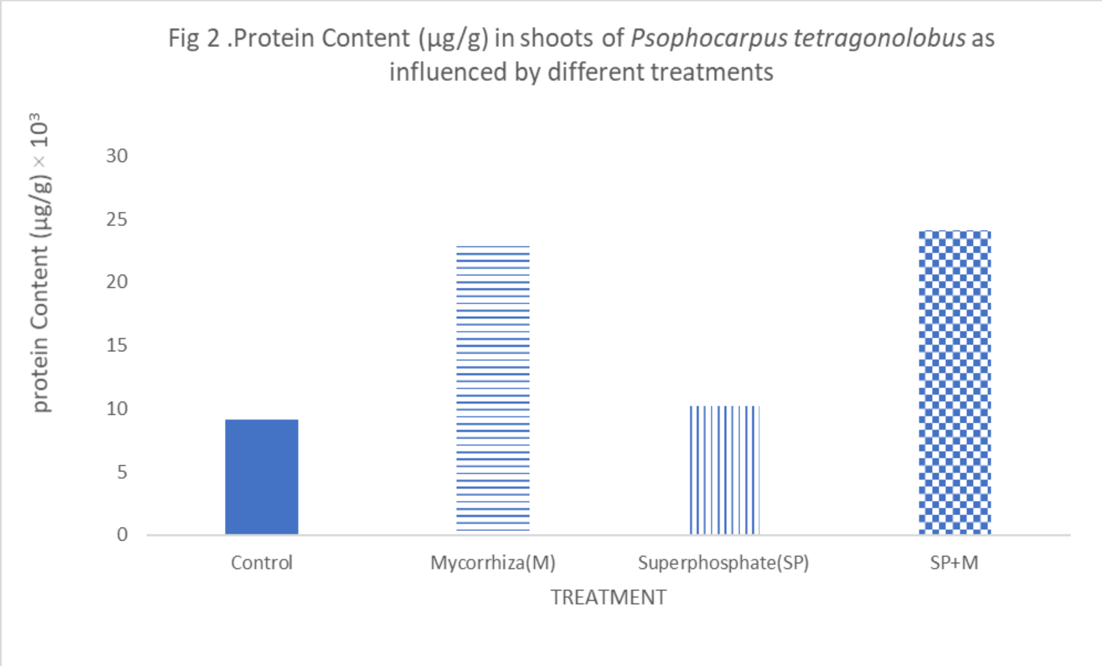
An increase in growth response due to AM inoculation has been earlier reported by several workers using different crop plants.(Ortas 2010, Olagunju et al.2014) .Increase in dry weight accumulation owing to mycorrhiza has also been reported by number of researchers(Thilagar and Bagyaraj 2015) .Generally mycorrhizal plants exhibit better growth than non-mycorrhizal plants when supplied with adequate amount of P (Bagyaraj et al. 2015, Sharif et al. 2011) .However, the response seems to vary with crop ,soil and mycorrhizal fungi used (Su et al. 2003). Further these fungi show a preferential colonization of the hosts, and consequently, the degree of benefits received by the host depends on the fungal species involved (Abbott and Robson, 1991).

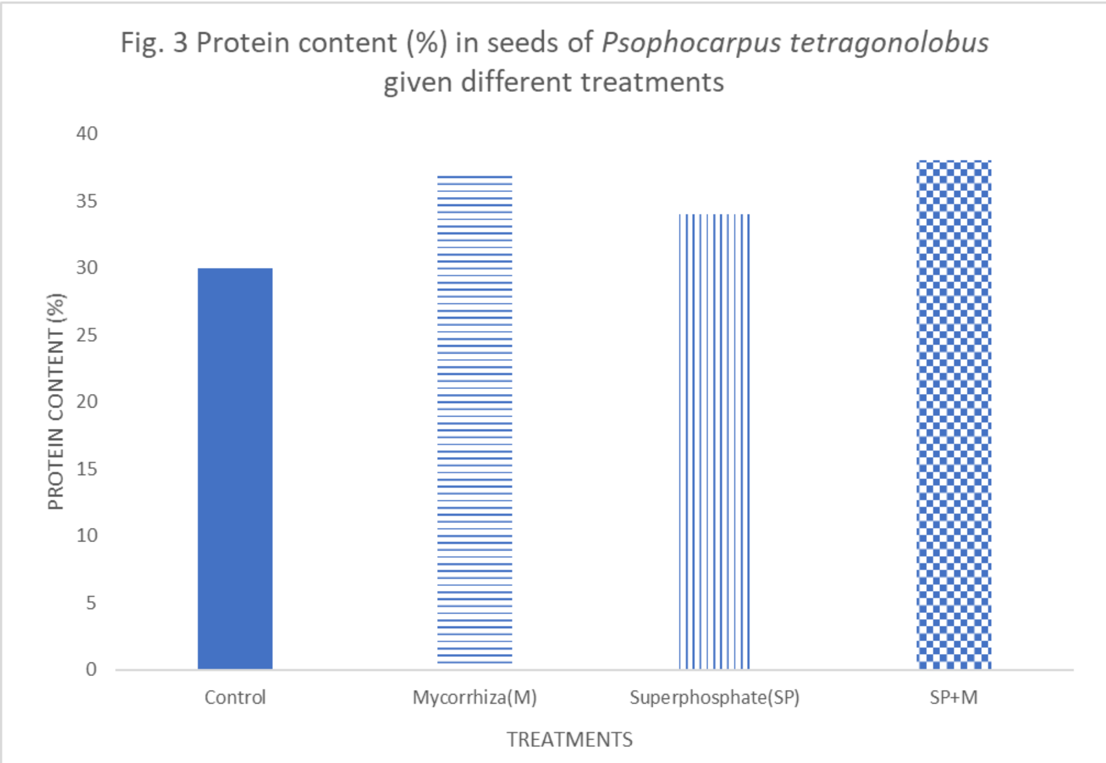
Table 2: Influence of different treatments on various reproductive growth parameters and yield of *Psophocarpus tetragonolobus* . (Naqvi)

Serial no.	Growth parameters	Treatment	Mean
1	Number of pods per plant	Control	4.6 ± 1.52
		Mycorrhiza(M)	13.4 ± 2.88
		Super Phosphate(SP)	9.6 ± 2.41
		SP+M	21.2 ± 5.72
2	Number of seeds per pod	Control	6.6 ± 0.55
		Mycorrhiza(M)	8.8 ± 0.45
		Super Phosphate(SP)	9.4 ± 0.89
		SP+M	10.4 ± 1.52
3	Length of pod (cm)	Control	8.64 ± 0.65
		Mycorrhiza(M)	10.4 ± 0.99
		Super Phosphate(SP)	11.18 ± 0.49
		SP+M	14.74 ± 1.36
4	Dry Weight of 20 seeds (g)	Control	2.819
		Mycorrhiza(M)	3.682
		Super Phosphate(SP)	3.744
		SP+M	4.287

Values are expressed as mean ± SD, where n=5







Mycorrhization

Since at the time of seed sowing, all the plots were sterilized by fumigation, therefore no colonization was observed in plants without mycorrhizal colonization. Presence of Superphosphate inhibited the mycorrhizal colonization in AM inoculated plants. Internal spread of AM fungus as well as arbuscule formation was slow and vesicle formation was suppressed and formed at later stage. However, formation of superficial mycelia and appressoria was not affected by P application. Suppression of vesicle formation with P amendment suggests that vesicles which are known for storage are not required because of continuous supply of P in the host tissue. High levels of soil Phosphorus are reported to be deleterious to AM fungi (Koide and Li 1990, Kuramshina et al. 2020)). Percent mycorrhizal colonization increased over a time interval. All the stages of root colonization by AM fungi were observed. Number of spores were initially high which later decreased but again increased at later stages.

Protein content

AM fungi as well as Superphosphate increased the content of protein in plant tissues as well as seeds as compared to control. In general, there was more protein in shoots than in roots. At 60 days, mycorrhizal inoculation resulted in five times increase in protein content in roots as compared to control (Fig.1). In shoots also, inoculation of AM fungi caused 2.5 times increase over control (Fig. 2). Protein content was greater in seeds of AM inoculated plants as compared to Superphosphate treated plants (Fig. 3).

CONCLUSION

Commercial use of Arbuscular mycorrhizal fungi may be an alternative to rising fertilizer costs. AM fungi appear to decrease the need of fertilizers by contributing to the demand of P to crop plants for optimum growth. The potential for employing AM fungi on a wide scale in agriculture is dependant on the soil conditions ,crop ,strain of AM fungi used .The results suggest that the use of eco-friendly and environmentally safe approaches such as AMF fungi will not only reduce fertilizer inputs but also contributes towards sustainable plant production system.

Conflicts of Interest: The author declares no conflict of interest

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