Influence of Inoculation with the Endomycorrhizal Fungi and *Trichoderma viride* on Morphological and Physiological Growth Parameters of *Rauwolfia serpentina* Benth. Ex. Kurtz

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Abstract Two arbuscular mycorrhizal fungi, *Glomus mosseae* and *Acaulospora laevis* either alone or in combination with *Trichoderma viride* showed the dependence of *Rauwolfia serpentina* on endomycorrhizal fungi. After 60 days, *G. mosseae* singly or in combination with *Trichoderma viride* showed enhanced height increment compared to control plants. Maximum phosphorus content was shown by plants treated with *G. mosseae* plus *T. viride* (0.444 ± 2.62) in roots and (0.437 ± 4.71) in shoots. Phosphorus content in roots was more than that in shoots. Chlorophyll content and stomatal conductivity also showed similar trend.

Keywords *Rauwolfia* · *Glomus* · *Acaulospora* · *Trichoderma* · Arbuscular mycorrhiza

Mycorrhizal associations in plants have been shown to enhance growth and produce higher yield. Their latent capacity to sustain the productivity of agro-ecosystem and recovery of degraded lands through nutrient cycling and

soil aggregation has been well documented [1]. They increase plant growth, improve tolerance to drought and provide protection against root borne pathogens, increase chlorophyll content and phosphorus content. Arbuscular mycorrhizal fungi (AMF) and *Trichoderma* can interact synergistically to stimulate plant growth through a range of mechanisms that include improved nutrition acquisition and inhibition of fungal plant pathogens. Keeping in view the above information we undertook the present study to understand how native AM fungi along with *Trichoderma* play their role in association with test medicinal plants so that their bio-fertilizing potential can be exploited accordingly.

Mass production of *Trichoderma viride* was done using wheat bran and saw dust mixed with distilled water in the ratio 3:1:4. The VAM fungi (*Glomus mosseae* and *Acaulospora laevis*) were multiplied using wheat as a host and soil:sand (3:1) as a substrate. 2 months old seedlings of *Rauwolfia serpentina* were maintained in the poly-house conditions. Hoagland nutrient solution (without KH_2PO_4) was given every 15 days. Total phosphorus content (%) was determined by Vanadomolybdo-phosphoric yellow colour method in nitric acid system [2]. Chlorophyll content and stomatal conductance ($\text{mmol}^{-2} \text{s}^{-2}$) were estimated by using Arnon's method [3] and Porometer. After 60 and 120 days, isolation of AM spores and root colonization were studied by wet sieving and staining technique [4, 5]. Data was statistically analyzed by ANOVA followed by post hoc test.

G. mosseae was found to be more efficient than *A. laevis* and *T. viride* when used singly (Table 1). *G. mosseae* singly or in combination with *T. viride* showed enhanced height increment, approximately eleven times increase in double combination (22.9 ± 3.00) as compared to control plants (2.3 ± 0.20) followed by *A. laevis* plus *T. viride*

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Table 1 Growth response of *Rauwolfia serpentina* after 60 days with single and double VAM inoculation and *T. viride*

S. No.	Treatment	Increase in height (cm)	Fresh shoot weight (g)	Dry shoot weight (g)	Root length	Fresh root weight (g)	Dry root weight (g)	Leaf number	Leaf area	Mycorrhizal spore count	Mycorrhizal root colonization (%)
1.	Control	2.3 ± 0.20 ^a	3.6 ± 0.06	0.7 ± 0.01	14.2 ± 0.16	2.4 ± 0.20	0.7 ± 0.10	8.0 ± 0	10.5 ± 1.18	9.67 ± 2.05	59.1 ± 11.1
2.	<i>Glomus mosseae</i>	20.0 ± 3.00	11.5 ± 0.84	2.8 ± 0.37	15.3 ± 0.16	5.4 ± 1.03	1.7 ± 0.11	11.7 ± 0.47	19.2 ± 1.81	78.6 ± 1.24	100.0 ± 0
3.	<i>Acaulospora laevis</i>	17.0 ± 4.06	6.7 ± 1.07	1.8 ± 0.52	14.7 ± 0.12	4.7 ± 0.86	1.5 ± 0.11	11.7 ± 0.47	16.0 ± 0.04	59.3 ± 2.05	91.6 ± 0.51
4.	<i>Trichoderma viride</i>	15.6 ± 0.18	8.2 ± 2.33	3.6 ± 1.04	14.8 ± 0.12	4.5 ± 0.71	0.8 ± 0.14	12.0 ± 0	16.1 ± 0.04	42.0 ± 1.63	90.0 ± 0
5.	<i>Glomus mosseae</i> ± <i>Trichoderma viride</i>	22.9 ± 1.56	13.8 ± 3.36	2.9 ± 0.42	16.3 ± 0.12	6.6 ± 2.48	2.0 ± 0.07	13.7 ± 0.47	21.6 ± 2.56	60.6 ± 2.49	100.0 ± 0
6.	<i>Acaulospora laevis</i> ± <i>Trichoderma viride</i>	18.4 ± 4.16	12.1 ± 1.38	2.6 ± 0.10	16.4 ± 0.12	5.2 ± 0.15	1.8 ± 0.66	14.0 ± 0	20.1 ± 1.46	59.0 ± 0.94	90.3 ± 3.29

According to one way analysis of variance (ANOVA) the mean difference is significant at 0.05 level

^a Each value is a mean of three replicates, ± standard deviation

Table 2 Growth response of *Rauwolfia serpentina* after 120 days with single and double VAM inoculation and *T. viride*

S. No.	Treatment	Increase in height (cm)	Fresh shoot weight (g)	Dry shoot weight (g)	Root length	Fresh root weight (g)	Dry root weight (g)	Leaf number	Leaf area	Mycorrhizal spore count	Mycorrhizal root colonization (%)
1.	Control	9.5 ± 1.53 ^a	5.9 ± 0.39	1.8 ± 0.17	18.2 ± 0.12	3.6 ± 0.08	2.0 ± 0.06	10.7 ± 0.47	14.8 ± 0.08	54.6 ± 3.68	79.5 ± 4.38
2.	<i>Glomus mosseae</i>	38.6 ± 7.22	25.2 ± 0.08	9.6 ± 2.37	20.4 ± 0.12	13.8 ± 2.16	5.8 ± 0.26	15.3 ± 0.47	64.5 ± 0.08	103.6 ± 5.31	94.8 ± 4.05
3.	<i>Acaulospora laevis</i>	29.4 ± 2.28	19.8 ± 1.79	5.9 ± 0.70	21.2 ± 0.08	12.5 ± 0.93	5.0 ± 0.11	14.7 ± 0.94	61.5 ± 0.12	84.6 ± 3.29	92.0 ± 2.71
4.	<i>Trichoderma viride</i>	34.7 ± 3.02	21.3 ± 1.43	6.8 ± 0.79	21.4 ± 0.04	9.8 ± 2.43	2.9 ± 0.59	15.0 ± 0	66.1 ± 0.12	67.3 ± 1.69	87.6 ± 4.44
5.	<i>Glomus mosseae</i> ± <i>Trichoderma viride</i>	62.1 ± 3.478	27.6 ± 1.11	12.1 ± 0.43	23.2 ± 0.08	21.5 ± 4.38	11.1 ± 3.07	17.3 ± 0.47	83.1 ± 0.04	204.0 ± 3.74	100.0 ± 0
6.	<i>Acaulospora laevis</i> ± <i>Trichoderma viride</i>	46.4 ± 4.94	23.4 ± 2.46	11.2 ± 0.14	25.4 ± 0.12	20.9 ± 1.93	8.4 ± 2.17	18.3 ± 0.47	71.4 ± 0.12	180.3 ± 6.54	90.1 ± 4.65

According to one way analysis of variance (ANOVA) the mean difference is significant at 0.05 level

^a Each value is a mean of three replicates, ± standard deviation

Table 3 Physiological parameters in *Rauwolfia serpentina* after 60 days

S. No	Treatment	Phosphorus content		Chlorophyll content		Stomatal conductance	
		Shoot P	Root P	Chl. a	Chl. b	Total Chl.	M A
1.	Control	0.352 ± 1.63 ^a	0.353 ± 4.96	0.232 ± 0.01	0.228 ± 0.02	0.471 ± 0.03	L 303.3 ± 2.35 471.66 ± 2.35
2.	<i>Glomus mosseae</i>	0.436 ± 2.62	0.438 ± 2.16	0.628 ± 0.06	0.688 ± 0.02	1.316 ± 0.05	U 88.66 ± 2.62 110.33 ± 0.47
3.	<i>Acaulospora laevis</i>	0.422 ± 2.16	0.425 ± 0.01	0.602 ± 0.05	0.391 ± 0.03	0.993 ± 0.04	L 531.66 ± 2.35 261.66 ± 2.35
4.	<i>Trichoderma viride</i>	0.408 ± 1.69	0.432 ± 3.85	0.464 ± 0.02	0.362 ± 0.02	0.826 ± 0.04	U 303 ± 2.35 122 ± 2.16
5.	<i>Glomus mosseae</i> ± <i>Trichoderma viride</i>	0.437 ± 4.71	0.444 ± 2.62	0.790 ± 0.03	0.618 ± 0.03	1.436 ± 0.04	L 471.66 ± 2.35 223 ± 0.081
6.	<i>Acaulospora laevis</i> ± <i>Trichoderma viride</i>	0.431 ± 8.17	0.440 ± 1.24	0.699 ± 0.05	0.670 ± 0.04	1.368 ± 0.03	L 581.66 ± 2.35 441 ± 0.81
							U 502.33 ± 6.23 535 ± 4.08
							L 588.33 ± 6.33 222 ± 1.63
							U 603.33 ± 2.35

M Morning (10.30 am), A afternoon (3.30 pm), L lower surface of leaf, U upper surface of leaf

^a Mean of three replicates, ± standard deviation

Table 4 Physiological parameters in *Rauwolfia serpentina* after 120 days

S. No.	Treatment	Phosphorus content		Chlorophyll content		Stomatal conductance	
		Shoot P	Root P	Chl. a	Chl. b	Total Chl.	M A
1.	Control	0.451 ± 4.71 ^a	0.480 ± 0	1.044 ± 0.01	0.557 ± 0.01	1.601 ± 0.03	L 320 ± 4.08 425 ± 4.08
2.	<i>Glomus mosseae</i>	0.464 ± 8.16	0.492 ± 1.63	1.185 ± 4.32	0.944 ± 0.02	2.128 ± 0.02	U 4.33 ± 0.24 14.83 ± 0.04
3.	<i>Acaulospora laevis</i>	0.462 ± 4.71	0.481 ± 8.16	1.098 ± 0.02	0.615 ± 0.02	1.715 ± 0.04	L 292 ± 2.16 435 ± 4.08
4.	<i>Trichoderma viride</i>	0.469 ± 8.16	0.493 ± 1.69	1.168 ± 0.02	0.782 ± 0.03	1.949 ± 0.05	U 24.1 ± 0.08 29.16 ± 0.12
5.	<i>Glomus mosseae</i> ± <i>Trichoderma viride</i>	0.472 ± 0.0	0.502 ± 1.24	1.431 ± 5.56	1.232 ± 9.88	2.663 ± 0.01	L 271 ± 2.94 440 ± 4.08
6.	<i>Acaulospora laevis</i> ± <i>Trichoderma viride</i>	0.472 ± 4.71	0.497 ± 1.24	1.380 ± 4.64	1.113 ± 0.02	2.492 ± 0.02	U 39.1 ± 0.08 45.5 ± 0.08
							L 563.66 ± 2.62 591 ± 0.81
							U 30.2 ± 0.16 40.43 ± 0.04
							L 635 ± 4.08 698.66 ± 5.79
							U 16.1 ± 0.08 26.1 ± 0.08
							L 595 ± 4.08 615 ± 4.08
							U 6.76 ± 0.12 14.06 ± 0.09

M Morning (10.30 am), A afternoon (3.30 pm), L lower surface of leaf, U upper surface of leaf

^a Mean of three replicates, ± standard deviation

Plate 1 Effect of VAM inoculation and *T. viride* on growth of *Rauwolfia serpentina*. A Control; B *Acaulospora laevis*; C *Glomus mosseae*; D *Trichoderma viride*; E *Acaulospora laevis* + *Trichoderma viride*; F *Glomus mosseae* + *Trichoderma viride*

Height of the plant initially

Height of the plant after 60 days of inoculation

Height of the plant after 120 days of inoculation

A - Control ; B - *Acaulospora laevis* ; C - *Glomus mosseae* ; D - *Trichoderma viride* ;
E - *Acaulospora laevis* + *Trichoderma viride* ; F - *Glomus mosseae* + *Trichoderma viride*

(18.4 ± 4.16), *A. laevis* (17.0 ± 4.06) and *T. viride* (15.6 ± 0.18) respectively. Other growth parameters also showed the same trend in the increase pattern except fresh shoot weight, root length and leaf number where *T. viride* in single inoculation proved to be better than *A. laevis* alone.

After 120 days, best response was shown by *G. mosseae* followed by *T. viride* and *A. laevis* alone for height

increment, biomass yield, root length, leaf number and leaf area (Table 2, Plate 1). VAM spore number, root colonization and root weight were increased by *G. mosseae* followed by *A. laevis* and *T. viride* alone. Dual inoculation of *G. mosseae* plus *T. viride* and *A. laevis* plus *T. viride* showed better results after 120 days as compared to 60 days response. Higher root colonization allows more host-fungal contact and exchange of nutrients and helps in

plant growth. Similar was the assessment by Gupta et al. [6] who studied the growth and development of menthol mint with reference to VAM.

The number of VAM spores ranged from 9.7 ± 2.05 to 78.6 ± 1.24 after 60 days and the range was 54.6 ± 3.68 – 204.0 ± 3.74 after 120 days. The efficiency *G. mosseae* attributed due to its ability to colonize roots extensively, as is observed from the highest infection percentage (100 ± 0) both singly as well as in combination. Gai et al. [7] also found *Glomus* species to be more effective than *Acaulospora*. Plant growth increase is also due to disease control by the application of antagonistic fungus particularly *Trichoderma* [8]. Srinath et al. [9] found maximum plant height, phosphorus content, biomass, spore number and root colonization when *G. mosseae* was inoculated with *Trichoderma harzianum* and *Bacillus coagulans* to *Ficus benjamina*.

Tables 3 and 4 indicate that the maximum phosphorus content was shown by plants treated with *G. mosseae* plus *T. viride* (0.444 ± 2.62) in roots and (0.437 ± 4.71) in shoots after 60 days followed by *A. laevis* plus *T. viride* (0.440 ± 1.24 ; 0.431 ± 8.17), *G. mosseae* (0.438 ± 2.16 ; 0.436 ± 2.62), *T. viride* (0.432 ± 3.85 ; 0.408 ± 1.69), *A. laevis* (0.425 ± 0.01 ; 0.422 ± 2.16) and control plants. The phosphorus content in roots was more than that in shoots. Gupta et al. [6] studied the maximum depletion of available N, P, K from the rhizosphere soil of *Glomus fasciculatum* inoculated menthol plants as compared to non-inoculated plants. *G. mosseae* proved to be most efficient in increasing the total chlorophyll content in double combination as compared to control after 60 and 120 days. The results are in conformity with the work of Alarcon et al. [10] on *Carica papaya*, who reported *Glomus* species to be the best strain in enhancing the chlorophyll contents and related compounds whether used singly or in combination. After 60 and 120 days, maximum stomatal conductance was monitored in *G. mosseae* plus *T. viride*. Auge [11] also observed that colonization by *G. fasciculatum* and *Glomus deserticola* increased stomatal opening relative to uncolonized plants. Colonization by *Glomus intraradices*,

Glomus etunicatum and *G. mosseae* also increased stomatal conductance or transpiration.

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