Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(2): 2496-2500
Received: 25-01-2018
Accepted: 26-02-2018

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Field evalualtion of native arbuscular mycorrhizal fungi in the management of *Striga* in sugarcane (*Saccharum officinarum* L.)

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Abstract
An attempt was made to evaluate the native arbuscular mycorrhizal fungal (AMF) isolates for *Striga* suppression under farmer's sugarcane field at Yeragatti village of Belgaum district, Karnataka. Selected levels of herbicides were also tested for comparison and their interactive effect with AMF. The results of the present investigations have revealed that *Striga* emergence was significantly inhibited in the treatment UASDAMF consortium (native) followed by UASDAMF9. The highest number of *Striga* infestation was recorded in the UIC plots. In addition, UASDAMF consortium (native) and AMF consortium (STD) stimulated plant growth, plant biomass and physiological parameters of sugarcane over non mycorrhized sugarcane plants in the presence of *Striga*. These findings confirm the effectiveness of AMF in protecting sugarcane against *Striga* infestation as well as promoting crop growth and reducing the soil *Striga* seed bank. It can be concluded that native isolates from *Striga* suppressive soils can be exploited as a biotic tool in the management of *Striga.*

Keywords: *Striga*, arbuscular mycorrhizal fungi, weed control, sugarcane

Introduction
Sugarcane is considered as the world's largest crop in case of production and consumption. Globally, India is considered as one of the main sugarcane producing country next to Brazil. In agriculture sector, sugarcane share was about seven per cent of the total value of agriculture output and occupied about 2.6 per cent of India's gross cropped area. Sugarcane provides raw material for the second largest agro-based industry after textile. There are nine states in India where sugarcane is grown on a larger extent. Karnataka stands fourth position and the statistical analysis for year wise productivity (t/ha) of sugarcane crop in Karnataka showed a reduction during 2012-13; 2013-14 and 2014-15 compared to 2010-11 (84.07, 69.84, 70.86 and 93.76 t/ha accordingly). 13.32 percent decrease in Kharif production in 2016-17 (305.25 million tonnes) vis-à-vis 2015-16 (352.16 million tonnes). The reported cane yield losses are due to poor growth of sugarcane resulting from weed infestation and it is the main cause for quality depression in sugarcane.
Among several weeds *Striga* has been identified as a major sugarcane weed and creates a great threat in sugarcane growing belts of northern Karnataka, especially in the districts of Belgaum, Bagalkot and Vijaypur. *Striga* is an obligate parasitic weed which attaches themselves to the roots of cereals and other plants, not only robbing them of nutrition but also causing various debilitating effects which have earned them their common name of "witchweeds".
The control of *Striga* is difficult to achieve because of its asynchronous seed germination and high fecundity. Therefore, management of *Striga* infestation needs an integrated approach including host plant resistance, cultural practices and chemical and biological treatments. Among all the components, biological control of *Striga* gives a demonstrable crop yield benefit. Reports of Jones *et al.*, 2011 [6] and 2014 demonstrated that certain soil microorganisms like arbuscular mycorrhizal fungi (AMF) can inhibit or suppress *Striga* germination. In this light, an experiment was undertaken to validate the *Striga* suppressive AMF isolates under farmer's sugarcane field and to assess their effect on growth response of sugarcane variety CO86032.

Materials and Methods
An investigation was carried out to evaluate the suppression of *Striga* by AMF along with different levels of herbicides under field conditions. The experiment was conducted in a previously surveyed *Striga* infested field located at 16.01.01.0″N latitude; 074.58.190″ E longitude, and at an altitude of 643 m above mean sea level; at Yaragatti village of Belgaum

district of northern Karnataka. Before conducting the experiment, an extensive survey was under taken in the sugarcane growing areas of Belgaum district for the *Striga* infested fields. Based on the severity and the sugarcane crop damage, the Yaragatti village was selected for conducting the present investigation. Sugarcane variety CO86032 was used in the present study.

The efficient native AMF isolates used in the study were selected based on our previous pot culture studies (Shubha *et al*., 2015) [13] and maintained at weed control scheme, University of Agricultural Sciences, Dharwad. The isolates were coded as UASDAMF5; *Acaulospora maarowe*, UASDAMF9; *Glomus leptotichum*, UASDAMF Consortium (native); *Acaulospora maarowe* + *Glomusl eptotichum* and AMF Consortium (STD); *Glomus macrocarpum*, *Gigaspora margarita* and *Acaulospora laevis.* As pre emergent, Atrazine 50 WP @ 2.5 kg ha^{-1} sprayed at different levels *viz*., 50, 75 and 100 per cent on soil surface two days after planting (DAP) and 2, 4-D sodium salt 80 per cent @ 2.5 kg ha^{-1} at 60 DAP was sprayed at different levels *viz.,* 50, 75 and 100 per cent as post emergent herbicide. The AMF cultures were applied at the rate of 16 kg^{-1} acre (100 IP g^{-1} soil) by mixing with vermicompost (1:3).

The experiment was laid out in randomized complete design with factorial concept. There were 20 treatment combinations as detailed in table 1 with three replications. There were five main factor and four sub factor consisting of combination of AM fungi and different levels of herbicides as given below:

I –Factor: AMF		II – Factor: Levels of Herbicide	
M_1	UASDAMF5	H_1	0 per cent RDH
M_2	UASDAMF9	H_2	50 per cent RDH
M_3	AMF Consortium (native)	H_3	75 per cent RDH
M_4	AMF Consortium STD	H_4	100 per cent RDH
M_5	UIC		

Number of *Striga* emerged were recorded in each pot. The shoot and root portions of *Striga* plants were separated and oven dried at 60°C to constant weight. The dry weights were then recorded separately for shoots and roots and average of three were expressed in grams.

The sugarcane plant height was defined as the average stem distance from the soil to the insertion of the Top Visible Dewlap leaf (TVD) (Dillewiijn, 1952) [2] on the stem and average of five were expressed in cm. The green tillers present in each plot were counted and recorded as number of tillers at 180 days after planting and expressed in tillers per plot. The cane girth was measured at fourth node from the bottom of each plant and expressed in cm. The chlamydospores in rhizosphere of sugarcane were determined by wet sieving and decantation method as outlined by Gerdemann and Nicholson (1963) [3] and examined under a stereo zoom microscope (Labomed). The spore counts were taken at 30, 60 and 90 DAP.

Statistical analysis was carried out based on mean values obtained. The level of significance used in 'F' and 'T' test was P = 0.05 (Gomez and Gomez, 1984) [4].

Results

The number of *Striga* per plot and their dry weight were recorded at 120 and 180 DAP respectively and presented in Table 1. In general mycorrhization reduced the *Striga* emergence compared to UIC. We observed that the application of UASDAMF consortium (native) as well as AMF consortium STD suppressed the *Striga* emergence significantly (0.33plot^{-1} and 0.75plot^{-1}, respectively) over UIC (8.91plot^{-1}). However, the treatments with single inoculation of UASDAMF5 and UASDAMF9 were also found to reduce the number of *Striga* emergence (7.16 plot^{-1}and 8.33 plot$^{-1,}$ respectively), which is significantly superior over UIC. Applications of herbicidal molecules, at different levels have shown varied responses on *Striga* emergence. Among the interactive effects, there was zero *Striga* emergence in the treatment which received UASDAMF Consortium native plus 75% and 100 per cent RDH (atrazine 50 WP 2.5 kg ha^{-1} at 3 to 4 DAP; 2,4-D sodium salt 80 per cent @ 2.5 kg ha^{-1} 60 DAP), although results of of all RDH levels tested were statistically on par with each other.

At 180 DAP, the highest the total dry biomass of *Striga* was recorded in UIC (26.95 g plot^{-1}) over the rest of the treatments. The lowest biomass of *Striga* was recorded with the plots which received UASDAMF 9 at 180days after planting (2.57 g plot^{-1}).With respect to the different levels of herbicide application, the plot received 100 per cent recommended dose of herbicide recorded significantly the least total dry biomass of *Striga* (6.01g/plot) over the plots which received 0, 50 and 75 per cent of herbicidal levels (10.37, 8.30 and 6.77 g plot^{-1}, respectively).

Table 1: Interactive effect of AM fungi at different levels of herbicides on emergence and dry matter of *Striga*

Treatment	*Striga* per plot at 120 DAP					Dry matter of *Striga (*g/plot) at 180DAP				
AM fungi	Herbicide levels					Herbicide levels				
	0% RDH	50% RDH	75% RDH	100% RDH	Mean of A	0% RDH	50% RDH	75% RDH	100% RDH	Mean of A
UASDAMF 5	11.3	8.66	5.00	3.66	7.16	17.79	11.00	6.24	4.20	9.80
UASDAMF 9	9.00	9.00	8.33	7.00	8.33	3.76	2.71	2.08	1.75	2.57
UASDAMF consortium (native)	1.00	0.33	*	*	0.33	*	*	*	*	*
AMF Consortium STD	2.00	0.66	0.33	*	0.75	*	*	*	*	*
UIC	21.66	10.00	2.33	1.66	8.91	30.34	27.81	25.53	24.13	26.95
Mean of B	9.00	5.73	3.20	2.46		10.37	8.30	6.77	6.01	
S.Em. ±	C.D. at 5%					S.Em. ±	C.D. at 5%			
C.D. of A (AM fungi)	0.35	1.14				0.25	0.83			
C.D. of B (Herbicides)	0.29	0.85				0.18	0.52			
C.D. of A x B (AM fungi + Herbicide)	0.66	2.00				0.43	1.30			

Note: (*) No emergence of *Striga*

Note: Mean of A - AM fungi. Mean of B - Herbicide. Mean of A x B - AM fungi + Herbicide.

Influence of AM fungal isolates on growth parameters of sugarcane

Plant height

Effect of AM fungi and different levels of herbicides on plant height, number of tillers and millable cane girth of sugarcane is as given in table 2. The plant height was found to increase steadily with number of days after planting due to various treatments (data not shown). The plants inoculated with the AM fungal isolates were found to be superior over uninoculated control plant at 120 DAP. However the plant height differed significantly among the plants inoculated with UASDAMF consortium (native), AMF consortium (STD) and single inoculation of native isolates over UIC.

Among the different levels of herbicide application, all treatments without herbicide recorded significantly the highest plant height compared to rest of the levels of herbicides. Plant height in interactive effects between the standard and native AMF consortium and/ or herbicidal application at different levels were on par with each other.

Table 2: Interactive effect of AM fungi and different levels of herbicides on plant height, number of tillers, millable cane girth of sugarcane

Treatment	Plant height (cm)					Number of tillers per plot					Cane girth (cm)				
	120 DAP					180DAP					320 DAP				
AM fungi	Herbicide levels					Herbicide levels					Herbicide levels				
	0% RDH	50% RDH	75% RDH	100% RDH	Mean of A	0% RDH	50% RDH	75% RDH	100% RDH	Mean of A	0% RDH	50% RDH	75% RDH	100% RDH	Mean of A
UASDAMF 5	123.00	120.33	116.67	103.00	115.75	117.20	113.67	110.43	107.00	112.07	12.16	11.33	10.50	9.66	10.91
UASDAMF 9	129.33	124.33	119.00	109.67	120.58	116.90	112.63	109.40	106.90	111.46	12.50	11.50	10.50	9.83	11.08
UASDAMF consortium (native)	141.67	142.67	140.00	136.00	140.08	125.17	120.33	116.97	116.30	119.69	14.50	13.50	12.50	11.50	13.00
AMF Consortium STD	142.67	141.00	137.00	131.33	138.00	127.97	117.23	111.73	108.30	116.31	14.16	13.50	11.83	11.16	12.66
UIC	88.33	81.67	73.67	72.00	78.92	70.77	68.77	64.97	60.67	66.29	6.83	6.00	5.50	5.00	5.83
Mean of B	125.00	122.00	117.27	111.40		111.60	106.53	102.70	99.83		12.03	11.16	10.16	9.43	
	S.Em. ±		C.D. at 5%			S.Em. ±		C.D. at 5%			S.Em. ±		C.D. at 5%		
C.D. of A (AM fungi)	0.71		2.33			0.65		2.14			0.08		0.26		
C.D. of B (Herbicide)	0.48		1.39			0.61		1.78			0.11		0.33		
C.D. of A x B (AM fungi + Herbicide)	1.17		3.56			1.36		4.06			0.23		0.69		

Number of tillers

The observation on number of tillers recorded at 180 DAP have revealed that, application of AMF in general significantly influenced the tiller numbers over the plants which did not receive the AMF. Further the application of AMF as consortium has significantly influenced the number of tillers compared to the plants which received single inoculation of AMF. Among the AMF cultures, UASDAMF consortium (native) significantly influenced the tiller numbers per plot (119.69) followed by AMF consortium STD (116.31). However, the least numbers of tiller were recorded with non mycorrhized plots (66.29).

Among different levels of herbicide application, the treatment which received zero percentage of RDH recorded significantly higher number of tillers per plot (111.60) compared to rest of the levels. Interactive effects between the AMF and herbicidal application revealed that application of UASDAMF consortium (STD) along with zero per cent RDH significantly influenced the number of tillers per plot (127.97) compared to other plots. In single AMF inoculation UASDAMF 5 was found to be better than UASDAMF9 with respect to influencing number of tiller as seen in table 2.

Cane girth

At 320 DAP, inoculation of UASDAMF consortium (native) recorded the highest mill able cane girth (13 cm) which is followed by AMF consortium STD (12.66 cm). However the results with single inoculation of other native isolates were statistically on par with each other. The lowest mill able cane girth was recorded on UIC (5.83 cm) as shown in table 2.

The treatment absolutely free from herbicidal molecules recorded significantly the highest mill able cane girth (12.03cm) compared to rest of the elevated levels of RDH *i.e.*, 50, 75 and 100 per cent of RDH (11.16, 10.16 and 9.43 cm, respectively).

The application of UASDAMF consortium (native) along with 0 per cent RDH significantly influenced the mill able cane girth (14.50 cm) as compared to other levels. On the other hand, single inoculation of UASDAMF9 along with 0, 50, 75 and 100 per cent of RDH influenced on mill able cane girth of sugarcane (12.50, 11.50, 10.50 and 9.83 cm, respectively).

Mycorrhizal spore count

The influence of different treatments on the AMF spore count is depicted in fig.1. The perusal of data on spore count varied due to the AMF inoculations at different levels of RDH. The sugarcane received AMF STD consortium, UASDAMF (native consortium) and individual application of native isolates *viz.*, UASDAMF9, UASDAMF5, recorded increased number of spores over UIC (Fig.1).

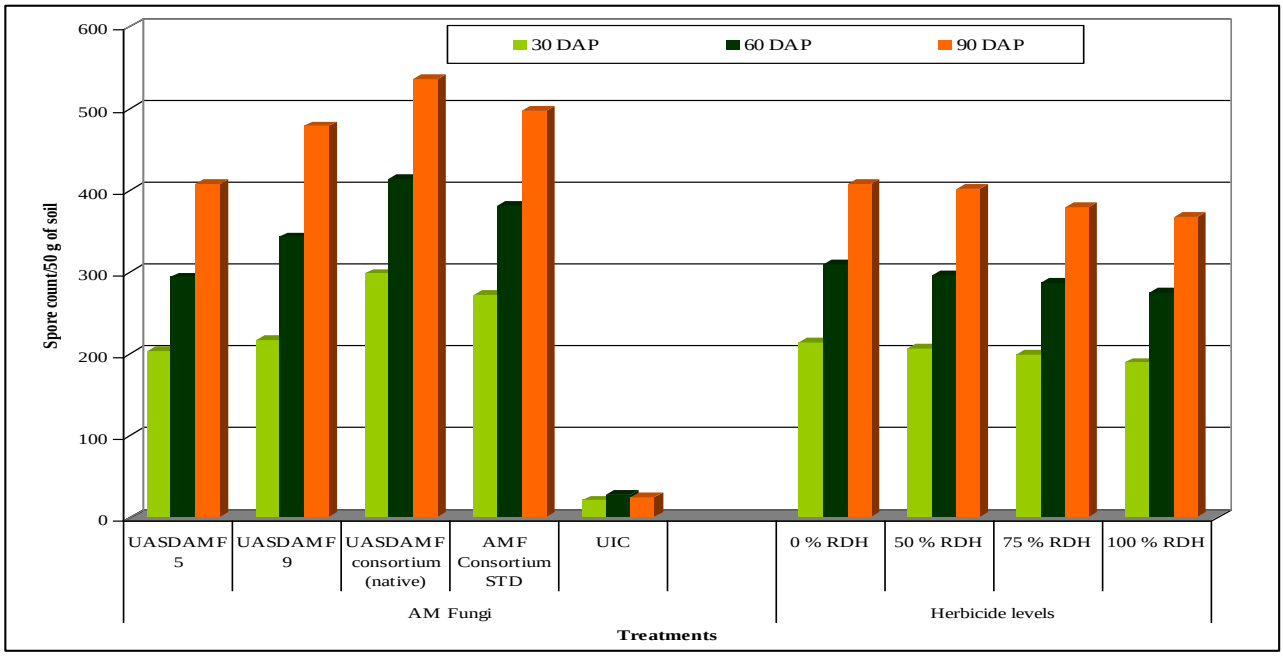

Fig 1: Interactive effect of AM fungi and different levels of herbicides on mycorrhizae spore load in sugarcane

The highest mycorrhizal spore count was recorded in the sugarcane rhizosphere of the plants inoculated with UASDAMF consortium (native) at 30, 60 and 90 DAP (297.67, 412.83 and 535.9/50^{-1} soil correspondingly).

At 90 DAP the spore load was highest in the treatment which received AMF consortium (native) (535.9 50^{-1}g soil) followed by AMF consortium (STD) (496.70/50^{-1} g soil). Among individual application of native isolates UASDAMF9 significantly influenced spore load (478.10/50^{-1}soil) compared to UASDAMF5 (407.9/50^{-1}soil). However, least numbers AMF spore was recorded in the rhizosphere of non mycorrhized sugarcane plants (24.8/50^{-1}soil).

In general, the plots received herbicidal application were found to have lesser AMF spore load compared to the plots received mycorrhizal application. Increased levels of RDH at 100 per cent reduced the spore load (367.00/50^{-1} g soil) compared to the herbicidal concentrations at 75, 50 and zero per cent (379.60, 400.90 407.20 /50^{-1} g soil, respectively).

Inoculation of UASDAMF consortium (native) along with 0 per cent RDH recorded significantly the highest mycorrhizal spore load (553.0/50 g soil) compared to the increased levels of RDH at 50, 75 and 100 per cent (543.0, 526.3 and 521.3 /50^{-1} g soil).

Discussion

The suppression of *Striga* by AM fungi is chiefly known to be due to depletion of strigolactones by them in the rhizosphere of the host plants. Strigolactones are signalling molecules that play a vital role as germination stimulants of the parasitic weeds *viz.*, *Striga*, *Orobanche* and *Phelipanche* sp. and the stimulants are exuded into the rhizosphere by the roots of their host plants. In the present field study, application of AMF STD consortium, AMF consortium native and single inoculation of native AMF isolates UASDAMF9 and UASDAMF5 inoculated plots have significantly reduced the emergence and biomass of *Striga* compared to UIC. This can be attributed to the fact that, the strigolactones are converted into another compound called mycoradicin in AMF colonized host plant and thereby strigolactone availability is nil for the emergence of *Striga* (Walter *et al.*, 2011) [14]. Arbuscular mycorrhizal fungi have been shown to be a promising bioagent in suppressing the emergence of *Striga*. In a study on the response of sorghum and *Striga* to VA-mycorrhizae fungus, *Glomus mosseae* revealed that the AM fungus significantly reduced the number of emerging *Striga* weed plants (62 per cent) and increased sorghum growth and yield (Gworgwor and Weber, 2003) [5].

These results suggest that AM fungal colonization likely induces resistance to plant parasitism by reducing the exudation of strigolactones (Lopez-Raez *et al.*, 2011) [10]. The interactive effect of UASDAMF consortium (native) as well as UASDAMF consortium (STD) at all the levels of RDH has totally suppressed the *Striga* emergence, indicating the compatibility of the bio agents with herbicides. In a similar study with herbicides alone, Kabambe *et al.* (2008) [9] evaluated the effects of seed dressing with imazethapyr significantly suppressed *Striga* emergence across all sites but did not increase yield. However, the literature with respect to the interaction between the AMF and different levels of recommended dosage of herbicide is not available and perhaps our study is first of its kind.

The results of the present study have indicated that mycorrhization in sugarcane influenced the growth attributes such as height, mill able cane girth and number of tillers over un inoculated control, which can be ascribed to positive interactions between the plant and AMF which promote balanced mobilization of nutrients *viz.*, P, K and micronutrients to the host crop. Also, plant growth promotion could be associated with early suppression of *Striga*, the nutrient robbing parasite. Similar results were also recorded by several workers (Salami *et al.*, 2005 and Shubha *et al.*, 2015) [12, 13].

The highest spore count at 90 DAP in the plots inoculated with UASDAMF consortium (native) along with 0 per cent RDH suggests that the bioagent is competent at *Striga* control when integrated with moderate levels of herbicides. Very high concentration of herbicide could be detrimental to AM fungi. High spore load could be due to the fungal preference by the host and due to the factors influencing the mycotrophy of sugarcane as reported previously by Devika *et al.* 2013 [1] and Mali *et al.*, 2009 [11]. Never the less, application of herbicides is an essential part of augmenting crop growth and yields,

excessive use of these chemicals lead to the microbial imbalance, environmental pollution and health hazards. Therefore, integration of this biotic *Striga*- suppressing strategy utilising arbuscular mycorrhizal fungi with admissible levels of chemical herbicides in to agronomic practices could help in maintaining ecological balance and augmenting sugarcane growth.

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