Original Research Article

Arbuscular mycorrhizal fungi as influenced by nutrient uptake and soil enzyme activity of tobacco rhizosphere under parasitic weed stress condition

ABSTRACT

The present study assessed the Arbuscular mycorrhizal fungi as influenced by nutrient uptake and soil enzyme activity of tobacco rhizosphere under parasitic weed stress condition. The experiment was carried out in *Orobanche* infested soils of Belagavi district during 2018-19. During the investigation different inoculation methods of mycorrhizal fungi were screened for their ability to improve the nutrient uptake and soil enzyme activity i.e. (pre inoculation of nursery seedlings; direct soil application and the combination of both). The present experimental results revealed that soil enzyme activities like dehydrogenase, phosphates and urease activity was highest in treatments received planting of pre colonized tobacco seedling with STD AMF along with soil application at the time of planting and second highest was recorded in the treatment received pre colonized UASDAMFT plus soil application. Furthermore nutrient uptake like N, P, K and micronutrients were found to be the highest in the plots received mycorrhization compare to the rhizosphere of non mycorrhized plants. In mycorrhizosphere the activity of mycorrhizal fungi and other beneficial microorganisms could be a source for different soil enzyme needed for biochemical reaction in the plant rhizosphere.

Key words: Mycorrhizal fungi, Nutrient uptake, Soil enzyme activity, and Pre inoculation

INTRODUCTION

Soil enzymes contribute to the total biological activities in the soil environment because they are intimately involved in catalyzing reactions necessary for organic matter decomposition, nutrient cycling, energy transfer, and environmental quality (Dick, 1994 and Dick, 1997). Enzyme activities often provide a unique integrative biological assessment of soil function, especially those catalyzing a wide range of soil biological processes, such as dehydrogenase, urease, phosphatase

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etc. (Nannipieri *et al*., 2002). Enzyme activities control rates of soil nutrient cycling and are valuable indicators of soil microbial diversity. Measurement of the activity of the soil micro flora provides indices of the biological state of the soil and hence the soil fertility.

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Among the diverse soil enzymes, dehydrogenase and phosphatase are important in the transformation of different nutrients for plants. The activity of dehydrogenase reflects the total oxidative capacity of the microbial biomass (Nannipieri *et al*., 1990). Phosphatases are a broad group of enzymes that are capable of catalyzing hydrolysis of esters and anhydrous of phosphoric acid (Schmidt and Lawoski, 1961). In soil ecosystems, these enzymes are believed to play critical roles in P cycles (Speir and Ross, 1978). AM fungi influence microbial population and activity and consequently nutrient dynamics in the soil through the release of organic compounds. AM fungi may directly or indirectly contribute to soil C and N dynamics and it could be source of different soil enzymes required for biochemical reactions. These reports indicating that soil enzymatic activities, such as dehydrogenases and phosphatases are increased by AM fungal inoculation (Kothari *et al*., 1990). When host plants are subjected to nutrient stress conditions at that time mycorrhizal fungi extend their extra radical, hyphal network and efficiently absorb mineral nutrients from the soil and deliver them to their host plants in exchange for carbohydrates. They facilitate nutrient uptake of N, P and other micronutrients.

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Hence, the present study envisaged the useful biotic interaction of Arbuscular mycorrhizal fungi as influenced by nutrient uptake and soil enzyme activity of tobacco rhizosphere under parasitic weed stress condition.

MATERIAL AND METHODS

A field experiment was conducted during *Kharif* 2018-19 in order to study the "Arbuscular mycorrhizal fungi as influenced by nutrient uptake and soil enzyme activity of tobacco rhizosphere under parasitic weed stress condition". These experiments were carried out in *Orobanche* infested soils of tobacco growing areas of Nipani in Belagavi district of northern Karnataka. During the investigation different inoculation methods of mycorrhizal fungi were screened for their ability to improve the nutrient uptake and soil enzyme activity i.e. (pre inoculation of nursery seedlings; direct soil application and the combination of both) with the use of following AMF culture UASDAMFT (Isolated from *Orobanche* suppressive soils in tobacco), UASDAMFS (Isolated

from *Striga* suppressive soils in sugarcane) and STD AMF Consortium (Department of Agricultural Microbiology, UAS Dharwad). The experiment was laid out in randomized complete design with factorial concept. There were 3 main factors and 3 sub factors consisting of combination of AM fungi and different methods of application and UIC outside the experiment run with RCBD as given below:

I factor: AMF cultures	
M_1	UASDAMFT consortium (tobacco native)
M_2	UASDAMFS consortium (sugarcane native)
M_3	STD AMF consortium (UASD reference)
II factor: Methods of application	
S_1	Pre colonization of the tobacco seedlings in the nursery beds @ 2 kg /m^2
S_2	Soil application (@ 6-8 kg/acre mixed with 200 kg of vermicompost)
S_3	Pre colonization + Soil application

UIC	UIC outside the experiment run with RCBD

Application of mycorrhizal cultures:

Pre colonization of the tobacco seedlings in the nursery beds with AMF @ 2 kg /m^2

Nursery beds were prepared and subjected for solarization (4 to 5 weeks) in order prevents the native AMF infective propagules. AMF culture along with vermicompost @ 2:25 was applied in the furrows prior to the sowing of tobacco seeds.

Soil application

AMF culture @ 8 kg per acre was applied along with 200 kg of vermicompost at the time of transplanting of tobacco seedlings.

Soil enzymes activity (45, 90 and 120 DAP)

Estimation of dehydrogenase activity

Dehydrogenase activity in the soil samples were determined by following the procedure as described by Casida *et al*. (1964).

Estimation of phosphatase activity

Phosphatase activity of soil samples were determined by following the procedure of Evazi and Tabatabai (1979).

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Estimation of urease activity

Urease activity of soil samples was determined by following the procedure of Tabatabai and Bremner (1972).

Chemical analysis of tobacco plants (120 DAP)

The chemical analysis was done by using shoot and leaf samples of tobacco plants.

Estimation of nitrogen

The total nitrogen content in the plant sample was estimated following the microkjeldahl method as outlined by Jackson (1973).

Estimation of phosphorus

Phosphorus was estimated by vanadomolybdate phosphoric yellow colour method (Jackson, 1973).

Estimation of Potassium

Potassium in the aliquot was estimated with the help of flame photometer after appropriate dilution (Tandon, 1998).

Micronutrients

Zinc, copper, iron and manganese were estimated in the aliquot of plant extract using atomic absorption spectrophotometer (Shimadzu model) as described by Tandon (1998).

Statistical analysis and data interpretation

The data collected at different growth stages of crop were subjected to statistical analysis. Based on mean values obtained, analysis and interpretation of data were studied using the Fischer's method of analysis of variance technique as described by Gomez and Gomez (1984). The level of significance used in 'F'and't' test was p = 0.05. Critical difference values were calculated wherever the 'F' test was significant.

Results and discussion

Dehydrogenase activity (µg TPF formed g^{-1}soil d^{-1})

At 120 DAP, Dehydrogenase activity was maximum in the treatment received both pre colonized tobacco seedling as well as soil applications of STD AMF (45.89 µg TPF formed g^{-1}soil d^{-1}) and second highest was recorded with treatment received pre colonized tobacco seedling with UASDAMFT (42.21 µg TPF formed g^{-1}soil d^{-1}) followed by pre colonized tobacco seedling with UASDAMFT as well as soil applications at the time of planting (41.18 µg TPF formed g^{-1}soil d^{-1}). However the least dehydrogenase activity was observed in the rhizosphere soil of non mycorrhized tobacco plants (25.83 µg TPF formed g^{-1}soil d^{-1}).

Phosphatase activity (µg pnp released g^{-1} soil h^{-1})

AMF cultures and methods of application revealed that phosphatase activity was found to be maximum in the treatment received pre colonization of tobacco seedling with STD AMF plus soil application (319.64 µg pnp released g^{-1} soil h^{-1}), which is followed by the treatment received pre colonized tobacco seedling with UASDAMFT alone (304.46 µg pnp released g^{-1} soil h^{-1}).However least phosphatase activity was observed in the rhizosphere soil of non mycorrhized tobacco plants (158.65 µg pnp released g^{-1} soil h^{-1}) at 120 DAP.

Urease activity (µg NH_4+Ng^{-1} day^1)

At 120 DAP the highest urese activity was observed in the treatment received planting of tobacco seedling pre colonized with STD AMF as well as (pre + soil) application of STD AMF at the time of planting (23.79 µg NH_4+Ng^{-1} day^1), followed by the tobacco seedling received pre

colonized with UASDAMFT (23.71 µg NH_4+Ng^{-1} day^1). However applications of AMF cultures were found to be superior over uninoculated control (14.19 µg NH_4+Ng^{-1} day^1).

In mycorrhizosphere the activity of mycorrhizal fungi and other beneficial microorganisms could be a source for different soil enzyme needed for biochemical reaction in the plant rhizosphere. Our findings are also similar to the findings of shubha *et al*. (2018); Asif *et al*. (2019) and Jones *et al*. (2012, 2014a). Pacovasky *et al*. (1991) documented that increased phosphatase activity in AM fungi associated roots, resulting in enhanced phosphate availability in mycorhizosphere. The activity of microorganisms and AM fungi in rhizosphere soil may be the source of different soil enzymes required for several biochemical reactions. Selvaraj (1998) reported that *Glomus fasciculatum* inoculated roots of *P. juliflora* improved the level of alkaline phosphatase activity Dubey and Fulekar (2011) reported that inoculation of soil based AMF in sorghum plants increased the phosphatase and dehydrogenase activities.

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Nutrients content in tobacco plants (120DAP)

Total Nitrogen content (%) in tobacco plats

The treatment received both pre colonization as well as soil applications of STD AMF (1.84%) recorded significantly the highest nitrogen uptake followed by pre colonized UASDAMFT (1.24%), pre colonized tobacco seedling with UASDAMFT plus soil application (1.20%). However minimum nitrogen uptake was recorded in the non mycorrhized treatment (0.70%).

Total phosphorous content (%) in tobacco plants

The treatment received both pre colonization as well as soil applications of STD AMF recorded significantly the highest phosphorous uptake (0.55%), followed by pre colonized tobacco seedling with UASDAMFT plus soil application (0.54%), pre colonized UASDAMFT alone (0.52%) were statistically with each other. However least phosphorous uptake was recorded in uninoculated control (0.32%).

Total potassium content (%) in tobacco plants

The treatment received as a both pre colonized tobacco seedling as well as soil applications of STD AMF recorded significantly the highest potassium uptake (2.94%) followed by pre colonized UASDAMFT (2.86%), pre colonized UASDAMFS plus soil application (2.74%). However least potassium uptake was recorded in uninoculated control (0.53%)

Micronutrients (Fe, Mn, Cu and Zn) content in tobacco plants as influenced by mycorrhizal native isolates.

Fe (mg/kg) micronutrients content in tobacco

The treatment received STD AMF as pre colonizing agent as well as soil application recorded significantly the highest Fe uptake(682.67 mg/kg) followed by UASDAMFT as pre colonized(663.00 mg/kg), UASDAMFT as pre colonized as well as soil application (613.33 mg/kg). However minimum Fe uptake was recorded in uninoculated control (307.67 mg/kg) compared to different methods of applications.

Mn (mg/kg) micronutrients content in tobacco

The treatment received pre colonized tobacco seedling as well as soil applications of STD AMF (215.53 mg/kg) recorded significantly the highest Mn uptake compared to the treatments received tobacco seedlings pre colonized with UASDAMFT (215.03 mg/kg) and tobacco seedling pre colonized with UASDAMFT as soil application of UASDAMFT(145.9 mg/kg). However minimum Mn uptake was recorded in uninoculated control (108.90 mg/kg).

Cu (mg/kg) micronutrients content in tobacco

The treatment received pre colonization as well as soil applications of STD AMF recorded significantly the highest Cu uptake (62.20 mg/kg) followed by pre colonized UASDAMFT as colonized seedling plus soil application (54.13 mg/kg), pre colonized tobacco seedling with UASDAMFT (52.10 mg/kg). However minimum Cu uptake was recorded in uninoculated control (31.37 mg/kg)

Zn (mg/kg) micronutrients content in tobacco

The treatment received pre colonization as well as soil applications with STD AMF recorded significantly the highest Zn uptake (72.33 mg/kg) followed with the treatment received pre colonized tobacco seedling UASDAMFT plus soil application (70.63 mg/kg), pre colonized tobacco seedling with UASDAMFT (69.43 mg/kg) and pre colonized tobacco seedling with STD AMF (53.45 mg/kg). However minimum Zn uptake was recorded in uninoculated control (33.75 mg/kg). Increased nutrient content was observed in tobacco plants received pre-colonization seedling followed by soil application of UASDAMF (native) isolates over uninoculated control. Preliminary studies by Lendzemo *et al*. (2005) observed that the symbiotic association between mycorrhizal fungi and roots of plants results in increased uptake of nitrogen phosphorus, potassium and micro-nutrient.

Conclusion

Mycorrhizal fungi improve the soil enzyme activity and the plant growth through the increased uptake of nutrients especially phosphorus, Zn, N, Cu, Mn, etc. due to exploration of higher soil volume by the external hyphe even beyond the root zone of nutrient absorption. Hence the present investigation is a promising strategy to develop an excellent biofertilizer for sustainable agricultural production.

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Table 1. Dehydrogenase activity in tobacco rhizosphere as influenced by AM fungi

Treatment	(µg TPF formed g^{-1} soil d^{-1})											
	45 DAP				90 DAP				120 DAP			
	Method of application				Method of application				Method of application			
AM Fungi	S_1	S_2	S_3	Mean	S_1	S_2	S_3	Mean	S_1	S_2	S_3	Mean
M_1	41.55	39.82	40.51	40.63	43.64	41.40	47.83	44.29	42.21	36.57	41.18	39.99
M_2	34.78	32.22	35.73	34.24	35.86	31.72	42.51	36.70	34.40	33.33	40.19	35.97
M_3	41.34	34.61	45.04	40.33	39.43	35.43	49.27	41.38	40.97	33.70	45.89	40.19
Mean	39.23	35.55	40.43		39.64	36.18	46.54		39.19	34.53	42.42	
UIC				29.93				26.43				25.83
	S.Em±		C D at 5 %		S.Em±		C D at 5 %		S.Em±		C D at 5 %	
M	1.01		3.96		0.66		2.60		0.34		1.36	

S	0.62	1.92	0.67	2.07	0.30	0.93
M at S	1.34	4.77	1.16	3.89	0.55	1.88
UIC	1.20	3.59	1.20	3.58	1.19	3.56

AM Fungi	**Method of application**
M_1: UASDAMFT (tobacco native)	S_1: pre-colonized
M_2: UASDAMFS (sugarcane native)	S_2: soil application
M_3: STD AMF	S_3: pre-colonized + soil application
UIC: Uninoculated control	

Table 2. Phosphatase activity in tobacco rhizosphere as influenced by AM Fungi

Treatment	**(µg pnp released g^{-1} soil h^{-1}).**											
	45 DAP				**90 DAP**				**120 DAP**			
	Method of application				**Method of application**				**Method of application**			
AM Fungi	**S_1**	**S_2**	**S_3**	**Mean**	**S_1**	**S_2**	**S_3**	**Mean**	**S_1**	**S_2**	**S_3**	**Mean**
M_1	283.33	224.40	287.50	265.08	305.65	241.07	292.85	279.86	304.46	285.41	301.48	297.12
M_2	166.96	161.31	233.33	187.20	171.12	172.85	243.74	195.90	181.84	177.30	272.32	210.49
M_3	274.26	149.10	293.75	239.04	280.06	149.40	320.50	249.99	294.05	175.59	319.64	263.09
Mean	241.52	178.27	271.52		252.28	187.77	285.70		260.12	212.77	297.81	
UIC				132.44				157.15				158.65
	S.Em±		**C D at 5 %**		**S.Em±**		**C D at 5 %**		**S.Em±**		**C D at 5 %**	
M	4.36		17.15		2.83		11.14		4.41		17.33	
S	4.07		12.54		5.21		16.06		3.51		10.83	
M at S	7.22		24.50		7.89		25.20		6.64		22.95	
UIC	6.92		20.58		8.39		24.93		7.29		21.67	

AM Fungi	**Method of application**
M_1: UASDAMFT (tobacco native)	S_1: pre-colonized
M_2: UASDAMFS (sugarcane native)	S_2: soil application
M_3: STD AMF	S_3: pre-colonized + soil application
UIC: Uninoculated control	

Table 3. Urease activity in tobacco rhizosphere as influenced by AM fungi

Treatment	($\mu g\ NH_4^+\ N\ g^{-1}$soil day^{-1})											
	45 DAP				90 DAP				120 DAP			
	Method of application				Method of application				Method of application			
AM Fungi	S_1	S_2	S_3	Mean	S_1	S_2	S_3	Mean	S_1	S_2	S_3	Mean
M_1	24.98	23.96	24.71	24.55	25.46	24.85	25.26	25.19	23.71	23.03	23.21	23.31
M_2	18.70	17.95	18.53	18.39	20.04	18.36	20.37	19.59	17.72	17.53	17.79	17.68
M_3	24.96	21.12	25.47	23.85	24.28	22.21	25.85	24.11	23.79	21.61	23.79	23.06
Mean	22.88	21.01	22.90		23.26	21.81	23.83		21.74	20.72	21.59	
UIC				15.55				16.43				14.19
	S.Em±		C D at 5 %		S.Em±		C D at 5 %		S.Em±		C D at 5 %	
M	0.48		1.90		0.37		1.46		0.15		0.58	
S	0.29		0.90		0.41		1.26		0.38		1.19	
M at S	0.63		2.27		0.69		2.29		0.56		1.78	
UIC	0.79		2.35		0.68		2.03		0.80		2.39	

AM Fungi	**Method of application**
M_1: UASDAMFT (tobacco native)	S_1: pre-colonized
M_2: UASDAMFS (sugarcane native)	S_2: soil application
M_3: STD AMF	S_3: pre-colonized + soil application
UIC: Uninoculated control	

Table 4. Effect of AM fungi on nutrient concentration in tobacco plants at 120 DAP

Treatment	N, P K, (%)											
	N				P				K			
	Method of application				Method of application				Method of application			
AM Fungi	S_1	S_2	S_3	Mean	S_1	S_2	S_3	Mean	S_1	S_2	S_3	Mean
M_1	1.24	0.85	1.20	1.10	0.52	0.32	0.54	0.46	2.86	2.17	2.20	2.41
M_2	0.73	0.79	1.01	0.84	0.36	0.32	0.43	0.37	0.80	0.71	2.74	1.42
M_3	1.09	0.92	1.84	1.28	0.49	0.36	0.55	0.47	2.33	1.07	2.94	2.11
Mean	1.02	0.85	1.35		0.46	0.33	0.51		2.00	1.32	2.62	
UIC				0.70				0.32				0.53
	S.Em±		C D at 5 %		S.Em±		C D at 5 %		S.Em±		C D at 5 %	
M	0.027		0.108		0.008		0.031		0.038		0.148	
S	0.028		0.087		0.009		0.026		0.041		0.127	
M at S	0.049		0.163		0.014		0.048		0.070		0.232	
UIC	0.047		0.14		0.014		0.04		0.098		0.29	

AM Fungi	**Method of application**
M_1: UASDAMFT (tobacco native)	S_1: pre-colonized
M_2: UASDAMFS (sugarcane native)	S_2: soil application
M_3: STD AMF	S_3: pre-colonized + soil application
UIC: Uninoculated control	

Table 5. Effect of AM fungi on micro nutrient concentration in tobacco at 120 DAP

Treatment	Fe (mg/kg)				Mn (mg/kg)				Cu (mg/kg)				Zn (mg/kg)			
	Method of application				Method of application				Method of application				Method of application			
AM Fungi	S_1	S_2	S_3	Mean	S_1	S_2	S_3	Mean	S_1	S_2	S_3	Mean	S_1	S_2	S_3	Mean
M_1	663.00	349.33	613.33	541.89	215.03	133.27	145.90	164.73	52.10	46.10	54.13	50.78	69.43	49.45	70.63	63.17
M_2	325.80	337.00	439.33	367.38	135.80	129.00	129.87	131.56	37.43	39.27	47.53	41.41	42.95	44.10	47.60	44.88
M_3	473.00	404.30	682.67	519.99	145.27	126.57	215.53	162.46	49.23	37.93	62.20	49.79	53.45	49.35	72.33	58.38
Mean	487.27	363.54	578.44		165.37	129.61	163.77		46.26	41.10	54.62		55.28	47.63	63.52	
UIC				307.67				108.90				31.17				33.75
	S.Em±		C D at 5 %		S.Em±		C D at 5 %		S.Em±		C D at 5 %		S.Em±		C D at 5 %	
M	5.77		22.66		2.54		9.99		0.98		3.87		0.59		2.34	
S	8.69		26.79		1.77		5.47		1.69		5.22		1.30		4.00	
M at S	13.58		43.93		3.57		12.54		2.59		8.30		1.93		6.11	
UIC	13.42		39.87		4.56		13.56		2.54		7.55		1.94		5.78	

AM Fungi	**Method of application**
M_1: UASDAMFT (tobacco native)	S_1: pre-colonized
M_2: UASDAMFS (sugarcane native)	S_2: soil application
M_3: STD AMF	S_3: pre-colonized + soil application
UIC: Uninoculated control	

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