

Stimulatory synthesis of saponin by mycorrhizal fungi in safed musli (*Chlorophytum borivillianum*) tubers

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The amount of saponin accumulated in the tuber of safed musli (*Chlorophytum borivillianum*) was evaluated with or without mycorrhizal inoculation in a green house experiment conducted over a 270 days period. Differences in the saponin concentration and the total amount in tuber between the treatment and between the developmental stages were examined. The mycorrhizal treatment included three species viz *Glomus fasciculatum*, *Glomus intraradices* and *Glomus mosseae*. The amount of saponin accumulated in tuber increased with growth and it was under maximum *Glomus mosseae* infected plant. Mycorrhizal inoculated compared to non inoculated plants resulted up to 25-fold improvement in saponin content at critical growth stage (45 days) and up to 4-fold improvement at crop harvest i.e. 270 days. *Glomus mosseae* was found to be most efficient mycorrhizal species. The present results suggest the possibility to stimulate the saponin content by treating the tubers with mycorrhizal inoculation, which improved the therapeutic value of safed musli (*Chlorophytum borivillianum*) tubers.

Keywords: Medicinal value, mycorrhizal fungi, safed musli, saponin build up.

INTRODUCTION

Chlorophytum borivillianum Santapau and Fernandes, popularly known as safed musli is an important medicinal plants due to its aphrodisiac properties (Singh and Chauhan, 2003). *Chlorophytum borivillianum* is the most commercially exploited and widely growing species. It is widely used as a general health promotive tonic, for various gynecological disorders, arthritic conditions and to control diabetes mellitus (Purohit and Prajapati, 2003). The tubers are rich in alkaloids, vitamins, minerals, proteins, carbohydrates, saponins, polysaccharides and steroids (Raghavendra et al., 2005). Roots of the plant are used both in antiviral (Siddiqui, 2005), anticancer (Arif, 2005), immunomodulatory (Singh et al., 2004), anti-diabetic (Govindrajan et al., 2005), antistress (Gopalkrishna and Patil, 2006), aphrodisiac (Thakur and Dixit, 2006), antimicrobial (Deore and Khadabadi, 2007) and anti-inflammatory (Deore and Khadabadi, 2008). Arbuscular mycorrhizae can colonize the roots of most vascular plants and can develop a complex system of extraradical hyphae under natural conditions. Mycorrhizae

may help plants to thrive in arid environment (Requena et al., 2001) by increasing the supply of nutrients to plants particularly phosphorus (Toro et al., 1997), reducing water stress (Auge, 2001) and increasing secondary metabolites (Kapoor et al., 2007). Increase in yield of naturally occurring saponin is an important area of investigations. Hence, in the present study attempts have been made to increase saponin concentration in the tubers of safed musli through mycorrhizal fungi inoculation.

MATERIALS AND METHODS

A pot trial was conducted using loamy sand (camborthid) soil collected from barren lands of Jodhpur. The preliminary analysis of soil indicated organic C content 0.03%, pH 7.9, EC 0.13 dSm⁻¹, Olsen P 3.5 mg kg⁻¹, organic P 72 mg kg⁻¹ and total P 445 mg kg⁻¹. Earthen pots (50 cm × 30 cm) of 25 kg capacity were filled with 20 kg sieved (2 mm) soil. There were four treatments viz. control (without inoculation) and inoculated with three different types of mycorrhizal fungi namely *Glomus fasciculatum*, *Glomus intraradices* and *Glomus mosseae*.

Table 1. Root length, percent root infection and spore number in soil of safed musli (*Chlorophytum borivilianum*) with or without AM inoculation at critical growth stage (45 days)

Treatment	Plant age (days)		
	Root length (cm)	Root infection (%)	Spore number (100 g ⁻¹ soil)
Control	260.2	46.9	46.0
<i>Glomus fasciculatum</i>	410.0	70.4	74.3
<i>Glomus intraradices</i>	767.3	91.9	150.0
<i>Glomus mosseae</i>	707.8	76.4	125.0
LSD (p = 0.05)	21.3	4.9	8.3

The experiment was laid out in a green house under sunlight for 10 h day and was water on alternate days to field capacity. There were nine replicates in each treatment. Three pots from each treatment were harvested after 45, 90 and 270 days (at maturity).

Healthy tubers of *Chlorophytum borivilianum* were purchased from the Rajasthan Seed Corporation, Indore, Madhya Pradesh, India and stored in sand until the experiment was laid on. Tubers weighing 8 to 10 g were sown in each pot. The pure cultures of mycorrhizal fungal species maintained on pearl millet and wheat simultaneously in our net house were used as inoculum. The inoculum containing 1500 spores, plant root and fungal propagules was placed below the tubers in each arbuscular mycorrhizal (AM) fungi treatment at the start of the experiment. No manure and fertilizers were applied at any stage of plant growth. The root system was lifted by emptying the pots after each harvest for detailed analysis.

To access the root colonization by VAM fungi, the standard staining technique of Phillips and Hyman (1970) was followed. The roots of plants were cleared with 10% (w/v) KOH at 90°C for 12-15 min in a water bath, rinsed three times, stained in 0.1% trypan blue (made in lacto phenol) at 90°C for 3-5 min, and mounted in lactic acid: glycerol (1:1). The recovery and quantification of VAM fungal propagules for spore build-up studies were done in different harvesting periods. Root length colonized by AM fungi was determined by the grid line intersect method (Tennant, 1975). Wet sieving and decanting technique (Gaur and Adholeya, 1994) extracted spores of different species. All the spores were examined using Medilux-20 TR compound microscope. The saponin content in the root was estimated using (Bordia et al., 1995) with the modification that instead of refluxed extraction we have used microwave-assisted extraction. The root powdered material was defatted using petroleum ether and extracted with ethanol, chloroform, and butanol in 20-ml closed vials, which were placed in a mechanically modified microwave oven. After cooling to room temperature, the extract was collected and kept at 20°C until analysis. Finally, the saponin was quantified by a

colorimetric reaction mixture using acetic acid and sulphuric acid and the absorbance at 530 nm. Organic carbon was estimated by the method of Walkley and Black (1934) using 1N potassium dichromate and back titrated with 0.5N ferrous ammonium sulphates solution. pH, EC and P was estimated as described by Jackson (1967). Microsoft Excel 2000 was used in statistical processing of the data. The least significant difference was calculated (p = 0.05) as described by Sokal and Rohlf (1981).

RESULTS AND DISCUSSION

The root length, per cent root infection and spore number in soil was significantly increased by inoculation with all the three mycorrhizal fungi, which was highest under *Glomus mosseae* followed by *Glomus intraradices* (Table 1). Among the three AM species a significantly higher spore build up, root length and per cent root infection was observed under *Glomus mosseae*. The microscopic images of root infection was reflected as Figure 1. In general, AM fungi enhanced 50 to 96% of root infection, 58 to 195% of root length and 62 to 226% of spore number than uninoculated control treatment. A tremendous improvement in tuber formation was observed under all the AM fungi inoculated treatment which was more due to *Glomus mosseae* inoculation (Figure 2).

In the mycorrhizal treatments (Table 2), the saponin concentration in tuber varied between 104.8 and 205.7 mg g⁻¹, which was significantly higher than control (75.3 to 92.7 mg g⁻¹). In general, saponin concentration has been increased with plant age in entire mycorrhizal plants. The more increase in saponin concentration was observed with the inoculation of *Glomus mosseae* (65.2 to 158.7%) followed by *Glomus intraradices* (61.5 to 125.2%) and *Glomus fasciculatum* (13.0 to 113.1%) as compared to control treatment under different crop age.

The results shown in (Table 2) strongly suggest that mycorrhizal fungi substantially contribute to saponin content in safed musli, depending upon the type of AM

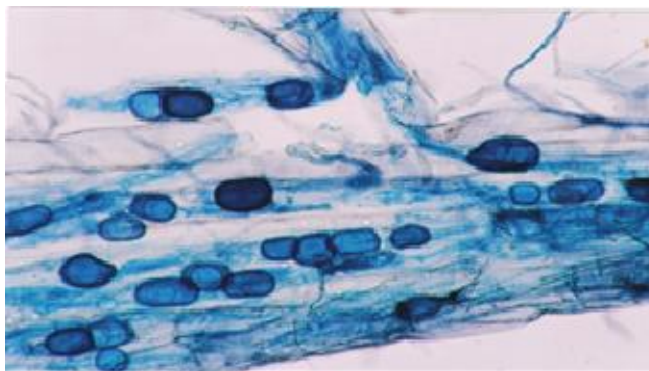


Figure 1. Images of root infection by AM fungi *Glomus mosseae* in safed musli (*Chlorophytum borivilianum*)



Figure 2. Differences in number of finger and size due to inoculation of *Glomus mosseae* in safed musli (*Chlorophytum borivilianum*)

Table 2. Saponin concentration (mg g⁻¹ dry weight) in tuber of safed musli (*Chlorophytum borivilianum*) at different crop age with or without AM fungi inoculation

Treatment	Plant age (days)		
	45	90	270*
Control	92.7	75.3	79.5
<i>Glomus fasciculatum</i>	104.8	125.9	169.4
<i>Glomus intraradices</i>	149.7	170.2	179.0
<i>Glomus mosseae</i>	153.1	166.9	205.7
LSD (p = 0.05)	0.62	0.34	0.39

* at crop harvest

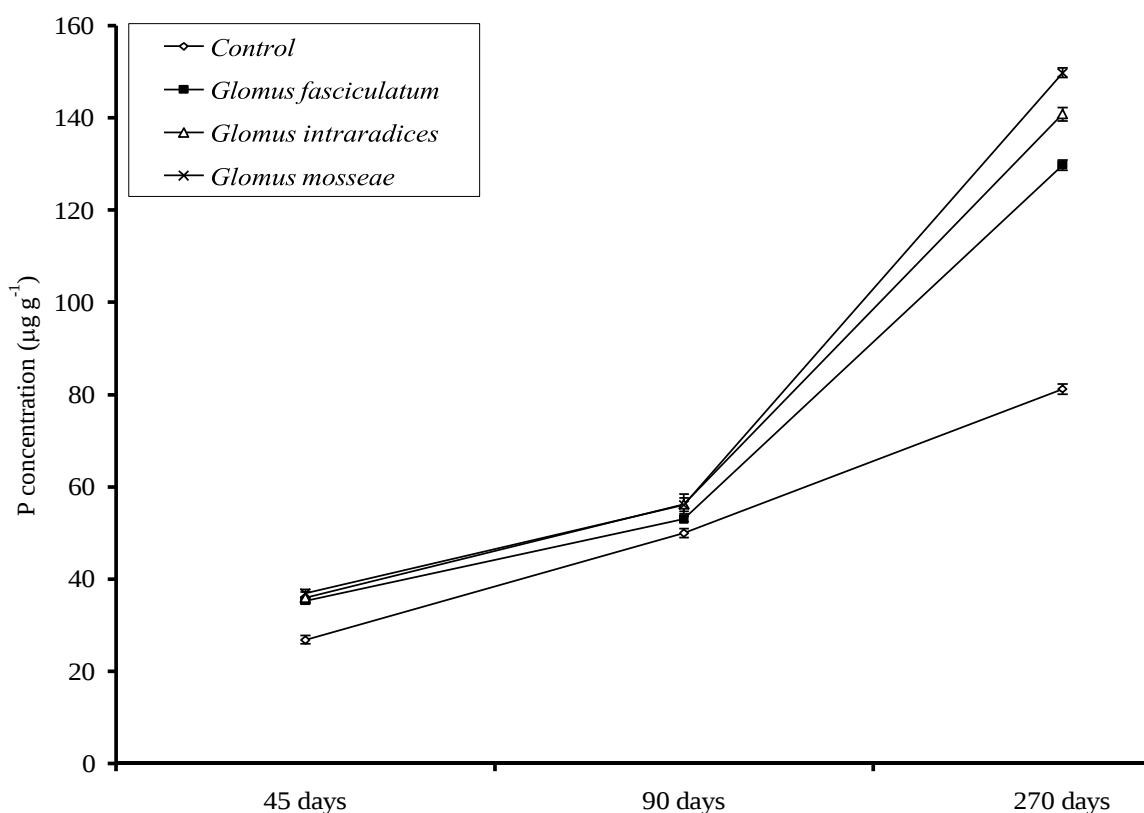
fungi supplied. At critical stage of plant growth (45 days) up to 25 times improvement in saponin content was observed by *Glomus mosseae* as compared to control. The contribution of saponin by AM fungi at crop harvest ranges between 2 and 4 fold as compared to control. In general, *Glomus mosseae* was found to be more effective followed by *Glomus intraradices* and *Glomus fasciculatum*.

The saponins, plant glycosides, are classified as

steroid or triterpenoid saponins depending upon the nature of the aglycone (Mahato et al., 1982). The aglycone of steroid saponins is usually a spirostanol or its modification. A third group of saponins that are called basic steroid saponin contains nitrogen analogues of steroid sapogenins as glycones. Saponins have potent antifungal activity (Deore and Khadabadi, 2009) and their natural role in plants is likely to be in protection against attack by pathogenic microbes (Osbourne, 2003). The

Table 3. Biomass (g plant⁻¹) at different stages of safed musli (*Chlorophytum borivillianum*)

Treatment	Plant age (days)					
	45		90		270	
	Shoot	Root	Shoot	Root	Shoot	Root
Control	1.05	0.41	-	4.58	-	7.02
<i>Glomus fasciculatum</i>	1.62	3.30	1.54	4.86	-	9.87
<i>Glomus intraradices</i>	3.51	3.96	4.99	14.34	-	14.86
<i>Glomus mosseae</i>	3.35	6.65	4.85	19.34	-	13.59
LSD (p = 0.05)	0.677	2.64	0.019	2.67	-	3.14

**Figure 3.** P concentration in safed musli (*Chlorophytum borivillianum*) under different crop age (I indicate the standard errors of mean)

mycorrhizal conditions used might have stimulated the intracellular concentrations of secondary metabolites like saponins, which is the most potential medicinal compound in the roots of safed musli. The higher rate of accumulation as saponins in mycorrhizal plants may be due to higher rate of photosynthesis as we have noticed, in general, 3-5 fold increase in shoot biomass (Table 3) in mycorrhizal plants. Since the vegetative portion of mycorrhizal plants persist longer (50 to 90 days), it may helps to accumulate more saponins in the tuber.

The more accumulation of saponins in later stage of plant growth (Table 2) may be due to restriction of shoot growth resulted the development of stress condition in safe musli plant. The results clearly demonstrated that safed musli should harvest after 270 days rather than 80 to 90 days as recommended (Singh and Chauhan, 2003) because the saponin concentration is increased in later stage of plant growth of mycorrhizal inoculated plants (Table 2) as well as the P concentration has improved many folds (Figure 3). The present results also clearly

shown that mycorrhizal fungi inoculated safed musli have higher potential of transfer of carbon to the tuber. The higher saponin content in tuber would have higher medicinal value as herbal Viagra. The present result also supports the formation of secondary metabolites like saponins. The isolation of genes for saponins may also have potential for the development of improved disease resistance in cultivated cereals. The study thus suggests that the increase in saponin content is attributed to the mycorrhizal inoculation in safed musli.

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REFERENCES

- Arif JM (2005). Effects of safed musli on cell kinetics and apoptosis in human breast cancer cell lines. International conference on promotion and developed biotanicals with international co-ordination exploring quality, safety, efficacy and regulation organized by school of Natural product study, Jadavpur University, Kolkata, India Feb 25-26.
- Augé RM (2001). Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. *Mycorrhiza* 11: 3-42.
- Bordia PC, Joshi A, Simlot MM (1995). Safed musli. *Adv. Hort.* 11: 429-451.
- Deore SL, Khadabadi SS (2007). In vitro antimicrobial studies of *Chlorophytum borivillianum* (Liliaceae) root extracts. *Asian J. Microbiol. Biotech. Environ. Sci.* 9: 807-809.
- Deore SL, Khadabadi SS (2008). Anti-inflammatory and antioxidant activity of *Chlorophytum borivillianum* root extracts. *Asian J. Chem.* 20: 983-986.
- Deore SL, Khadabadi SS (2009). Larvicidal activity of the saponin fractions of *Chlorophytum borivillianum* santapan and Ferrandes. *J. Entomol. Nematol.* 15: 64-66.
- Gaur A, Adholeya A (1994). Estimation of VAMF spores in soil: a modified method. *Mycorrhiza News* 6: 10-11.
- Gopalkrishna B, Patil SH (2006). Preliminary phytochemical investigation and in-vitro anti-stress activity of safed musli – *Chlorophytum borivillianum*. *Indian Drugs* 43: 878-880.
- Govindrajan R, Sreevidya N, Vijaykumar M, Thakum M, Dixit VK (2005). In-vitro antioxidant activity of ethanolic extract of *Chlorophytum borivillianum*. *Nat. Prod. Sci.* 11: 165-169.
- Jackson ML (1967). *Soil Chemical Analysis*. Prentice-Hall of India. New Delhi.
- Kapoor R, Chaudhary V, Bhatnagar AK (2007). Effects of arbuscular mycorrhiza and phosphorus application on artemisinin concentration in *Artemisia annua* L. *Mycorrhiza* 17: 581-587.
- Mahato SB, Ganguly AN, Sahu NP (1982). Steroid saponins. *Phytochem.* 21: 959-978.
- Osborn AE (2003). Saponins in cereals. *Phytochem.* 62: 1-4.
- Phillips J, Hayman DS (1970). Improved procedures for clearing roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Br. Mycol. Soc.* 55: 158-61.
- Purohit SS, Prajapati ND (2003). *Agro's colour atlas of medicinal plants*, Agrobios Publications, Jodhpur pp 43.
- Raghavendra VB, Lokesh S, Kumar TV, Shethy HS (2005). Compatibility of phyton with common fungicides and their role on the yield of safed musli. *World J. Agril. Sci.* 1: 62-64.
- Requena N, Pérez-Solis E, Azcón-Aguilar C, Jeffries P, Barea JM (2001). Management of indigenous plant-microbe symbioses aids restoration of desertified ecosystems. *Appl. Environ. Microb.* 67: 495-498.
- Siddiqui YM (2005). Potent antiviral effect of safed musli on poliovirus replication, international conference on promotion and development of botanicals with international co-ordination exploring quality, safety, efficacy and regulation organized by school of natural product study, Jadavpur University, Kolkata, India, Feb 25-26.
- Singh A, Chauhan HS (2003). Safed musli (*Chlorophytum borivillianum*): distribution, biodiversity and cultivation. *J. Med. Aromatic Pl. Sci.* 25: 712-719.
- Singh P, Gupta P, Singh R (2004). Safed musli as a Divya Aushad, *Chem. Biol. Interface, Synergistic New Frontiers*, New Delhi, India: Nov. 21-26.
- Sokal RR and Rohlf FJ (1981). *Biometry- The Principal and Practice of Statistics in Biological Research*. 2nd Edn. W.H. Freeman and Co., New York.
- Tennant D (1975). A test of a modified line intersect method of estimating root length. *J. Ecol.* 63: 995-1001.
- Thakur M, Dixit VK (2006). Effect of *C. borivillianum* on androgenic and sexual behaviour of male rats. *Indian Drugs* 43: 300-306.
- Toro M, Azcón R, Barea JM (1997). Improvement of arbuscular mycorrhiza development by inoculation of soil phosphate solubilizing rhizobacteria to improve rock phosphate bioavail (³²P) and nutrient cycling. *Appl. Environ. Microb.* 63: 4408-4412.
- Walkley A, Black IA (1934) An examination of the Degtiareff method for determining soil organic matter and proposed modification of the chromic acid titration method. *Soil Sci.* 37: 29-38.