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Isolation, screening and selection of efficient native arbuscular mycorrhizal fungi for suppression of *Striga* in sugarcane

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ABSTRACT

The control of *Striga* is difficult to achieve because of its high fecundity and asynchronous seed germination. Thus, an attempt was made to control *Striga* in its subterranean stage of development using native arbuscular mycorrhizal fungi (AMF) spp. In this investigation, 16 AMF spp. were isolated, grouped and mass multiplied according to their morphological differences from the *Striga* suppressive soil of sugarcane growing area. Further, these 16 native AMF isolates (coded as UASDAMF), native AMF consortium (including 16 native AMF spp.), standard AMF consortium and uninoculated control- UIC (without AMF spp.) were tested against *Striga* under pot experiment. Significant inhibition of *Striga* emergence was observed with standard AMF consortium, native AMF consortium, UASDAMF-2, UASDAMF-5, UASDAMF-9 and UASDAMF-12. While, the UIC recorded highest number of *Striga* infestation. Chlorophyll content in sugarcane leaves (43.36 and 42.72 at 90 and 120 DAP respectively) were recorded highest with native AMF consortium. The physiological parameters such as photosynthetic rate and stomatal conductivity of sugarcane also recorded highest (18.16 and 0.55 µmol/ m^2/sec respectively) with native AMF consortium. The results indicated that the native AMF can efficiently compensate the negative effect of *Striga* infestation on sugarcane plants. An overall improvement in the biochemical and physiological attributes of the *Striga*-susceptible sugarcane variety CO86032 upon AMF colonization, clearly suggests the biocontrol and growth promotional potential of AMF consortium.

INTRODUCTION

Sugarcane (*Saccharum officinarum*) is an important commercial crop of India occupying around 3.8 million hectares of land with an annual cane production of around 270 million tones (2012-13). In Karnataka, suga rcane is cultivated in 4.4 lakh ha area with a productivity of 90 t/ha during 2015-16 season. The total cane crushed in the state during 2015-16 was 376.65 lakh tones compared to cane crushed during 2014-15 (450.92 lakh tonnes) in Karnataka. The 16.6 % reduction in productivity was caused due to the deficiency of soil fertility, lack of nutrition supply, disease incidence (rust, leaf spot), insect incidence (ex. Shoot borer) and parasitic weed infestation (ex. *Striga*).

Striga, a root parasite of cereals and legumes, has attracted much attention off late, as it is the main cause for serious loss in crop production in the semi-arid tropics. The life cycle of *Striga* is mainly dependent on its host. Approximately 75% of the overall *Striga* damage to the host is made during its subterranean stage of development (Parker and Riches 1993). Rank *et al.* (2004) demonstrated that *Striga* exerts a potent phytotoxicity effect on the host. Managing *Striga* below ground is therefore a crucial task for successful *Striga* management.

The control of *Striga* is difficult to achieve because of its high fecundity and asynchronous seed germination. Therefore, management of *Striga* infestation needs an integrated approach including host plant resistance, cultural practices, and chemical and biological treatments. Among all the components of this integrated *Striga* management, biological control gives a demonstrable crop-yield benefit within one growing season (Ahonsi *et al.* 2002). Thus, in order to prevent the weed menace as well as to prevent the environmental pollution by herbicides, the biotic interaction is required for effective and

sustainable management of weed infestation and which will be a boon to sorghum and sugarcane growing farming community of northern Karnataka wherein devastating losses of yield due to *Striga* infestations are recorded in recent times. In this regard, an attempt was made to use the beneficial microbial community for the control of *Striga* weed in the sugarcane crop.

The germination stimulant for *Striga* seeds is a chemical exuded by the host roots known as strigolactones (SLs). However, the same chemical SL functions as a signal for recruitment of AM fungi in the host roots in P-deficient soils and is also known to induce hyphal branching.

Several species of mycorrhizal fungi have also been shown to increase plant biomass and compensate for damage by *S. asiatica* and their metabolites either stimulate or inhibit weed germination in sugarcane variety CO86032. Recent studies have shown that AM fungal colonization is likely to induce resistance to plant parasitism by converting strigolactones into mycorradicin, which is accumulated in mycorrhized roots and thereby reducing availability of strigolactones for *Striga* to germinate. The previous reports (Jones *et al.* 2014 and Sagarkar *et al.* 2017) also demonstrated the effectiveness of AM fungi against *Striga* emergence in sugarcane and sorghum. The present study envisaged the useful biotic interaction of AM fungi with plant roots for effective and sustainable integrated management of *S. asiatica* infestation for the resource-poor farming situations.

MATERIALS AND METHODS

Soil samples were collected from *Striga* infested site located at 16.01.01.03 N latitude; 074.58.1903 E longitude, and at an altitude of 643 m above mean sea level; and *Striga* suppressive sites located at 16.01.00.93 N latitude; 074.58.18.63 E longitude, and at an altitude of 642 m above mean sea level at Yergatti village of Belgaum district. The soil samples recovered from *Striga* infested soil was used to carry out the pot experiment while native AMF isolates were isolated from *Striga* suppressive soils. All the experiments were conducted at weed control scheme, MARS and Department of Agricultural Microbiology, University of Agricultural Sciences, Dharwad during the year 2014-15. There were nineteen treatments with five replications. The treatment details are as follows: UASDAMF1 to UASDAMF16; consortium AMF (standard) containing *Glomus macrocarpum, Gigaspora margarita, Acaulospora laevis;* consortium AMF (native) containing all 16 native isolates; and uninoculated control. AM spores were identified as described by Rodrigues and Muthukumar (2009). The pots were filled with *Striga* infested soil prior to the planting equal sized sugarcane sets (CO-86032). AMF inoculum at 150 g/pot was mixed thoroughly with the top 10 to 15 cm of the soil. The data were subjected to analysis following Completely Randomized Design (CRD) as defined by Gomez and Gomez (1984)

The number of *Striga* emerged was recorded in each pot. The shoot and root portions of uprooted *Striga* plants were separated and oven dried at 60°C to constant weight. The dry weights (n=5) were then recorded separately for shoots and roots.

The sugarcane leaf chlorophyll content was determined using a single photoelectric analyzing diode (SPAD) meter (SPAD-502 KONICA-Japan).

Measurement of photosynthetic rate, stomatal conductance, rate of transpiration and leaf temperature were made on the top fully expanded leaf of sugarcane at different locations by using a portable photosynthesis system (LI-6400 LICOR, Nebraska, Lincoln USA). The chlamydospores in rhizosphere of sugarcane were determined by wet sieving and decantation method as outlined by Gerdemann and Nicholson (1963). Spores counts were taken under a stereo zoom microscope.

Mycorrhizal root colonization was determined as per the procedure proposed by Philips and Hayman (1970). The percentage of roots colonized by mycorrhizae was calculated by the formula

$$\% \text{ root colonization} = \frac{\text{Root bits positive for colonization}}{\text{Total number of root bits}} \times 100$$

RESULTS AND DISCUSSION

The structural and morphological features of native AMF spores is as outlined in **Table 1** and microphotographs shown in **Figure 1**. *Glomus* was the predominant genus followed by *Acaulospora*.

No emergence of *Striga* was recorded in treatments received AMF consortium (STD), AMF consortium (native) and native AMF isolates UASDAMF2, UASDAMF5, UASDAMF9 and UASDAMF12. The shoot and root dry weight of *Striga* was found to be higher in UIC compared to all other isolates (18.02 and 2.60 g/plant respectively) at 120 DAP (**Table 2**). 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

Table 1. Identification of native AM fungal morpho-types from *Striga* suppressive soils

Isolate code no.	Shape	Colour	Spore mean size (µm)	Spore wall size mean (µm)	Spore surface	Size of hyphae mean (µm)	Species
UASD AMF1	Oval	Dark brown	104.83	5.60	Smooth	9.1	*Glomus ambisporum*
UASD AMF2	Oval	Dark brown	96.62	10.50	Smooth	12.3	*Glomus etunicatum*
UASD AMF3	Ellipsoid	Brown	137.60	11.90	Rough	-	*Glomus mossae*
UASD AMF4	Oval	Light yellow	104.60	9.40	Smooth	63.6	*Glomus spp.*
UASD AMF5	Oval	Light yellow	120.80	5.52	Smooth	71.8	*Acaulospora maarowe*
UASD AMF6	Oval	Brown	165.52	10.8	Granular	47.8	*Glomus deserticola*
UASD AMF7	Oval	Yellow	179.32	10.60	Laminated	53.3	*Glomus phansihalos*
UASD AMF8	Oval	Yellow	118.9	13.00	Smooth	-	*Acaulospora spinosa*
UASD AMF9	Round	Light brown	107.8	7.60	Smooth	-	*Glomus leptotichum*
UASD AMF10	Oval	Brown	78.58	7.90	Smooth	12.6	*Glomus aggregatum*
UASD AMF11	Ellipsoid	Dark yellow	149.56	12.75	Granular	-	*Glomus lacteum*
UASD AMF12	Oval	Dark yellow	73.7	11.9	Granular	51.4	*Glomus fasciculatm*
UASD AMF13	Ellipsoid	Yellow	111.27	8.90	Smooth	-	*Glomus radiata*
UASD AMF14	Oval	Brown	105.72	7.50	Rough	-	*Glomus retuculatum*
UASD AMF15	Oval	Dark yellow	121.65	5.40	Smooth	-	*Acaulospora bisporus*
UASD AMF16	Oval	Brown	131.45	10.02	Granular	-	*Acaulospora lacunosa*

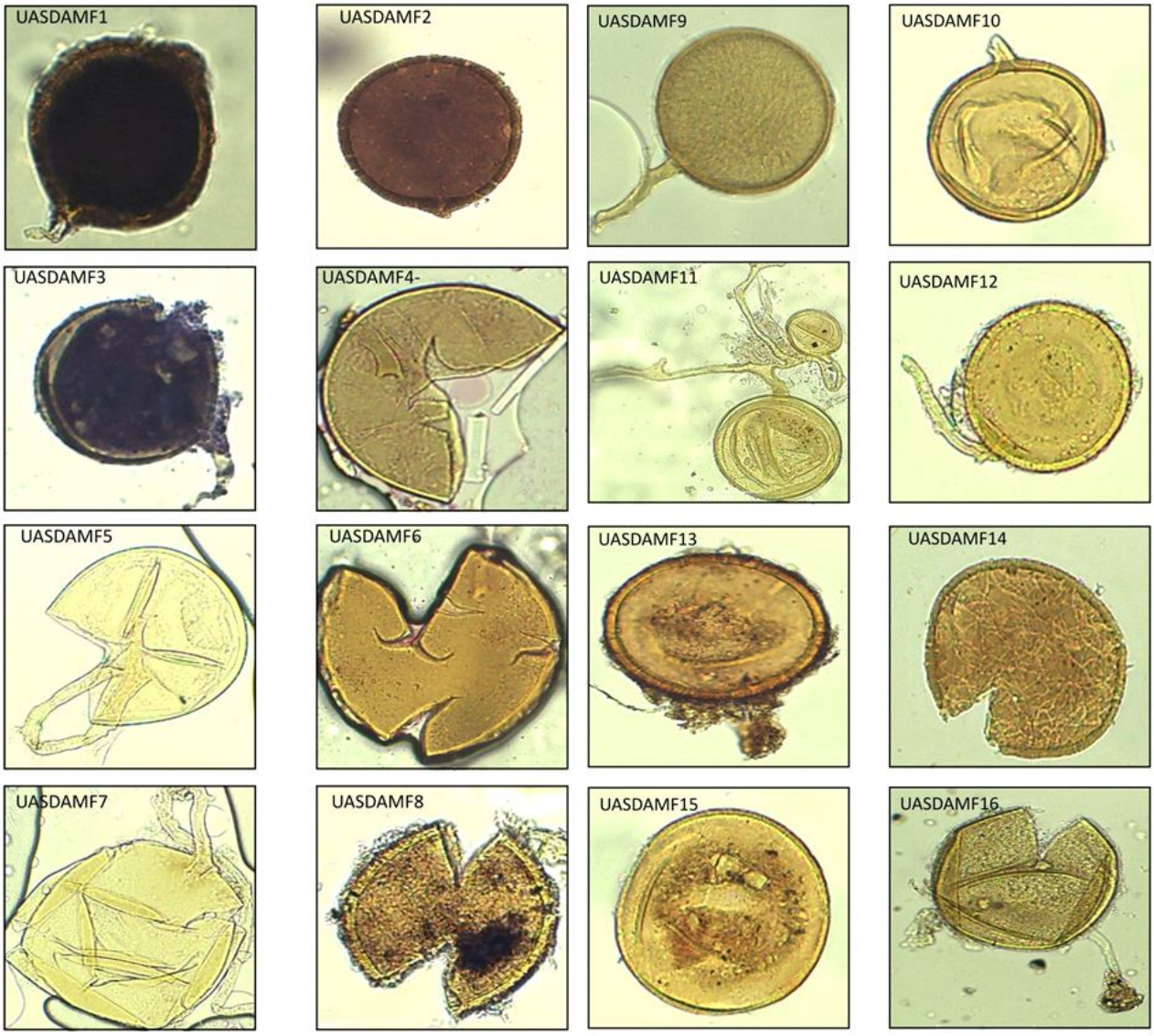

Figure 1. Microphotographs of chlamydospores of native AM fungi

Table 2. Influence of AM fungal isolates on *Striga* parameters

Treatment	No. of *Striga* per pot	Shoot dry matter (g)	Root dry matter (g)	Total dry matter (g)
UASD AMF1	40	12.2	1.53	13.9
UASD AMF2	0	0	0	0
UASD AMF3	41	13.5	1.71	15.2
UASD AMF4	39	15.2	1.65	16.8
UASD AMF5	0	0	0	0
UASD AMF6	42	13.2	1.53	14.7
UASD AMF7	42	12.2	1.62	14.0
UASD AMF8	43	13.5	1.50	15.0
UASD AMF9	0	0	0	0
UASD AMF10	40	14.7	1.53	16.2
UASD AMF11	44	15.9	1.71	17.3
UASD AMF12	0	0	0	0
UASD AMF13	47	15.3	1.92	17.5
UASD AMF14	41	11.5	1.41	12.9
UASD AMF15	41	13.4	1.54	14.9
UASD AMF16	45	17.6	1.72	19.1
AMF consortium (STD)	0	0	0	0
AMF consortium (native)	0	0	0	0
UIC	51	18.0	2.6	20.6
LSD (p=0.05)		0.62	0.07	0.65

Table 3. Sugarcane chlorophyll content as influenced by native AM fungal isolates in *Striga* infested soil

Treatment	Chlorophyll content of sugarcane plants			
	30 DAP	60 DAP	90 DAP	120 DAP
UASD AMF1	34.9	42.2	39.0	37.7
UASD AMF2	40.0	44.1	42.1	40.5
UASD AMF3	35.5	40.7	35.4	34.1
UASD AMF4	34.9	40.5	34.8	35.2
UASD AMF5	42.9	45.9	43.1	41.8
UASD AMF6	35.5	39.6	37.6	40.7
UASD AMF7	34.9	37.6	35.2	40.5
UASD AMF8	35.4	41.5	40.8	39.8
UASD AMF9	43.1	46.0	43.3	42.7
UASD AMF10	36.4	40.4	35.9	35.5
UASD AMF11	34.1	40.3	35.1	34.5
UASD AMF12	41.0	44.9	42.9	41.8
UASD AMF13	32.7	36.9	34.4	34.1
UASD AMF14	35.4	41.0	35.5	34.6
UASD AMF15	33.9	38.1	36.9	37.3
UASD AMF16	32.6	36.1	33.9	32.5
AMF consortium (STD)	46.7	49.8	46.3	45.8
AMF consortium (native)	43.4	46.5	43.4	42.7
UIC	29.8	35.4	32.0	25.3
LSD (p=0.05)	1.34	2.34	2.29	2.30

plants. Interestingly, AM fungi and parasitic weeds respond to strigolactones for their germination. Mycorrhizal colonization induces mycorrhizosphere effects that negatively impact on *Striga* germination (Lendzemo *et al*. 2007).When plants are subjected to a shortage in the available phosphate the production and release of strigolactones into the rhizosphere are increased. AM fungi perceive this signal and respond with extensive hyphal branching. This process increases the chance of encountering the roots of the host plant and hence assists in establishing the symbiosis. The *Striga*, *Orobanche* and *Phelipanche* spp. have likely evolved a mechanism to hijack this communication signal and turn it into a germination inducing signal to respond in the presence of a suitable host.

Field experiments have shown that AM symbiosis delayed the emergence and reduced the number of *Striga* parasites on sorghum (Lendzemo *et al*. 2007). In tomato, the decrease in parasitism by *Phelipanche ramosa* upon AM colonization also correlated with a lower induction of germination of seeds of this parasite by the root exudates. Subsequent LC-MS analysis showed that the root exudates of colonized plants indeed contained lower amounts of strigolactones (Lopez-Raez *et al*. 2011). These results suggest that AM fungal colonization likely induces resistance to plant parasitism by reducing the exudation of strigolactones.

The AMF consortium (STD), AMF consortium (native), and single native AMF isolates UASDAMF9, UASDAMF5, UASDAMF12 and UASDAMF2 significantly improved the chlorophyll content compared to the uninoculated plants as outlined in **Table 3**. AMF induced increase in chlorophyll content was also observed by Franco and Garza (2006).

Table 4. The influence of AMF on physiological parameters of sugarcane plants

Treatment	PR (µmol/ m^2/ sec)	SC (µmol/ m^2/ sec)	TR (µmol/ m^2/sec)	LT (C^o)
UASD AMF1	14.54	0.51	4.99	29.5
UASD AMF2	16.75	0.52	4.51	29.3
UASD AMF3	14.02	0.51	4.74	30.1
UASD AMF4	15.35	0.50	5.53	29.9
UASD AMF5	17.29	0.53	4.46	29.1
UASD AMF6	15.36	0.51	4.54	29.3
UASD AMF7	15.58	0.49	4.94	29.9
UASD AMF8	14.21	0.47	5.28	29.3
UASD AMF9	17.91	0.54	4.40	28.3
UASD AMF10	14.57	0.45	4.50	30.2
UASD AMF11	15.20	0.48	4.80	29.9
UASD AMF12	17.10	0.52	4.50	29.3
UASD AMF13	14.46	0.50	5.26	30.7
UASD AMF14	14.15	0.50	4.86	31.1
UASD AMF15	16.35	0.49	5.12	28.7
UASD AMF16	15.15	0.47	5.40	29.0
AMF consortium (STD)	18.91	0.58	4.16	28.0
AMF consortium (native)	18.16	0.55	4.36	28.0
UIC	13.59	0.45	5.54	31.7
LSD (p=0.05)	0.99	0.04	0.39	2.44

PR = Photosynthestic rate; SC = Stomatal conductance; TR = Transpiration rate; LT = Leaf temperature

Table 5. Mycorrhizal spore count in sugarcane rhizosphere and Mycorrhizal root colonization as influenced by AM fungal isolates

Treatment	Mycorrhizal spore count (no. of spores/50 g)				Per cent root colonization (%)
	30 DAP	60 DAP	90 DAP	120 DAP	
UASD AMF1	156.5	210.0	281.5	540.0	51.0
UASD AMF2	220.5	271.5	310.5	615.0	66.0
UASD AMF3	162.5	220.5	273.5	564.5	47.0
UASD AMF4	183.0	227.5	288.5	594.0	51.5
UASD AMF5	230.0	282.5	366.5	625.5	67.5
UASD AMF6	193.5	238.0	283.5	572.0	47.5
UASD AMF7	219.5	233.0	271.5	584.5	53.5
UASD AMF8	164.5	195.0	268.0	528.5	52.0
UASD AMF9	238.5	292.0	373.0	641.5	68.0
UASD AMF10	150.5	209.0	288.5	592.5	58.0
UASD AMF11	154.0	194.0	267.5	527.0	51.0
UASD AMF12	226.0	277.5	349.5	623.5	67.5
UASD AMF13	141.5	191.5	254.0	497.5	50.5
UASD AMF14	155.0	199.0	274.5	556.0	51.0
UASD AMF15	158.5	206.5	285.0	551.5	52.5
UASD AMF16	139.5	191.5	234.0	454.5	40.5
AMF consortium (STD)	255.0	305.0	384.5	687.5	72.5
AMF consortium (native)	227.0	300.0	379.0	668.0	70.0
UIC	137.0	146.5	167.0	211.0	25.5
LSD (p=0.05)	17.18	18.81	13.24	30.33	3.64

The physiological parameters such as photosynthetic rate and stomatal conductivity of sugarcane in the present study showed maximum values where AMF was inoculated in comparison with UIC (**Table 4**); this is in agreement with the reports of Selvaraj and Chellapan (2006), who also reported an increased photosynthetic activity in the leaves of *Prosopis julifera* inoculated with *G. fasciculatum*.

The highest mycorrhizal spore load was recorded with AMF consortium (STD) (687.5/50 g soil) followed by AMF consortium (native) (668/50 g soil) at 30, 60, 90 and 120 DAP. Least number of spore load was recorded with non mycorrhized sugarcane plants, UIC (211/50 g soil). Percentage root colonization by native AMF in the presence of *Striga* is given in **Table 5**.

Devika *et al*. (2013) reported AM fungal colonization in the roots of sugarcane may be due to fungal preference by the host and due to the factors influencing the mycotrophy of sugarcane. AM fungi can colonize many host plants. But it has a preferred host which exhibits maximum symbiotic response when colonized by that particular AM fungal species.

Thus, the native AM fungal species isolated in the present study could form an efficient and inexpensive *Striga* control agent, which should be integrated with other *Striga* management strategies to help sugarcane farmers of north Karnataka.

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REFERENCES

Ahonsi MO, Berker DK, Emechebe AM and Lagoke STO. 2002. Selection of rhizobacterial strains for suppression of *Striga hermonthica* (Del.). Benth. *Biological Control* **24**: 143–152.

Devika V, Prabudoss V and Sangeetha D. 2013. Influence of *Glucano acetobacter diazotrophicus* on the root colonization of *Glomus fasciculatum* and growth of sugarcane. *International Journal of Pharmaceutical and Biological Archives* **4**(6): 1019–1027.

Gerdemann JW and Nicholson TH. 1963. Spores of mycorrhizal endogone species extracted from soil by wet sieving and decanting. *Transactions of the British Mycological Society* **46**: 235–244.

Gomez KA and Gomez AA. 1984. *Statistical Procedures for Agricultural Research*, 2nd Edition, A Wiley- International Sciences Publisher, New York (USA), pp.125-130.

Franco DA and Garza CI. 2006. Arbuscular mycorrhizal colonization and growth of buffel grass (*Cenchrus ciliaris)* genotypes. *Revista Fitotecnia Mexicana* **29**: 203–206.

Jones PN, Meti N, Sagarkar MA, Ramesh Babu and Jagadeesh KS. 2015. Effectiveness of arbuscular mycorrhizal fungi against *Striga* emergence in sugarcane and sorghum. p. 133. *In. Proceedings Volume II (Oral Papers) of 25th Asian-Pacific Weed Science Conference on "Weed Science for Sustainable Agriculture, Environment and Biodiversity"*.

Lendzemo VW, Kuyper TW, Matusova R, Bouwmeester HJ and Vanast A. 2007. Colonization by arbuscular mycorrhizal fungi of sorghum leads to reduced germination and subsequent attachment and emergence of *Striga hermonthica*. *Plant Signalling and Behaviour* **2**(1): 58–62.

Lopez-Raez JA, Charnikhova T, Fernandez I, Bouwmeester H and Pozo MJ. 2011. Arbuscular mycorrhizal symbiosis decreases strigolactones production in tomato. *Journal of Plant Physiology* **168**: 294–297.

Parker C and Riches CR. 1993. Parasitic Weeds of the World: Biology and Control. CAB International, *Wallingford*, UK.

Phillips JM and Hayman DS. 1970. Improved procedures for clearing and staining parasites and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society* **55**: 158–161.

Rank C, Rasmussen LS, Jensen SR, Pierce S, Press MC and Scholes JD. 2004. Cytotoxic constituents of *Alectra* and *Striga* species. *Weed Research* **44**(4): 265–270.

Rodrigues BF and Muthukumar T. 2009. *Arbucular Mycorrhizae of Goa*-A Manual of Identification Protocols. Published by Goa University,Goa-India. **1**: 37-38.

Sagarkar MA, Jones PN and Meti N. 2017. Striga (*Striga asiatica*), a parasitic weed inhibition by arbascular mycorrhizal fungi in sugarcane (*Saccharum officinarum*) *International Journal of Applied and Pure Science and Agriculture* **3**(3): 1–9.

Selvaraj T and Chellapan. 2006. Arbuscular mycorrhizae: a diverse personality. *Journal of Central European Agriculture* **7**(2): 58–65.