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Influence of AM Fungi on the Growth and Biomass Production of *Catharanthus Roseus* (L.) G. Don

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Abstract:
In addition to their applications in folk medicine for therapeutic purposes, plants serve as significant sources of diverse pharmaceutical drugs. While numerous compounds can be synthesized in laboratory settings, the complexity of the synthesis leads to low yields and renders economic production unfeasible. *Catharanthus roseus* (L.) G. Don (periwinkle) is notable for containing over 130 distinct terpenoid indole alkaloids, which are cyclic and nitrogen-containing compounds.
The results of the present investigation bring out the important role of AM fungi in enhancing the growth and biomass of periwinkle. It may be concluded that all the medicinal plants screened in the present study were mycorrhizal inspite of possessing secondary substance like alkaloids. The AM inoculated plants showed improved plant growth such as plant height, dry weights of shoot and root. The biomass and dry matter production also significantly enhanced in the mycorrhizal plants over control. Mycoorhizal plants also showed increased levels of nitrogen, phosphorus, potassium, zinc, iron and copper in their shoot and root tissues. Of the three treatments, *G.aggregatum* was the most effective AM fungus followed by *G.mosseae* and native inoculum. In addition to plant growth improvement, mycorrhizal plants also exhibited higher levels of alkaloid content in root and shoot tissues.

Keywords: G.mosseae, G.aggregatum, Catharanthus roseus (L.) G. Don, Biomass, A.M Fungi

Introduction
Organisms conducive to the growth of various medicinal plants and agricultural crop production systems encompass free-living, associative, and symbiotic nitrogen fixers, phosphate solubilizers, mycorrhizal fungi, cellulolytic agents, and hormone producers. Soil bio-inoculants exhibit distinct functions, including biological nitrogen fixation, nitrification, de-nitrification, bio-control of root pathogens, phosphate solubilization and mobilization, decomposition of cellulose and lignin, and the production of growth-promoting hormones within the rhizosphere of plants. Previous studies indicated that the inoculation of arbuscular mycorrhizal fungi (AM fungi) with agricultural crops may enhance the growth of root and shoot biomass. Additionally, the agricultural significance of AM fungi has been documented by various authors (Anuradha et al, 2001; Karagiannidis et al, 2012).
In addition to their applications in folk medicine for therapeutic purposes, plants serve as significant sources of diverse pharmaceutical drugs. While numerous compounds can be synthesized in laboratory settings, the complexity of the synthesis leads to low yields and renders economic production unfeasible.

Catharanthus roseus G. L Don (periwinkle) is notable for containing over 130 distinct terpenoid indole alkaloids, which are cyclic and nitrogen-containing compounds. Several of these alkaloids, including ajmalicine, which is extracted from the roots, have significant pharmaceutical applications, particularly as antihypertensive agents (Almagro et al., 2015). Plants frequently synthesize alkaloids as a defense mechanism against unfavorable environmental conditions and external biological stimuli (Zeng et al., 2013).
The synthesis of secondary plant metabolites is significantly influenced by growth conditions, including mineral nutrition and associations with microorganisms (Ramakrishna and Ravishankar, 2011; Andrade et al., 2013). The inoculation of plants with arbuscular mycorrhizal fungi (AMF) can modify the production of secondary metabolites (Pedone-Bonfim et al., 2015). Several authors indicate that mycorrhizae positively influence the accumulation of alkaloids in medicinal plants (Yu et al., 2010; De la Rosa-Mera et al., 2011).
Arbuscular mycorrhizal fungi are obligate biotrophs that depend on carbon supplied by their host plant instead of utilizing dead organic matter (Smith and Smith, 2011). They are essential for plant nutrition and growth in various agricultural systems. The AMF hyphal network plays a crucial role in facilitating plant access to ions situated at considerable distances from the root surface. Research employing 15N tracer methodologies has demonstrated that AM hyphae are capable of transporting nitrogen from the soil to plant roots (Tanaka and Yano, 2005; Jackson et al., 2008). Smith and Smith (2011) indicated that arbuscular mycorrhizal fungi (AMF) contribute minimally to nitrogen nutrition in plants. AMF contributed to a 28.2% increase in rice grain yield and a 7.4% enhancement in protein content, while also reducing the C/N ratio, as AMF inoculation significantly elevated nitrogen accumulation in rice plants (Zhang et al., 2017).
Significance of the current research work is to identify the plant biomass increment after using AM fungus.

Materials and Methods:

Plant growth and nutrient uptake (Pot culture experiments)

A controlled pot culture experiment was conducted to assess the influence of Arbuscular mycorrhizal (AM) fungus on plant development and nutrient absorption. The study included two kinds of Arbuscular mycorrhizal (AM) fungus, viz.,*Glomus aggregatum* and *Glomus mosseae*. The plant species employed as the host in the present study was *Catharanthus roseus* (L.) G. Don, commonly referred to as *Catharanthus roseus* or pink flowered periwinkle.
The plants were systematically uprooted at regular intervals of 30, 60, 90, and 120 days throughout the growth period. This was done to examine the colonization patterns, determine the height of the plants, and evaluate their biomass.
The nutritional condition of both mycorrhizal and non-mycorrhizal (control) plants was evaluated in plants that had reached a maturity of 120 days.

Plant dry weights

Shoot dry weight (g): The plants were uprooted and shoots of each plant was cut and oven dried at 75°C for 48hr till constant weights were obtained.
Root dry weight (g): The roots were cut for each plant and washed thoroughly under running tap water to remove adhesive soil particles, oven dried at 75°C for 48hr till constant weights were obtained.

Dynamics of growth (cm)
The growth characteristics in respect of biomass increment and mean rate of dry matter production were calculated.

Biomass increment:
As an index of growth character, increase in biomass (W) was expressed in terms of dry weight (Sestak et al., 1971).

Nitrogen (estimation by kjeldhal method)
For estimation of available nitrogen, mineralizable nitrogen alkaline permanganate method (Singh 2017) was followed.

Potassium (estimation by Photometer method)
The determination of potassium was conducted using the flame photometer method, as described by Banerjee and Prasad (2020).

Estimation of micronutrients
Micronutrients were estimated by Atomic Absorption sSpectroscopy on GBC 906 AA model

Copper
Copper content was estimated in the diluted sample. The sample was fed into AAS set at 327.4nm wavelength. The O.D reading was observed and calculated accordingly.

Iron
The processed dried plant material sample was transferred to AAS set at 372.0nm and readings were noted.

Zinc
Zinc was estimated in the same way as described above at 213.9nm.

Results and Discussion

Effect of AM inoculation on biomass and dry matter production
A series of studies were undertaken to examine the effectiveness of Arbuscular mycorrhizal (AM) fungal inoculation on the biomass and dry matter production of periwinkle at different growth stages (30, 60, 90, and 120 days). The results were presented in Table 1.

The study demonstrated a considerable increase in biomass for *G. aggreagtum* and *G. mosseae* amendments as the plants' age progressed. Nevertheless, the utilization of indigenous (hybrid) inoculum also yielded substantial improvements in the growth of both shoot and root biomass over a 90-day timeframe. The root biomass of plants that were inoculated with *G. aggregatum* had an initial period of growth within the first 90 days, which was then followed by a subsequent decline.

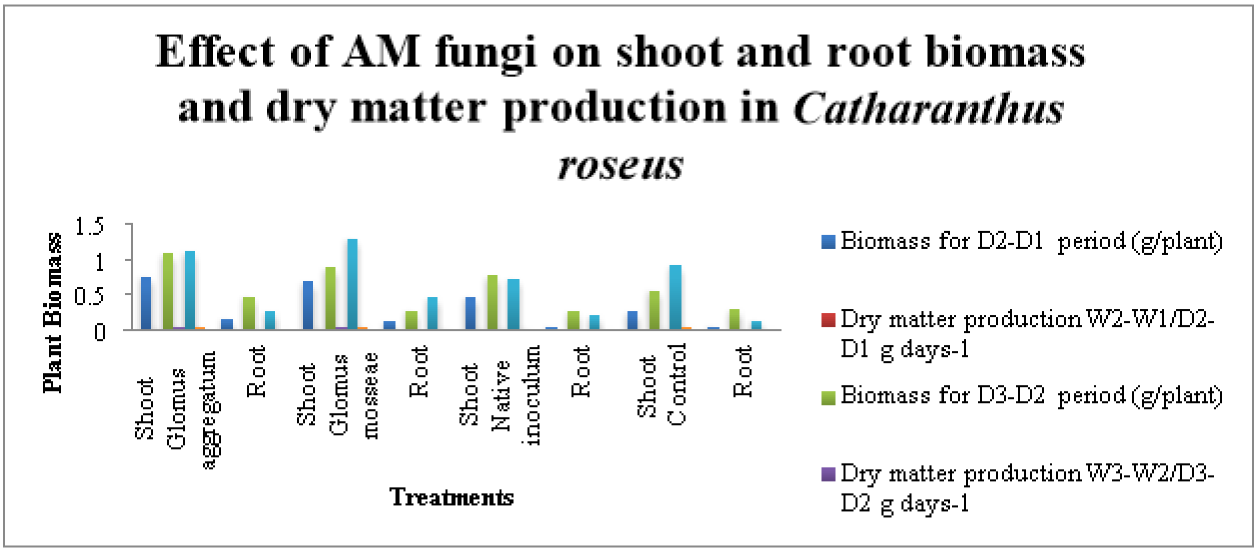
The introduction of *G. mosseae* through inoculation led to a significant augmentation in both biomass and dry matter output. The effectiveness of indigenous inoculum in enhancing biomass and dry matter production was found to be restricted.

The results of this investigation suggest that *G. mosseae* demonstrates effectiveness in promoting the growth of biomass and dry matter.

TABLE-1: Effect of AM fungi on shoot and root biomass and dry matter production in *Catharanthus roseus*

S.NO.	Treatments		Biomass for D_2-D_1 period (g/plant)	Dry matter production W_2-W_1/D_2-D_1 g $days^{-1}$	Biomass for D_3-D_2 period (g/plant)	Dry matter production W_3-W_2/D_3-D_2 g $days^{-1}$	Biomass for D_4-D_3 period (g/plant)	Dry matter production W_4-W_3/D_4-D_3 g $days^{-1}$
1	*Glomus aggregatum*	Shoot	0.765	0.0255	1.086	0.0362	1.112	0.0370
		Root	0.160	0.0005	0.474	0.0158	0.268	0.0008
2	*Glomus mosseae*	Shoot	0.684	0.0228	0.889	0.0296	1.293	0.0431
		Root	0.125	0.0004	0.275	0.0009	0.466	0.0155
3	Native inoculum	Shoot	0.468	0.0156	0.768	0.0256	0.727	0.0242
		Root	0.031	0.0001	0.266	0.0008	0.209	0.0006
4	Control	Shoot	0.262	0.0008	0.567	0.0189	0.918	0.0306
		Root	0.042	0.0001	0.291	0.0009	0.138	0.0004

W_1 to W_4 represent dry weights of shoot / root corresponding to different growth stages (D_1 to D_4).
D_1 = 30 days
D_2 = 60 days
D_3 = 90 days
D_4 = 120 days



Mycorrhizal dependency of Periwinkle

The mycorrhizal dependency percentage (MD%) of periwinkle was determined by calculating the ratio of the total dry mass of mycorrhizal plants to the total dry mass of non-mycorrhizal plants, multiplied by 100. The obtained data are displayed in Table-2.

The findings indicate that periwinkle is a plant that relies on mycorrhizal associations. The degree of mycorrhizal dependency exhibited a notable decline as plants progressed through several stages of growth, with a higher reliance observed during the initial phases. The level of mycorrhizal dependence reached its peak at 60 days of plant growth in the treatment involving *G. aggregatum*. The level of mycorrhizal reliance exhibited a decrease to 209.5 percent by the 90th day, followed by a further reduction to 173.6 percent in plants observed at the 120th day. Plants that have been inoculated with *G. mosseae* have demonstrated a significant reliance on mycorrhizal associations in 90-day-old plants. The level of mycorrhizal dependence shown a decrease to approximately 167.0 starting from the 90-day mark. However, the treatment using native (mixed) inoculums exhibited a higher level of mycorrhizal dependence, but only during the initial 30 days of growth. The degree of mycorrhizal reliance exhibited a significant decrease after a period of 60 days of plant growth.The findings of this study demonstrate the extent of periwinkle's reliance on mycorrhizal associations during the initial 60 days of growth.

TABLE-2: Mycorrhizal dependency % (MD%) of *Catharanthus roseus* inoculated with mycorrhizal fungi (Pot experiments)

S.NO.	Treatments	Mycorrhizal Dependency (%)			
		D_1	D_2	D_3	D_4
1	*Glomus aggregatum*	249.4	275.6	212.5	176.4
2	*Glomus mosseae*	215.0	240.0	168.5	165.2
3	Native inoculums	256.0	161.0	135.7	114.5
4	Control	100	100	100	100

D_1 = 30 days D_2 = 60 days
D_3 = 90 days D_4 = 120 days

NPK Levels

At 120 days of plant growth, the NPK levels of periwinkle inoculated with AM are depicted in Table-3. In general, NPK concentrations were lower in the roots than in the stems. *G.aggregatum* inoculated plants had the highest nitrogen content in the shoots, followed by *G.mosseae* and native (mixed) inoculants. The level of phosphorus in the branches of *G.aggregatum* and *G.mosseae* inoculated plants was nearly identical. Both regimens (*G.aggregatum* and *G.mosseae*) produced identical root nitrogen content. Phosphorus was more abundant in the root tissue of *G.aggregatum*-inoculated plants. Potassium content was also increased in the shoots and roots of *G.aggregatum*-inoculated periwinkle. Shoots and roots from *G.mosseae* and native inoculums contained the same amount of potassium.

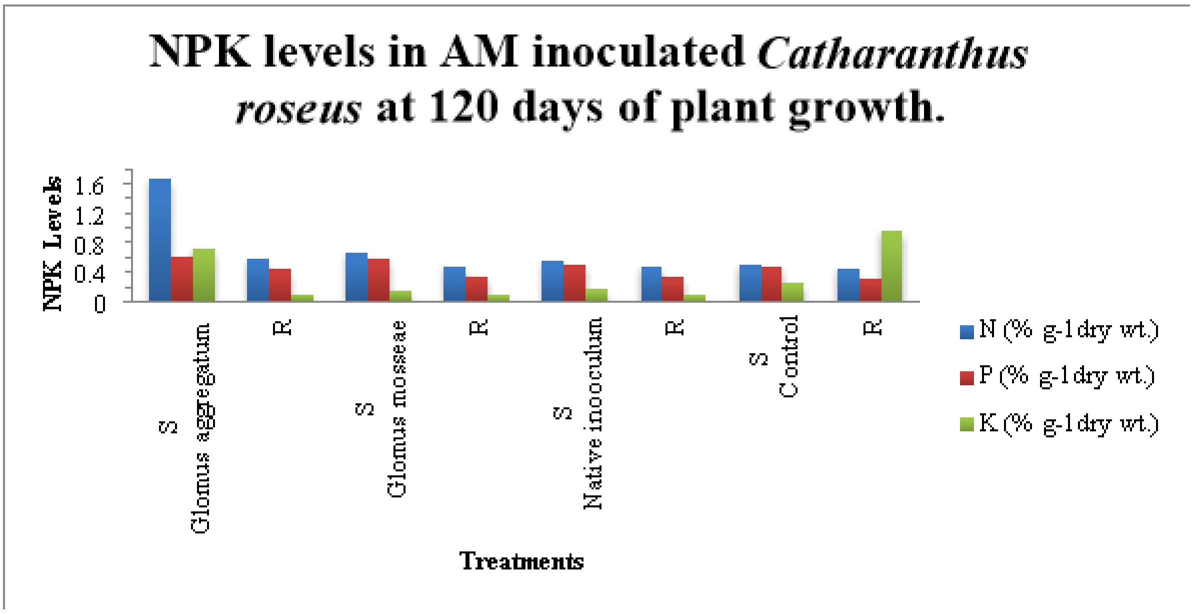
The inoculation of *G.aggregatum*, *G.mosseae*, and native inoculum resulted in an increase in all three elements (N,P,K) compared to the control. *G.aggregatum* was more efficient than alternative interventions. Tissue analysis reveals an increase in phosphorus absorption and accumulation. Phosphorus levels in mycorrhizal plants were nearly double when compared to control plants.

TABLE-3: NPK levels in AM inoculated *Catharanthus roseus* at 120 days of plant growth. (Pot experiments)

Treatments		N (% g^{-1}dry wt.)	P (% g^{-1}dry wt.)	K (% g^{-1}dry wt.)
Glomus aggregatum	S	2.653	1.596	1.671
	R	1.447	1.405	1.091
Glomus mosseae	S	1.651	1.580	1.151
	R	1.047	1.345	1685

Native inooculum	S	1.534	1.501	1.155
	R	1.466	1.330	1.086
Control	S	1.477	1.457	1.247
	R	1.456	1.310	1.205

S = Shoot **R = Root**



Organic Carbon

The present study investigated the impact of Arbuscular mycorrhizal (AM) fungi, specifically *Glomus aggregatum*, *Glomus mosseae*, and a mixed native inoculum, on the levels of Organic Carbon in the shoots and roots of periwinkle plants.

Micronutrients

The present study investigated the impact of Arbuscular mycorrhizal (AM) fungi, specifically *Glomus aggregatum*, *Glomus mosseae*, and a mixed native inoculum, on the levels of micronutrients (zinc, iron, and copper) in the shoots and roots of periwinkle plants. The quantification of micronutrients was conducted using an Atomic Absorption Spectrophotometer (AAS) on plants that had reached a growth period of 120 days. The obtained data is furnished in Table-4.

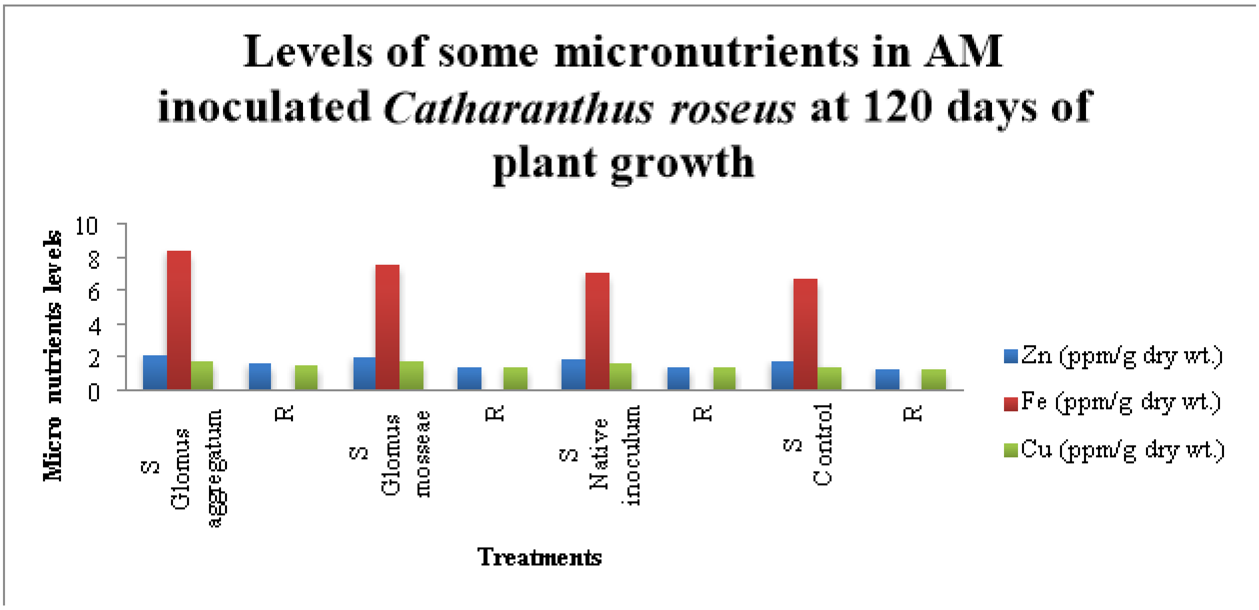
The application of mycorrhizal inoculation resulted in a significant enhancement in zinc, iron, and copper mobilization in both the above-ground shoots and below-ground roots. The shoots frequently exhibited higher zinc concentration compared to the roots across all treatments. The AM fungus *G.aggregatum* demonstrated the most efficacy in enhancing the zinc concentration in both the roots and shoots, with *G.mosseae* exhibiting a somewhat lower effectiveness in comparison. The addition of native inoculums resulted in a significant enhancement of zinc content compared to the control group.

The presence of *G.aaggregatum*, *G.mosseae*, and native inoculum resulted in enhanced levels of iron in the shoots of periwinkle. In contrast, the root tissue had a higher concentration of copper compared to zinc and iron. All treatments resulted in a significant increase in copper levels in both the shoot and root tissues. *G. aggrigatum* demonstrated the highest efficacy among the studied fungi in augmenting copper levels. In each of the treatment groups, the concentration of copper was found to be higher in the roots compared to the shoots. The findings suggest that *G. aggrigatum* exhibited efficacy in facilitating the mobilization of micronutrients such as zinc, iron, and copper.

TABLE-4: Levels of some micronutrients in AM inoculated *Catharanthus roseus* at 120 days of plant growth. (Pot experiments)

Treatments		Zn (ppm/g dry wt.)	Fe (ppm/g dry wt.)	Cu (ppm/g dry wt.)
Glomus aggregatum	S	2.108	8.324	1.752
	R	1.524	-	1.425
Glomus mosseae	S	1.998	7.542	1.754
	R	1.352	-	1.365
Native inoculum	S	1.845	6.989	1.564
	R	1.385	-	1.302
Control	S	1.742	6.704	1.398
	R	1.242	-	1.236

S = Shoot - = not estimated
R = Root



Discussion:

The levels of nitrogen, phosphate, and potassium in periwinkle plants that were infected with *G.aggregatum*, *G.mosseae*, and native inoculums were significantly increased compared to the control group. The findings indicate a greater influx of phosphorus (P) into both the shoot and root tissues, leading to the eventual buildup of this nutrient. The study conducted by Hernández-Dorrego and Mestre-Parés (2010). On evaluation of some fungicides on mycorrhizal symbiosis between two *Glomus* species from commercial inoculum and *Allium porrum* plants that form mycorrhizal associations exhibited elevated levels of phosphorus and potassium, while displaying reduced levels of nitrogen. In their study, Hoeksema et al., (2010) observed meta-analysis of context-dependency in plant response to inoculation with mycorrhizal fungi. The inoculation of *Gigaspora calospora* and *Glomus fasciculatum* resulted in phosphorus level increase comparable to the application of phosphate at a rate of 8 kg/ha. In the current experiment, both *G.aggregatum* and *G.mosseae* demonstrated increased nitrogen (N) and phosphorus (P) levels in both shoot and root tissues compared to the control group. Arbuscular mycorrhizal (AM) fungi have the capacity to augment the levels of macronutrients, such as potassium, in addition to nitrogen (N) and phosphorus (P). In the current study, it was observed that *Glomus aggregatum* and *G. mosseae* exhibited more efficacy in promoting plant development, biomass, and nutrient absorption in periwinkle compared to the indigenous (mixed) inoculum derived from rhizosphere soils. This observation highlights the significance of employing specific strains of Arbuscular mycorrhizal (AM) fungi in contrast to utilizing natural, mixed strains.

In the present investigation micronutrient levels in *C.roseus* were estimated at 120 days of plant growth from mycorrhizal and non-mycorrhizal plants. The results reveal the increased intake of zinc, iron and copper in the mycorrhizal plants. Higher levels of zinc, iron and copper were recorded in plants inoculated with *G.aggregatum* than the plants inoculated with *G.mosseae* the present results are in conformity with the observations made by earlier workers with reference to other crops (Garg, N. and Chandel, S. 2010).

Conclusion

The results of the present investigation bring out the important role of AM fungi in enhancing the growth and biomass of periwinkle. It may be concluded that all the medicinal plants screened in the present study were mycorrhizal inspite of possessing secondary substance like alkaloids. The AM inoculated plants showed improved plant growth such as plant height, dry weights of shoot and root. The biomass and dry matter production also significantly enhanced in the mycorrhizal plants over control. Mycoorhizal plants also showed increased levels of nitrogen, phosphorus, potassium, zinc, iron and copper in their shoot and root tissues. Of the three treatments,*G.aggregatum* was the most effective AM fungus followed by *G.mosseae* and native inoculum. In addition to plant growth improvement, mycorrhizal plants also exhibited higher levels of alkaloid content in root and shoot tissues.

These results emphasize the role of AM fungi in improving the yield of vegetable matter in periwinkle which may be exploited in the intensive cultivation of this medicinal plant. Application of AM fungi would supplement the chemical fertilizers in achieving higher yields. AM inoculation would be of special significance in tropical soils which are poor in nutrients like phosphorus. AM fungi may be involved in the improvement of alkaloid contents indirectly through enhancing the uptake of nutrients and plant growth.

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