

ISOLATION AND SCREENING OF ARBUSCULAR
MYCORRHIZAL FUNGI FOR SUPPRESSION OF
STRIGA, A PARASITIC WEED IN SUGARCANE

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MYCORRHIZAL FUNGI FOR SUPPRESSION OF
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By
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CERTIFICATE

This is to certify that the thesis entitled "ISOLATION AND SCREENING OF ARBUSCULAR MYCORRHIZAL FUNGI FOR SUPPRESSION OF *STRIGA*, A PARASITIC WEED IN SUGARCANE" submitted by Mis. SHUBHA CHIMMALAGI, for the degree of MASTER OF SCIENCE (AGRICULTURE) in AGRICULTURAL MICROBIOLOGY, to the University of Agricultural Sciences, Dharwad is a record of research work done by her during the period of her study in this university under my guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles.

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1. INTRODUCTION

Sugarcane belongs to the family Poacea and the genus *Saccharum*, native to warm temperate to tropical regions of South Asia. 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. The sugarcane cultivation and sugar industry in India play a vital role towards socio-economic development in the rural areas by mobilizing rural resources and generating higher income and employment opportunities. About 7.5 per cent of the rural population, covering about 45 million sugarcane farmers, their dependents and a large number of agricultural labour are involved in sugarcane cultivation, harvesting and ancillary activities. 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 (84.07 t/h) compared to 2010-11 (93.76 t/ha). The reported cane yield losses is due to poor growth of sugarcane resulting from weed infestation and it is the main cause for quality depression in sugarcane.

As a thumb rule, first 1/4th -1/3rd of the growing period in many crops is considered as critical period. Likewise, in sugarcane the initial 120 days considered as a critical period of crop weed competition. 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 Bijapur.

The word *Striga* is derived from the Latin word, which means witch weed. *Striga* often referred to the word 'witch', presumably because plants diseased by *Striga* display a stunted growth and an overall drought-like phenotype. *Striga* species are annual and most of their life cycle occurs below ground. *Striga* is an obligate parasitic weed which attach themselves to the

roots of several field crops, thereby robbing them for nutrition and also causing various debilitating effects on the host. The two most important species are *S. asiatica* and *S. hermonthica* which parasitize particularly sugarcane, maize, sorghum, millets and upland rice. *Striga* produces numerous tiny seeds which remain viable in the soil for many years and do not germinate unless a suitable crop host grows nearer to them.

The control of *Striga* is difficult to achieve because of its high fecundity. In addition, seed germination is asynchronous. 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. In this context, it has been recently demonstrated that certain soil microorganisms like arbuscular mycorrhizal fungi (AMF) can inhibit or suppress *Striga* germination (Berner *et al.*, 1995 and Jones *et al.*, 2014)

Strigolactones are secondary metabolites, which are exuded into the rhizosphere by the roots of their host plants. They are important in stimulating the seeds of the parasitic weed *Striga*. However, it was unclear why plants would produce these compounds considering that they promote plant parasitism. The solution to this dilemma came when strigolactones were reported to induce hyphal branching of AM fungi. The root parasitic weeds took advantage of the cues that are released by plants to attract symbiotic arbuscular mycorrhizal fungi, and developed the ability to detect the adjacent and living hosts roots by sensing the strigolactones (Akiyama *et al.*, 2006).

AM fungi establish a symbiotic interaction with the vast majority of land plants. In the symbiosis, the fungus supplies water and nutrients, which it can obtain very efficiently from the soil to plant. In exchange, the plant provides photo assimilates to the fungus. Phosphate is one of the most important elements in this symbiotic interaction. AM fungi extend their hyphae far beyond the root rhizosphere zone and explore the soil with a fine network of mycelium that can absorb not only inorganic but also organic forms of phosphate. 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 parasitic *Striga* has 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 (Lendzemo *et al.*, 2009).

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 reduces availability of strigolactones for *Striga* to germinate.

Hence, in this regard an approach has been made to study the effect of native as well as standard AMF isolates maintained at UAS, Dharwad on the emergence and colonization of *Striga* in sugarcane for the first time with the following objectives:

1. Isolation and morphological characterization of native AM fungal isolates from the *Striga* suppressive soils of sugarcane growing areas.
2. To screen these native AM fungal isolates for their ability to suppress *Striga* under pot culture studies.
3. To assess the plant growth promotional abilities of these isolates in presence of *Striga*.

2. REVIEW OF LITERATURE

Striga is an obligate parasitic weed which attaches themselves to the roots of several field crops like, sorghum, upland rice, sugarcane and maize, thereby robbing them for nutrition and also causing various debilitating effects on the host. Strigolactones are important in stimulating the seeds of the parasitic *Striga*. When crop plants are subjected to a shortage in the available P, the production and release of strigolactones into the rhizosphere are increased. AM fungi perceive this signal and respond with extensive hyphal branching. AM fungal colonization likely induce resistance to plant parasitism by converting strigolactones into mycorradicin, which is accumulated in mycorrhized roots and there by reduced availability of strigolactones for *Striga* to germinate. With this back ground, the literature pertaining to isolation and screening of arbuscular mychorrhizal fungi for the control of *Striga*, a parasitic weed in sugarcane were reviewed in this chapter

2.1 Isolation and morphological characterization of native AM fungal isolates from the *Striga* suppressive soils of sugarcane growing areas

2.1.1 Arbuscular mycorrhiza

The term 'mycorrhiza' (Greek *mykes*, fungus + *rhiza*, root) implies an association of fungi and plant roots. However, the mycorrhizal associations, recognized as nutrient absorptive formations in soil, as well as in without root system belonging to bryophyte and pteridophytes (Smith and Read, 1997).

It is accepted widely that mycorrhiza, not plant root, is the principal organ of nutrient uptake from the soil (Smith and Read 1997). The important features of the AM fungi are their presumed asexuality, production of large soil-borne spores harboring several nuclei and the multinucleate mycelium without true septa (Smith and Read, 1997). The symbiotic association itself is ancient, the first spore fossils originating from the Ordovician (Redecker *et al.*, 2000). It has been hypothesized that the first land plants, with no roots but with protostelic rhizomes, were arbuscular mycorrhizal (Pirozynski and Malloch, 1975). Based on molecular, morphological, and biochemical data, the AM fungal species are placed in four orders i.e. *Archaeosporales*, *diverseosporales*, *Glomerales* and *Paraglomerales*

which compose of 13 families and 19 genera of fungi (Schüßler *et al.* 2001). The number of AM fungal species has been suspected to be much larger than 150, based on selectivity between fungal and plant species (Helgason *et al.*, 2002).

2.1.2 Soil trap cultures

Stutz and Morton (1996) Studied AMF species diversity rely on the extraction and identification of fungal spores because they are quantifiable and identifiable to a species level (Morton *et al.*, 1995). AMF diversity based on soil samples alone may be underestimated for two reasons: spores extracted from soil are often impossible to identify due to parasitism and another reason is all AMF species may be sporulating at the time the soil sample is collected. The establishment of successive trap cultures have been successful in detecting non-sporulating AMF and can provide more on complete representation of species present in the soil. In order to stimulate sporulation and determine if additional AMF species could be detected from each soil sample, two generations of successive trap cultures were established in the greenhouse following a modification of the protocol used by Stutz and Morton (1996).

Spores extracted directly from the rhizosphere soil can be used for the identification. However, identification of the AM fungi is found to be better after trap pot culturing as not all AM fungal species sporulate at the time soil sampling, resulting in reduced spore number in the soil. Furthermore, spores from trap culturing are in better state i.e., higher viable percentage of the spores (Patrícia *et al.*, 2009).

2.1.3 Spore extraction and identification

Daniel and Skipper (1982) extracted spores from each soil samples of successive generation of trap culture by wet sieving and sucrose density gradient centrifugation.

Identification to a species level was made based on characteristics of spore cell walls and comparison to voucher specimens. Species richness was determined as a count of the different AMF species detected at each site. Classical techniques

for AMF identification are based on spore morphology. This approach is time consuming and difficult to master. Spore characteristics, especially spore wall structure, morphology, and ontological changes are the most important criteria for AMF identification (Morton and Bentivenga, 1994). An illustrated hard copy (Schenck and Perez, 1990) and online electronic taxonomic guides/keys (<http://invam.caf.wvu.edu/>) explain and apply the systematics. Approximately 150 species of AMF are described to date. Undoubtedly, majority of the AMF species remain undescribed (Bever *et al.*, 2001). For example, the International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi (INVAM) maintain approximately 40 isolates that do not belong to any of the currently described species. In field samples, low spore number, parasitization of spores, age and environmental alteration of spores (e.g., discoloration) are known hinder accurate identification (Bever *et al.*, 2001).

The development of molecular biology methods has allowed the circumvention of many of the above limits, first by applying isozyme (Rosendahl and Sen 1992), immunology methods (Sanders *et al.*, 1992), and nucleic acids-based approaches (Clapp *et al.* 2001). Amplification of AM fungal genes from environmental samples with the aid of primers of different specificities now allows fairly accurate detection and identification of AM fungi both in the soil and in plant roots.

Starting with the work of Simon *et al.* (1992), different sets of primers, mostly amplifying nuclear ribosomal genes of AM fungi, have been developed. For research into the natural communities of AM fungi, primers amplifying all AM fungi, but excluding other fungi and plant hosts, are desirable. Fitting these requirements the best is the primer pair NS31 coupled with AM1 (Simon *et al.*, 1992; Helgason *et al.*, 1998), designed to amplify (AM) the fungal and exclude the plant small subunit nuclear ribosomal rRNA gene (SSU rDNA). The AM1 primer was later shown to exclude the deeply branching Archaeosporaceae and Paraglomaceae families (Daniell *et al.*, 2006), but attempts to develop better AM fungi-specific primers have not been successful. However, this primer pair and the SSU region are the most widely used targets in AM fungal community studies. A range of group specific primers is available (Geue and Hock, 2004).

Other nuclear ribosomal DNA regions have been used in AM research, including the internal transcribed spacer (ITS) flanking the

5.8S gene, and the large ribosomal subunit (LSU) gene. The ITS region is considered to be an appropriate tool for studying AMF at similar taxonomic level. AM fungi possess divergent copies of the ITS region within an individual spore (Jansa *et al.*, 2002 and Jones *et al.*, 2010). Species-specific primers of the LSU region have been useful for detection of AM fungi (Turnau *et al.*, 2001).

Amplification of AM fungal genes from environmental samples with the goal of detection or identification, if using general primers, needs to be followed by an amplicon separation and/or screening step, because the amplicon consists of amplified gene fragments of multiple co-existing organisms. The separation can be achieved via electrophoresis or cloning of PCR products. Cloning followed by clone screening with restriction enzymes and sequencing of representatives of RFLP groupings has been widely used in natural AM fungal community studies (Husband *et al.* 2002a; Vandenkoornhuyse *et al.*, 2002).

DGGE is widely used in environmental microbiology for microbial community monitoring and is useful for screening of numerous samples. The method was applied in this study by Kowalchuk *et al.* (2002) to describe natural root-inhabiting AM fungal communities of *Pulsatilla* spp. and *Ammophila arenaria*, respectively. The second, Single Strand Conformation Polymorphism (SSCP), is a sensitive and invaluable method for distinction of DNA fragments differing only by a few base pairs, and therefore has been applied as a pre-sequencing screen of samples in extensive population studies of AM fungi (Jansa *et al.*, 2002a and Jansa *et al.*, 2003).

Jones *et al.* (2012) explored the diversity of AM fungi and revealed that, a total of fifty nine species of AM fungi were identified from four ecological zones of northern Karnataka, among them twenty one species of AMF were recorded in the western ghats and followed by northern dry zones (19 species). In North eastern dry zone, a total number of ten species were identified. The lowest composition of AM species was recorded in transitional zones (9 species). The diversity analysis have indicated that the Shannon-weiner diversity index for AM fungi was higher in

western ghats followed by northern dry zone; Transitional zone and North eastern dry zone recorded poorer with respect to AMF diversity. The Margalef's index of species richness for AMF was highest in western ghats and lowest species richness was recorded in northern dry zone. The jaccards similarity index between the four ecological zones revealed that northern dry zone and northeastern dry zone showed maximum similarity. While, the AMF diversity was least similar between Western Ghats and Northeastern dry zone.

Abdullahi *et al.* (2014) conducted an experiment to study the suitable host plant for mass production of indigenous arbuscular mycorrhizal fungi. Lemongrass and onion were compared for mass multiplication of *Glomus sp.* *Glomus mossea*, *Glomus geosporum* and *Glomus etunicatum*. *Glomus mossea* recorded the highest spore number and root colonization followed by *Glomus geosporum* in both plant species.

Manimegalai *et al.* (2011) studied the variability in natural infection rates, spore density and species richness of AM fungi associated with medicinal plant *Solanum viarum*, which was culture from herbal garden at Thanjavur. The efficient of AM fungi and their mass production of *Sorghum bicolor* roots by pot culturing techniques were assessed. The physico-chemical characteristics of the soil at the study site were, analysed. The observations recorded on the influence on growth were also described.

Reena and Alok (2013) studied the AMF diversity and habitat relationships, they characterized and enumerated spores in 55 fields of wheat from 11 agro-climatic regions of India, varying in climatic and edaphic characteristics. The AM fungal spore count, species richness, most frequent species, and intra-radical colonization were studied in various samples drawn from these regions. A total of 165 samples were collected at the time of the wheat harvest. These samples were used as trap cultures and multiplied in a greenhouse for a period of one year, which yielded 34 species scattered over 6 genera. The genera *Glomus* Tulasne and Tulasne occurred most frequently, constituting 89.1 per cent of the total species. The number of species in a given region ranged from 1-9. *Glomus albidum* Walker and Rhodes and *G. macrocarpum* Tulasne and Tulasne were found to be the most common occurring species.

Sarah *et al.* (2014) reported that the AMF are known to facilitate the growth and vigour of many plants, particularly in arid ecosystems. In a survey of AMF in a date palm plantation at two natural sites of a desert in Oman, they generated many single spore derived cultures of AMF. They identified these number of isolates based on their spore morphology. They presented the four important native AMF species which are boon for conservational and sustainable agriculture.

2.2 Screening of native AM fungal isolates for their ability to suppress *Striga* under pot culture studies

Lenzemo *et al.* (2001) studied the interaction between arbuscular mycorrhizal (AM) fungi and the root hemi-parasite *Striga hermonthica* in sorghum and revealed that in the absence of AM fungi, *S. hermonthica* reduced biomass of sorghum, while Arbuscular mycorrhizal fungi cancelled out damage by *S. hermonthica*.

Nuhu *et al.* (2003) screened five *Glomus sp* i.e., *G. intraradices*, *G. albidum*, *G. mosseae*, *G. fasciculatum*, and *G. etunicatum* against *Striga* under controlled conditions. The results of the experiment revealed that *G. mosseae* significantly reduced the number of *Striga* emerged per plant, while increased the plant growth parameters, total dry matter and yield of sorghum compared to *Striga* alone.

Vierheilig *et al.* (2004) reviewed the regulatory mechanisms during the interaction between host plants and AM fungi and noted that AM plants show auto regulation, i.e., plants that are colonized by an AM fungus suppress subsequent colonization by AM fungi through altered root exudation and also repress soil pathogens that are attracted to the root by the same compounds in the exudates.

Lenzemo *et al.* (2005) investigated the impact of field inoculation of AMF on the performance of parasite *Striga hermonthica* in cereals and observed a significant reduction in the emergence of *Striga hermonthica* as well as the *Striga* shoot biomass with AMF inoculation.

Arnaud *et al.* (2006) reported that the strigolactones stimulates AM fungi by activating mitochondria as well as they are known to stimulate the parasitic plants *Striga* and *Orabanche*. They showed that sorghum also contains strigolactones.

Strigolactones strongly and rapidly stimulated cell proliferation of the AM fungus *Gigaspora rosea* at concentration as low as 10^{-13} M. This effect was not found with other sesquiterpene lactones known as germination stimulants of parasitic weeds. Within 1 hr. of treatment, the density of mitochondria in the fungal cells increased, and their shape and movement changed dramatically. Strigolactones stimulated spore germination of two other phylogenetically distant AM fungi *Glomus intraradices* and *Glomus claroideum*. This was also associated with a rapid increase of mitochondrial density and respiration as shown with *Glomus intraradices*. They conclude that strigolactones are important rhizospheric plant signals involved in stimulating both the pre-symbiotic growth of AM fungi and the germination of parasitic plants.

Veniasius *et al.* (2007) studied the effect of AM fungi on two sorghum cultivars *viz.*, S35 (*Striga*-tolerant) and CK60B (*Striga*-sensitive) and concluded that root exudates from AM sorghum plants induced lower germination of *S. hermonthica* seeds than exudates from non-mycorrhizal sorghum. Furthermore, they have also recorded lower number of *S. hermonthica* seedlings attached on both sorghum cultivars in mycorrhizal than in non-mycorrhizal plants. However this reduction was more pronounced with S35 than with CK60B plants.

Arnaud *et al.* (2009) reported that natural strigolactones or the synthetic strigolactone analogue GR24 elicited hyphal branching of AMF there by reducing the *Striga* parameters in cereals.

Lenzemo *et al.* (2009) observed that root colonization by arbuscular mycorrhizal fungi reduced stimulation of seed germination of the plant parasite *Striga* resulting in a lower parasite incidence. They studied influence of AM fungi in root colonization and the seed germination activity of the *Striga hermonthica* in nonhost plants like cowpea, cotton and *Striga gesnerioides* in host plant cowpea. They found that the Mycorrhization not only results in a lower seed germination of *S. hermonthica* and *S. gesnerioides* in host and nonhost plants and also better root colonization of AM fungi.

Catarina *et al.* (2011) studied the Strigolactones and root infestation by plant-parasitic *Striga*, Orobanche and revealed that strigolactones are the important

compounds for the stimulation of parasitic weeds as well as AMF. In presence of AMF the availability of strigolactones to parasitic weeds is very less and hence it helps in preventing weeds and enhance growth parameters in host plants.

The haustoria are the exclusive structures of all parasitic plants via which nutrients and water are absorbed from host plants, the fact that inoculation with some AM fungi significantly suppress formation of haustoria, and opens up the possibility of using these beneficial fungi in biocontrol of parasitic weeds. AM fungi have been found to reduce strigolactones production by host plants (Lopez *et al.*, 2011).

Walter *et al.* (2011) reported that an intermediate of the strigolactones biosynthetic pathway can also be converted into mycorradicin, an apocarotenoid that accumulates in mycorrhized roots. Possibly, these two biosynthetic pathways compete with each other, which could result in reduced strigolactone production upon AM colonization and resulted in less *Striga* emergence.

Ai-rong *et al.* (2012) reported that AMF suppress the initiation of haustorium in the root hemiparasite *Pedicularis tricolor*. They tested with two AM fungal species, *Glomus mosseae* and *Glomus intraradices*, on haustorium initiation in *P. tricolor* grown alone or with *Hordeum vulgare* "Fleet" (barley) as the host plant. Results revealed both AM fungal species dramatically suppressed intraspecific haustorium initiation in *P. tricolor* at a very low colonization level. The suppression over-rode inductive effects of the parasite's host plant on haustoria production and caused significant growth depression of *P. tricolor*. They conclude that AM fungi had strong and direct suppressive effects on haustorium formation in the root hemiparasite. The significant role of AM fungi in haustorium initiation of parasitic plants was demonstrated for the first time. This study provides new clues for the regulation of haustorium formation and a route to development of new biocontrol strategies in management of parasitic weeds.

Jones *et al.* (2014) evaluated arbuscular mycorrhizal fungi for suppression of *Striga* in sorghum crop and revealed that the application of *Glomus macrocarpum* and *Sclerocystis dussi* not only suppressed the *Striga* emergence and also increased

the root colonization and spore load per 50 g of soil compared to other AMF isolates and control (*Striga* alone) where highest *Striga* plants were emerged.

2.3 Plant growth promotional abilities of native AMF isolates in presence of *Striga*

Allen *et al.* (1982) identified the ability of *Glomus sp.* to significantly improve maize shoot dry matter and confirms previous reports on the ability of AMF to enhance growth of untreated and *Striga* infected plants through enhanced nutrient uptake and synthesis of plant growth promoting hormones.

Amelia and Cinta (1996) conducted an experiment on Isolation and screening of mycorrhizal fungi from citrus nurseries and orchards and revealed that the *Glomus intraradices* was most efficient AMF which enhanced the all growth parameters along with root colonization and soil enzyme activities.

Aditya *et al.* (2009) analysed the effect of two AM fungi (*Acalospora laevis* and *Glomus mosseae*) along with *Trichoderma viride*. Inoculation of AMF along with *T. viridae* significantly increased of the growth parameters after 45 and 90 days after inoculation. Among all treatments, dual combination of *A. laevis* plus *T. viridae* was most effective in increasing shoot length, leaf area, root length, root weight AM spore number and percent root colonization.

Jones *et al.* (2011) studied the influence of native AM fungi from four different ecological zones of northern Karnataka and their outstanding efficacy in P uptake and growth promoting potential in *Vitis vinifera* cv. *Cabernet sauvignon* poly house studies and response of grapevine to the native AM fungal strains viz., AMF G3 and AMF 528 recorded substantial increase in plant growth parameters, physiological parameters, total dry matter production, mycorrhizal parameters and soil enzyme activities.

Anju *et al.* (2013) conducted a pot experiment to test the influence of sugarcane bagasse as a substrate for the inoculum production of *Funneliformis mosseae* in terms of AM root colonization, spore number using onion as test plant. The results showed that the supplementation of compost bogasse promoted higher AM root colonization and sporulation, followed by dry and fresh bagasse. Maximum

AM spores, vesicles, arbuscules and 100 per cent colonized roots. This treatment also influenced significantly increase in plant growth.

Jones *et al.* (2014) evaluated the arbuscular mycorrhizal fungi for suppression of *Striga hermonthica* in sugarcane crop and revealed that the application of suppressed the *Striga* emergence to a greater extent than other AMF isolates and they significantly increased the plant growth parameters, soil enzyme activities, mycorrhizal parameters and nutrient content of sugarcane plants

2.3.1 Soil enzyme activity

Soil enzymatic activities are closely related to microbial activity or biomass as they catalyse biochemical reactions and nutrient cycling in the soils (Burns, 1982). They also integrate information on the one hand from microbial status, and on the other hand from soil physico-chemical conditions, thus being a candidate “sensors” of ecosystem stress, changing soil properties and soil degradation (Badiane *et al.*, 2001 and Vepsalainen *et al.*, 2001). Soil enzymes can represent mineralization rates of organic matter as well as creating a longer term legacy of decomposition processes (Frankenberger and Dick, 1983).

The major parameters of soils fertility are their biological properties. Among these, special emphases is given to the enzyme activity. Many researches established that the activity of soil enzymes can be an additional diagnostic index of soil fertility and its changes as a result of human activity (Evgenia and Dadenko, 2006).

Phosphatase and dehydrogenase are recognized as important soil enzymes. Measurement of dehydrogenase activity provides on index of catabolic activity of the soil. As microorganisms are important sources of soil enzymes, the measurement of activities of these enzymes have often been used as an index of microbial activity (Dadenko, 2005).

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).

Zhao *et al.* (2010) reported the effects of arbuscular mycorrhizae on microbial population and enzyme activity in replant soil used for watermelon production and In each replant soil, the inoculation with AM fungus *Glomus versiforme* enhanced soil enzyme activities along with bacteria and actinomycete population, and decreased the fungal numbers when compared with controls.

Dubey and Fuleka (2011) studied the influence of AMF application on soil enzymes and rhizosphere microflora, revealed that AMF application significantly increased the soil nitrogen, soil carbon, C: N ratio along with dehydrogenase and phosphatase activity, viable AMF count and rhizosphere micro flora.

2.3.2 Mycorrhizal parameters

Geeta *et al.* (2013) conducted an experiment to study the co inoculation effect of arbuscular mycorrhizal fungi (*Glomus fasciculatum*), *Azospirillum brasilense* and PSB on plant height, dry weight of root and shoot per cent root colonization, spore number, P and N uptake. Single inoculation of AM fungi and combined inoculation of AM fungi with *Azospirillum brasilense* or PSB was found to be have highest root colonization, spore load as well as growth parameters of sorghum plants.

Suresh *et al.* (2015) studied the diversity and distribution of AMF in the rhizosphere soil of sugarcane in different sites of Tamil Nadu. The results have indicated variation in AMF spore load, root colonization and number of AM fungal species in different sampling season. Higher spore population and number of AM

fungal species was recorded in the rainy season, minimum were recorded in the winter season and lowest were recorded in the summer season. Altogether twenty five different AM fungal species belonging to four genera viz., *Glomus*, *Gigaspora*, *Acaulospora* and *Scutellospora* were characterized. They showed variable distribution pattern. It was observed that *Glomus* was the most dominant genus in the rhizospheric soil of sugarcane. *Glomus aggregatum*, *Glomus mosseae*, and *G. fasciculatum* were identified in all the sampling season.

Devika *et al.* (2013) studied the occurrence of AM fungi in sugarcane rhizosphere. Thirteen location samples were collected from Vallampadugai recorded the highest root colonization percentage and spore number. Five different AM fungi were isolated and were identified as *Glomus mosseae*, *Glomus fasciculatum*, *Glomus vermiform*, *Acaulospora laevis*, and *Gigaspora margarita*. Among the five different AM fungal isolates, *Glomus fasciculatum* recorded highest root colonization percentage, spore number, acid and alkaline phosphatase enzyme activities.

2.3.3 Plant physiological parameters

It has been reported that species of AM fungi differ significantly in their ability to improve plant growth and other aspect of plant performance (Liu and Luo, 1988; Liu and Luo, 1989).

Mbogo and Osoro (1992) screened eighteen sugarcane clones for their field reaction to *Striga hermonthica* Benth. The effect of *Striga* on cane was first noted two months after planting and exhibited leaf burn, wilting, stunted growth and small pointed leaves, which contributed to poor canopy formation and tiller mortality. Some clones were significantly more productive in the presence of *Striga* and were rated as resistant.

Gworgwor *et al.* (2003) observed reduced *Striga hermonthica* emergence due to AM fungi inoculation (62%). There were resulted in about 30 per cent increase in total dry matter yield of sorghum over control, while the total loss in dry matter yield of sorghum due to *Striga hermonthica* infestation was 36 per cent.

Lendzemo *et al.* (2004) reported that the mutualistic symbiosis between AMF and crop plants results in increased uptake of phosphorus, potassium, nitrogen, and other nutrients, increased growth at high soils, more efficient water utilization, increased levels of cytokinins, and also increased photosynthetic rates and stomatal conductance.

Gogoi and Singh (2011) evaluated the potential for arbuscular mycorrhizal fungi (AMF) to influence the host species. Twenty three different AMF strains from nearby localities were evaluated for their symbiotic response with *Piper longum*. Saplings were raised in 10 cm sand-soil mix inoculated with AMF isolates. Almost all the AMF strains showed increase in plant growth, biomass and nutrient content over the control. While retarded growth response was observed with the inoculation of 6 different AMF species. Considering the shoot length, total biomass, nutrient content, chlorophyll content and root colonization. Among six native AMF species viz; *Glomus fasciculatum*, *G. versiforme*, *G. clarum*, *Glomus* sp, *G. mosseae* and *G. etunicatum* appeared to be the most promising AM fungi medicinal plant.

Murgan *et al.* (2011) attempted to know the combining ability of *G. diazotrophicus* with AM fungi on sorghum. The AM fungi *Glomus fasciculatum* in association with *G. diazotrophicus* were evaluated on the basis of root colonization and revealed that the combination of these two inoculum significantly increased plant N and P content, fresh and dry matter yield, soluble sugars and photosynthetic pigments in leaves of *Sorghum bicolor*. Fresh weight and dry weight was significantly higher in dual inoculated plants.

2.3.4 Chemical Analysis of plants

The association of plants with mycorrhizal fungi can have strong on P fertilization, due to the fact that these fungi are able to enhance P availability to and uptake by plants inoculated with mycorrhiza (Smith and Read, 1997).

Jamal *et al.* (2004) made an comparative study on the relationship between plant and leaf chemical elements of sugarcane plants with low and high per cent mycorrhization. Results from high per cent mycorrhization plants indicated enhanced relationships between soil pH and leaf Ca, soil pH and leaf N, soil Ca/Mg and leaf N, and soil Na. Further they have revealed that Ca exists in the middle

lamella as calcium-pectate, which helps with resistance to fungal infection. Nitrogen and Mg are required for photosynthesis, while Na may increase stomatal regulation under water limiting conditions. AMF colonisation therefore, may play an important role in plant physiology in terms of resistance to bacterial and fungal pathogens, increased photosynthetic rates and enhanced stomatal regulation under water stressed conditions.

Othira *et al.* (2012) investigated the effect of AMF on two maize varieties Nyamilambo (*Striga* –susceptible) and KSTP94 (*Striga*-tolerant). Three species of AMF *Glomus etunicatum*, *Scutellospora fulgida*, *Gigaspora margarita* and *Striga hermonthica* seeds were used in a greenhouse experiment. The results of the investigation revealed that the AMF reduced *Striga* plant incidence and biomass significantly lower levels in both the varieties compared to non mycorrhizal treatments of same variety. Mycorrhizal Nyamilambo and KSTP94 had higher nitrogen and phosphorus content compared to non mycorrhizal cultivars in presence and absence of *Striga* infestation. *Glomus spp.* had most significant positive effect compared to both *Gigaspora* and *Scutellospora spp.*

Lenin *et al.* (2010) made an effort to study the effects of arbuscular mycorrhizal fungi on the morphological and biochemical changes of four different vegetable seedlings grown under nursery condition. The symbiotic association between AM fungi and plant roots provides a significant contribution to plant nutritional growth. Hence, an attempt has been made to examine the effects of AM on the four vegetable crops such as Tomato (*Lycopersicum esculentum* L.), Brinjal (*Solanum melongena* L.), chill (*Capsicum annum* L.) and Bhendi (*Abelmoschus esculentus*). The maximum increase in four plant's morphological parameters like root length, shoot length, fresh weight, dry weight, number of leaves, total leaf area, and biochemical parameters like chlorophyll content, nutrient content of plants (Nitrogen, Phosphorus, Potassium) were found to be highest with AM fungi treated seedlings when compared to non-mycorrhizal seedlings.

3. MATERIAL AND METHODS

An investigation was carried out to isolate native AM fungal isolates from the *Striga* suppressive soils of sugarcane growing areas in order to screen them for their ability to suppress *Striga* as well as to promote plant growth under pot culture studies. These experiments were conducted at weed control scheme, MARS and Department of Agril. Microbiology, UAS, Dharwad. The materials used and the methods followed are described below.

3.1 Collection of soil samples

Soil samples were collected from *Striga* infested and *Striga* suppressive sites of at Yergatti village of Belgaum district (Plate 1). 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. The soil samples were analyzed for physicochemical properties of soil.

The details of the study area are given below:

Geographical details of the study area.			
	Altitude	position	East
<i>Striga</i> infested soil	643	N- 16.01.01.0"	074.58.190"
<i>Striga</i> suppressive soil	642	N- 16.01.00.9"	074.58.18.6"

Initial soil physico-chemical properties		
Properties	<i>Striga</i> Suppressive soil	<i>Striga</i> infested soil
Available nitrogen (kg/ha)	229	227
Available phosphorus (kg/ha)	34	33
pH	7.5	7.4

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Plate 1 Soil sampling in a sugarcane field infested with Striga (Yergatti village Belgaum district)

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The samples were taken after removing the top litter layer (2 cm) and digging out an appropriate amount of soil close to the sugarcane roots from a depth of 10cm. The soil samples were packed in polythene bags and were stored at 4–8°C to sustain the viability of arbuscular mycorrhizal spores.

3.1.2 Study of native AM fungal community by following trap pot culturing

Spores extracted directly from the rhizosphere soil can be used for the identification. However, identification of the AM fungi is found to be better after trap pot culturing (Plate 2) as not all AM fungal species sporulate at the time soil sampling, resulting in reduced spore number in the soil. Furthermore, spores from trap culturing are in better state *i.e.*, higher viable percentage of the spores (Patrícia *et al.*, 2009).

The test *Striga* suppressive soil sample at one location was mixed with equal amount of sterilized sand: soil mixture(1:1) and planted suitable host plant (mixture of sorghum and cow pea). The plants were watered twice in a week. Hogland nutrient solution was added bi-weekly. After 3 months, the potting mix was wet sieved and the spores were counted under a stereomicroscope and grouped according to their morphological characteristics.

3.2 Extraction of AM spores from the soil

3.2.1 Wet sieving and decanting method

AM fungal spore extraction was done as outlined by Gerdemann and Nicholson (1963) AM spores were isolated using wet sieving and decantation method. One hundred gram of soil was taken in one liter beaker, made up to 1000 ml with water, stirred well and allowed for heavier soil particles to settle down for a few seconds. Then the suspension was passed through a series of different sized sieves (500µm,250 µm, 106 µm, 75 µm, 45 µm,and 37 µm) arranged in the descending order of their mesh size. Again water was added up to 1000 ml, stirred well and allowed for few seconds. This was repeated for five to six times, till the suspension appeared clear. Sievings were collected from each sieve separately in beakers. The collected sievings was subjected to the density gradient centrifugation method.



Plate 2 AMF trap cultures using sorghum and cowpea as trap crops in *Striga* suppressive soil

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3.2.2 Sucrose density gradient centrifugation

Density gradient centrifugation was done as outlined by Ohms (1957).

Density gradient centrifugation method is a most commonly used technique for AM fungal spore extraction. Large or small quantities of soil can be processed rapidly with reasonable efficiency and very little debris remains with the spores. Soil sieving was added to centrifuge tubes and centrifuge (Eppendoff) at 2000 rpm for five min. The suspension floating on top was removed carefully and 50 per cent sucrose solution was added in the centrifuge tube containing sample and centrifuged at 2000 rpm for two minutes. The suspension was passed through 37 μm sieve and AMF spore samples were collected by washing with distilled water and filtered through Whatman No.1 filtered paper.

3.3 Funnel technique

Morphologically similar spores were picked and a single spore as representation of each morphotype (as distinguished by colour or size) was brought in to funnel technique (Plate 3) after surface sterilization of spore with 200 ppm streptomycin sulphate and 2% chloramine T. Sand and soil was mixed in 1:1 proportion and the isolated spore was placed inside the mixture and Ragi seeds were sown and maintained for 45 days. Hoagland's solution (Appendix-1) was applied at weekly intervals.

3.4 Spore identification

The single spore was extracted from the respective funnels and mounted on clean glass slides in lacto phenol. The spore identification was mainly based on morphological features, viz., color, size, wall structure and hyphal attachment as per the outline given by Schenk and Perez, (1990); Rodrigues and Muthukumar, (2009) and INVAM website <http://invam.caf.wvu.edu> by Joe Mortan. Finally the spores were photographed under Olympus research stereomicroscope connected to a computer with digital image analysis software.



Plate 3 Mass multiplication of single spores of native AMF isolates using funnel technique

3.5 Screening of AMF spores

Once roots start emerging from the stem-tips of the funnels, the contents were transferred to small plastic cups containing sterilized sand: soil (1:1) after confirming the roots for AM colonization (Plate 4). Cultures were maintained and spores were multiplied up to 45 days in cups and later on they were transferred to plastic pots for the further scaling up of AMF cultures and they were used in the pot experiment (Plate 5).

3.6 Treatments

Based on the characterization sixteen identified native AMF isolates were selected and screened for their ability to suppress *Striga*. The pots were filled with about fourteen kg's of *Striga* infested soil prior to the planting equal sized two sugarcane sets (CO-86032) collected from Yaragatti village of Saudatti. AMF inoculum @ 150 g pot⁻¹ (150 spores/10 g of soil) was mixed thoroughly with the top 10 to 15 cm of the soil. There were nineteen treatments with five replications.

3.6.1 Treatment Details

Treatments no.	Code no. for treatments	AMF species
T1	UASDAMF1	<i>Glomus ambisporum</i>
T2	UASDAMF2	<i>Glomus etunicatum</i>
T3	UASDAMF3	<i>Glomus mosseae</i>
T4	UASDAMF4	<i>Glomus spp</i>
T5	UASDAMF5	<i>Acaulospora maarowe</i>
T6	UASDAMF6	<i>Glomus deserticola</i>
T7	UASDAMF7	<i>Glomus phansihalos</i>
T8	UASDAMF8	<i>Acaulospora spinosa</i>
T9	UASDAMF9	<i>Glomus leptotichum</i>
T10	UASDAMF10	<i>Glomus aggregatum</i>
T11	UASDAMF11	<i>Glomus lacteum</i>
T12	UASDAMF12	<i>Glomus fasciculatum</i>
T13	UASDAMF13	<i>Glomus radiata</i>
T14	UASDAMF14	<i>Glomus reticulatum</i>
T15	UASDAMF15	<i>Acaulospora bisporus</i>
T16	UASDAMF16	<i>Acaulospora lacunosa</i>
T17	Consortium AMF (STD)	<i>Glomus macrocarpum</i> , <i>Gigaspora margarita</i> <i>Acaulospora laevis</i>
T18	Consortium AMF (native)	Native isolates from 1 to 16.
T19	<i>Striga</i> alone as control(UIC)	-



Plate 4 AMF single spore culture in plastic cups using ragi as host plant



Plate 5 Mass multiplication of native AMF isolates in small pots

3.7 Observations recorded on *Striga*

3.7.1 Relative number of days for *Striga* emergence

The number of days taken for the emergence of *Striga* was recorded from sixty days after planting of sugarcane sets (DAP).

3.7.2 No. of *Striga* emerged pot⁻¹

Number of *Striga* emerged were recorded in each pot.

3.7.3 Biomass of *Striga*

The shoot and root portions uprooted *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 five were expressed in grams.

3.8 Observations recorded on sugarcane (30, 60, 90 and 120 DAP)

3.8.1 Plant growth parameters:

3.8.1.1 Plant height

The sugarcane plant height was defined as the average stem distance from the soil to the insertion of the Top Visible Dewlap leaf (TVD) (Dillewijn, 1952) on the stem and average of five were expressed in cm.

3.8.1.2 Root and shoot biomass

The uprooted plants were partitioned into root and shoot at harvest stage. The samples were dried at 60 °C till a constant weight was achieved. The dry weights were then recorded separately for shoots and roots and average of five were expressed in grams.

3.8.1.3 Stem girth

Stem girth was measured at fourth node from the bottom of each plant and expressed in cm.

3.8.1.4 Chlorophyll content (SPAD readings)

The single photoelectric analyzing diode (SPAD) meter (SPAD-502 KONICA-Japan) was used for recording the chlorophyll reading. The reading was taken between 10.00 and 12.00 hours of the day.

3.8.1.5 Sugarcane biomass

The shoot and root portions of the uprooted 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 the three plants were expressed in grams per plant.

3.8.2 Biophysical parameters (Infra Red Gas Analyser)

Measurement of photosynthetic rate, stomatal conductance, rate of transpiration and leaf temperature were made on the top fully expanded leaf at different locations by using a portable photosynthesis system (LI-6400 LICOR, Nebraska, Lincoln USA,). These measurements were made between 10.00 am to 12.00 noon on all the sampling dates.

Parameters	:	units
Photosynthetic rate	:	μ mole CO ₂ /m ² /s
Transpiration rate	:	m mole of H ₂ O /m ² /s
Stomatal conductance	:	μ mole/m ² /s
Leaf temperature	:	°C

3.9 Mycorrhizal parameters

3.9.1 Spore count

The chlamydospores in rhizosphere of sugarcane were determined by wet sieving and decantation method as outlined by Gerdemann and Nicholson (1963).

AM spores were isolated using wet sieving and decantation method. One hundred gram of soil sample was taken in one liter beaker, made up to 1000 ml with

water, stirred well, heavier soil particles were allowed to settle for a few seconds. Then the suspension was passed through a series of different size sieves (500 µm, 250µm, 106 µm, 75 µm, 45 µm, 37 µm) arranged in the descending order of their mesh size. Again water was added up to 1000 ml, stirred well and allowed for few seconds this was repeated for five to six times, till the suspension appeared clear. Sievates were collected from each sieve separately in beakers. The supernatant from each beaker was separately filtered through Whatman No. 1 filter paper and the content of the filter papers were examined for spores under a stereo zoom microscope (Labomed).

3.9.2 Per cent root colonization.

Mycorrhizal root colonization was determined as per the procedure proposed by Philips and Hayman (1970). Fresh root samples were cut into 1 cm pieces and placed in screw cap vials. The clearing of the roots was achieved by treating them with 10 percent KOH and leaving them overnight. The KOH solution was poured off and the roots were rinsed with tap water. Later, the roots were treated with 10 per cent HCl for 10 minutes to neutralize the residual effect of alkali and create an acidic environment required for further staining. The root bits were stained with 0.05 per cent trypan blue in lactoglycerol (lactic acid, glycerol and water in the ratio of (40:20:20 respectively) by boiling them at 90° C for 30 minutes. Excess stain was decanted and the root samples were immersed in lactoglycerol for de staining. The stained root bits were placed on a clean glass slide and observed under microscope for colonization. The percentage of roots colonized by mycorrhizae was calculated by the formula

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

3.10 Soil enzymes

3.10.1 Estimation of dehydrogenase activity

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

Ten grams of soil and 0.2 g CaCO_3 were thoroughly mixed and dispensed in test tubes. To each tube, one ml of 3 per cent aqueous solution of 2, 3, 5- Triphenyl tetrazolium chloride (TTC), one ml of one per cent glucose solution and eight ml of distilled water were added. This was sufficient to leave a thin film of water above the soil layer. The tubes were stoppered with rubber cork and incubated at 30°C for 24 h. At the end of incubation, the contents of the tube were rinsed down into a small beaker and slurry was made by adding 10 ml methanol. The slurry was filtered through Whatman No. 50 filter paper. Repeated rinsing of soil with one ml methanol was continued till the filtrate ran free of red color. The filtrate was pooled and made up to 50 ml with methanol in a volumetric flask. The intensity of red color was measured at 485 nm against methanol as blank using UV- vis spectrophotometer. (Thermo Scientific, USA). The concentration of formazan in soil samples were determined by referring to a standard curve prepared using graded concentration of formazan. The results were expressed as μg of triphenyl formazan (TPF) formed g^{-1} soil per day.

3.10.2 Estimation of phosphatase activity

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

One gram of soil sample was placed in 50 ml Erlenmeyer flask to which 0.2 ml toluene followed by four ml of modified universal buffer (pH 7.5) were added. One ml of para-nitrophenol phosphate solution made in modified universal buffer was added to the flasks and contents of the flasks were mixed by swirling for 2 minutes. The flasks were stoppered and incubated at 37°C for one hour. After incubation, one ml of 0.5 M CaCl_2 and four ml of 0.5 M NaOH were added to the flask, swirled and filtered through Whatman No. 42 filter paper. The intensity of yellow colour developed was measured at 420 nm against the reagent blank using spectrophotometer. Controls were maintained for each soil sample and were analyzed by following the same procedure described above except that the para nitrophenol phosphate solution was added after the addition of 0.5 M CaCl_2 and 0.5 M NaOH and just before filtration. The phosphatase activity in the soil samples was expressed as μg para nitrophenol formed per gram soil per hour with reference to the standard curve prepared by using graded concentrations of p-nitrophenol phosphate.

1.11 Chemical analysis of sugarcane plants at harvest:

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

3.11.1 Estimation of nitrogen

The total nitrogen content in the plant sample was estimated following the microkjeldahl method as outlined by Jackson (1973). The analysis was done with 500 mg of oven dried finely ground samples which were digested with five ml of concentrated H_2SO_4 in the presence of 200 mg catalyst mixture (containing potassium sulphate, copper sulphate and selenium in 100:10:1 ratio). The samples were digested on a microkjeldahl digestion unit till a clear solution was obtained. The digest was cooled and diluted with distilled water. The digested samples were distilled after adding 20 ml of 40 per cent NaOH to make the digest alkaline in a semi microkjeldahl distillation unit. The evolved ammonia was absorbed in four per cent boric acid solution and titrated against 0.05N H_2SO_4 . A standard was run by using 1 mg of nitrogen per five ml solution of ammonium sulphate and the titre values were converted to mg of nitrogen and per cent nitrogen was calculated.

3.11.2 Estimation of phosphorus

3.11.2.1 Pre digestion

Five hundred mg of plant sample was taken in a 250 ml conical flask and 2.5 ml of concentrated HNO_3 was added. The flasks were swirled to moisten the entire sample and then placed on a hot sand bath for 30 min followed by keeping on electric hot plate maintained at 1800C to 2000C, until the suspension dried.

3.11.2.2 Wet oxidation

Five ml of tri acid mixture (Nitric acid, 60% perchloric acid and sulphuric acid in the ratio 10:1:4) was added to predigested sample and further digestion was carried out at 180-2000C on a digestion mantle until the content in the flasks turned clear white. The contents in flasks were cooled and 10-15 ml of 6N HCL added and stirred well. The acid digest was transferred to 50 ml volumetric flask and the volume was made up to 50

ml with distilled water. From this, wet oxidized digest sample, P was estimated by vanadomolybdate phosphoric yellow colour method (Jackson, 1973).

Ten ml of the wet digested sample was taken in a 50 ml volumetric flask and 10 ml of vanadomolybdate reagent was added.

The volume was made upto 50 ml with distilled water and allowed to react for 30 min. The intensity of the yellow colour developed was read at 490 nm using spectrophotometer. The P content in the sample was determined by referring to the standard curve.

3.11.2.3 Standard curve

For obtaining the standard curve, 0.439g of KH_2PO_4 was dissolved in distilled water and the volume was made upto 1000 ml in volumetric flask. Aliquots of 1-10 ml were transferred to 50 ml volumetric flask and 10 ml of vanadomolybdate reagent was added to each flasks including blank. The volume was made upto 50 ml with distilled water. The yellow color developed was read after 10 min in spectrophotometer at 490 nm. The standard curve was obtained by plotting a graph with concentration along X axis and corresponding absorbance along Y axis.

4. EXPERIMENTAL RESULTS

The present investigation encompasses isolation, morphological characterization, and screening of native AM fungi against *Striga* under pot culture conditions. The wet lab experiments were conducted at weed scheme laboratory, whereas pot trials were conducted in the mesh house at Department of Agricultural Microbiology, University of Agricultural Sciences, Dharwad. The results obtained during the investigations are documented and presented below.

4.1 Isolation and morphological characterization of native AM fungal isolates from the *Striga* suppressive soils of sugarcane growing areas

Native AM fungal isolates were isolated from *Striga* suppressive soil. These isolates were identified up to species level based on morphological characteristics of the chlamydospores.

The AM fungal species identified during the investigations are given in Table 1 and Plate 6. Sixteen species were identified from the *Striga* suppressive soils, among them twelve species belonged to the genera *Glomus* and four were representing *Acaulospora*. These native isolates were used in the pot experiment and the general view of the experiment were depicted in Plate 7 and 8.

4.2 Influence of AM fungal isolates on *Striga* parameters

4.2.1 Relative number of days for *Striga* emergence

The number of days taken for the emergence of *Striga* was recorded from sixty days after planting of sugarcane sets (DAP) and presented in Table 2 Plate 9 and 10)

The results have revealed that the first batch of *Striga* emergence was recorded at 63 DAP in the uninoculated control (UIC) pots. However, at 64 DAP, the *Striga* emergence was observed in the treatment received single inoculation of UASDAMF1, UASDAMF4, UASDAMF6, and UASDAMF16, while zero emergence of *Striga* were noticed with the treatment received the native isolates viz., UASDAMF2, UASDAMF5, UASDAMF9, and UASDAMF12, AMF consortium (STD) and AMF consortium (native) recorded zero emergence of *Striga*.

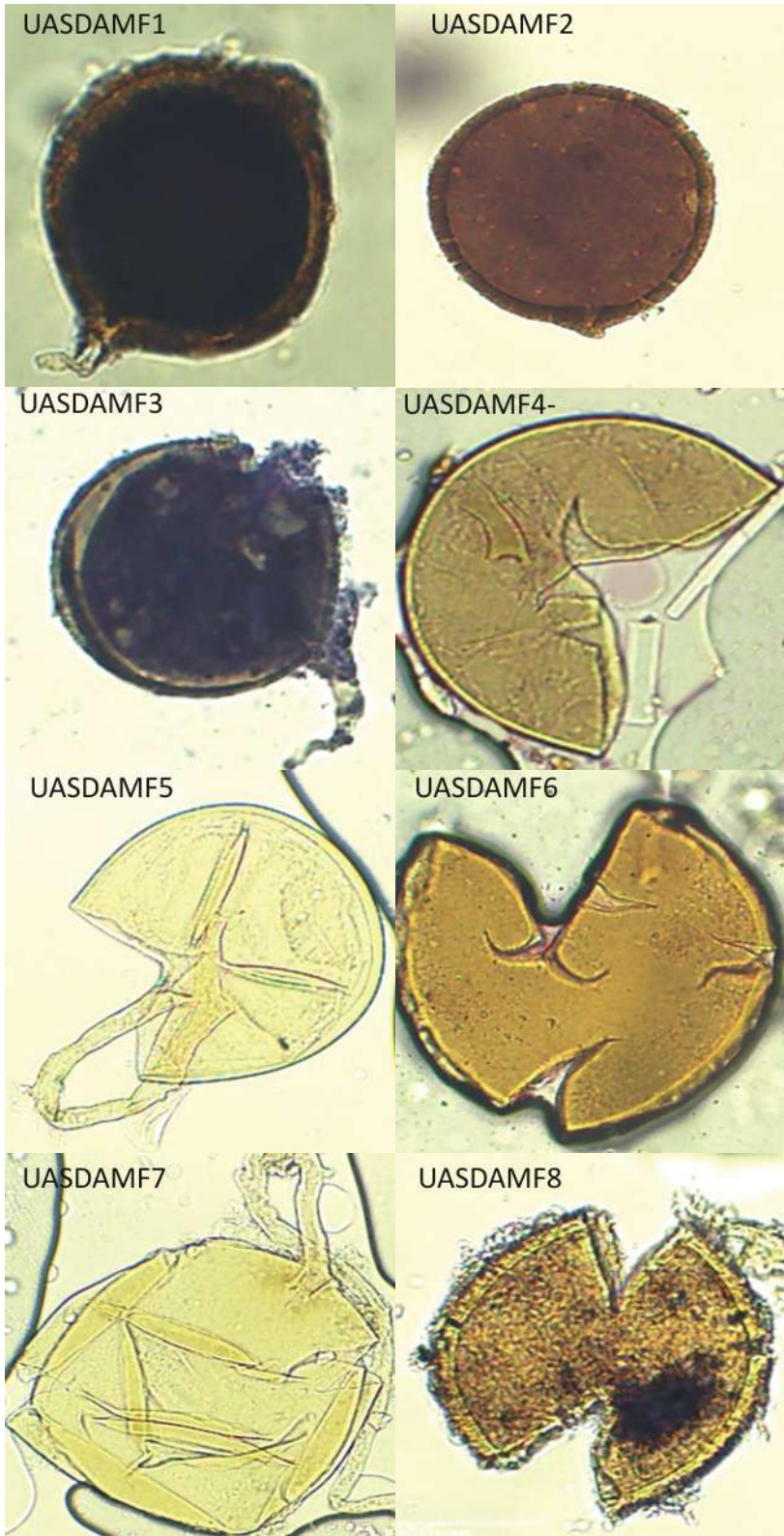


Plate 6 Chlamydospores of native AM fungal isolates from *Striga* suppressive soils of sugarcane

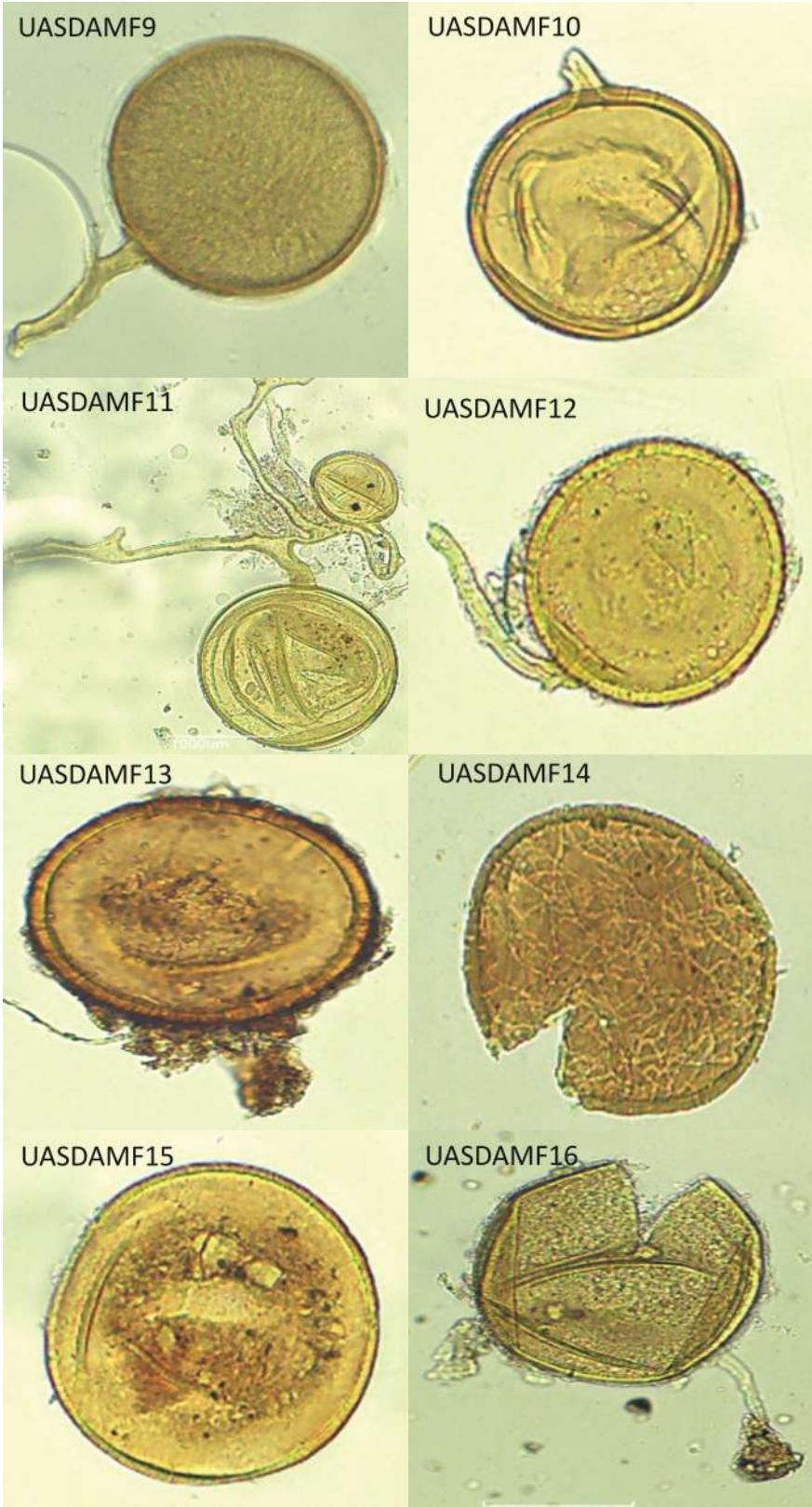


Plate 6 Contd...



Plate 7 General view of the experiment at 60 DAP

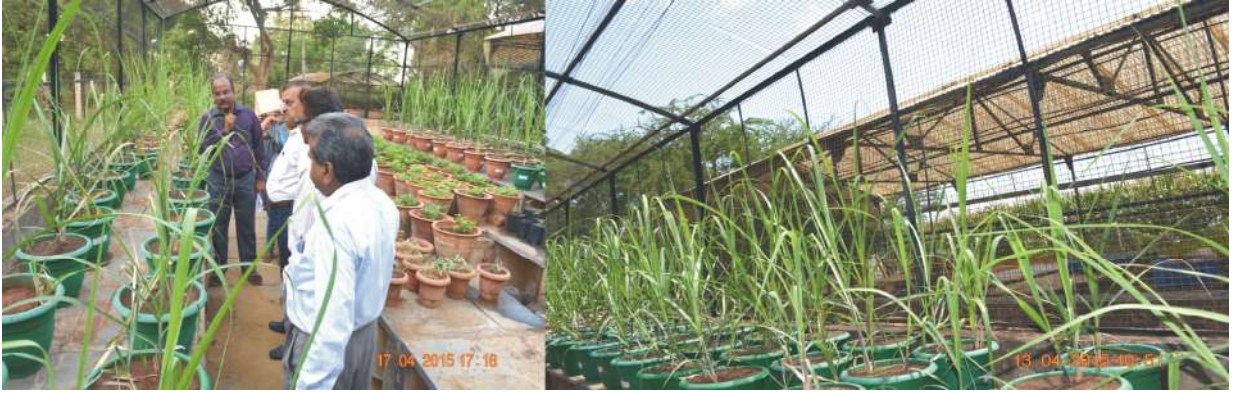


Plate 8 General view of the experiment at 90 DAP

Table 1: Tentative identification of native AM fungal morpho types from *Striga* suppressive soils

Isolates	Code no. of the isolates	Shape	colour	Spore mean Size (μm)	Spore wall thickness mean(μm)	Spore surface	size of Hyphae mean(μm)	AMF Species
1	UASD AMF1	Oval	Dark brown	104.83	5.60	Smooth	9.1	<i>Glomus ambisporum</i>
2	UASD AMF2	Oval	Dark brown	96.62	10.50	Smooth	12.3	<i>Glomus etunicatum</i>
3	UASD AMF3	Ellipsoids	Brown	137.60	11.90	Rough	-	<i>Glomus mosseae</i>
4	UASD AMF4	Oval	Light yellow	104.60	9.40	smooth	63.6	<i>Glomus spp.</i>
5	UASD AMF5	Oval	Light yellow	120.80	5.52	Smooth	71.8	<i>Acaulospora maarowe</i>
6	UASD AMF6	Oval	Brown	165.52	10.8	Granular	47.8	<i>Glomus deserticola</i>
7	UASD AMF7	Oval	Yellow	179.32	10.60	Laminated	53.3	<i>Glomus phansihalos</i>
8	UASD AMF8	Oval	Yellow	118.9	13.00	Smooth	-	<i>Acaulospora spinosa</i>
9	UASD AMF9	Round	Light Brown	107.8	7.60	Smooth	-	<i>Glomus leptotichum</i>
10	UASD AMF10	Oval	Brown	78.58	7.90	Smooth	12.6	<i>Glomus aggregatum</i>
11	UASD AMF11	Ellipsoid	Dark yellow	149.56	12.75	Granular	-	<i>Glomus lacteum</i>
12	UASD AMF12	Oval	Dark yellow	73.7	11.9	Granular	51.4	<i>Glomus fasciculatum</i>
13	UASD AMF13	Ellipsoidal	Yellow	111.27	8.90	Smooth	-	<i>Glomus radiata</i>
14	UASD AMF14	Oval	Brown	105.72	7.50	Rough	-	<i>Glomus reticulatum</i>
15	UASD AMF15	Oval	Dark yellow	121.65	5.40	Smooth	-	<i>Acaulospora bisporus</i>
16	UASD AMF16	oval	Brown	131.45	10.02	Granular	-	<i>Acaulospora lacunosa</i>

Table 2. Screening of arbuscular mycorrhizal fungi against *Striga* emergence per pot (relative number of days taken)

Treatments	UASD AMF1	UASD AMF2	UASD AMF3	UASD AMF4	UASD AMF5	UASD AMF6	UASD AMF7	UASD AMF8	UASD AMF9	UASD AMF10	UASD AMF11	UASD AMF12	UASD AMF13	UASD AMF14	UASD AMF15	UASD AMF16	Std. AMF consortium	native AMF consortium	UIC
Number of days after sugarcane planting																			
60 DAP
61
62
63
64	+	.	.	+	.	+	+	.	+	.	.	+
65	+	.	+	+	.	+	.	.	.	+	.	.	.	+	.	+	.	.	+
67	+	+	.	.	.	+	.	.	.	+	.	+	.	.	+
68	+	.	+	+	.	+	+	+	.	+	.	.	.	+	.	+	.	.	+
69	+	.	+	+	+	.	+	.	.	+
70	.	.	+	+	.	+	+	.	.	+	+	.	+	+	.	+	.	.	+
71	+	.	+	+	.	.	+	+	.	.	+	.	+	+	.	+	.	.	+
72	.	.	+	.	.	+	.	+	.	+	+	.	+	.	.	+	.	.	+
73	+	.	.	+	.	+	+	.	.	+	+	.	+	+	+
74	+	.	+	+	.	.	+	.	.	.	+	.	+	+	.	+	.	.	+
75	+	.	+	.	+	.	.	+	.	.	+	.	.	+
76	+	.	+	+	.	+	+	+	.	+	+	.	.	+	+
77	+	.	+	+	+	+	.	+	.	.	+
78	+	+	.	.	+	+	.	+	.	.	+	.	.	+
79	+	.	.	+	.	.	.	+	.	.	+	.	.	+	+
80	+	.	+	+	+	+	.	+	.	.	+
81	+	.	.	+	.	.	.	+	.	.	+	.	+	.	.	+	.	.	+
82	.	.	+	.	.	+	.	+	.	+	+	+
83	+	.	+	.	.	+	+	.	.	+	.	.	+	+	+
84	+	.	.	+	.	.	.	+	.	.	+	.	+	+	.	+	.	.	+
85	.	.	+	+	+	.	.	+
86	.	.	+	+	.	+	+	+	.	+	+
87	+	.	+	+	.	.	.	+	+	+	+
88	+	.	+	.	+	+	.	+	.	.	+	.	.	+
89	+	.	.	+	.	+	.	+	.	+	+	.	.	+	.	+	.	.	+
90	+	.	+	+	+	.	+	.	.	+

91	-	-	-	-	-	+	-	-	-	+	-	-	+	-	-	+	-	-	+
92	+	-	+	-	-	-	-	+	-	-	+	-	-	+	-	-	-	-	-
93	-	-	+	-	-	-	+	+	-	-	-	-	+	-	-	+	-	-	-
94	-	-	-	-	-	+	+	-	-	-	+	-	+	-	-	-	-	-	-
95	-	-	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
96	-	-	-	+	-	+	-	+	-	+	-	-	+	-	-	-	-	-	+
97	+	-	-	-	-	-	+	+	-	-	+	-	-	+	-	-	-	-	-
98	+	-	-	-	-	+	+	-	-	+	-	-	-	-	-	+	-	-	+
99	+	-	-	-	-	-	+	+	-	-	-	-	-	+	-	-	-	-	-
100	-	-	+	-	-	-	+	+	-	-	-	-	-	-	-	+	-	-	-
101	-	-	-	-	-	+	+	-	-	+	-	-	+	-	-	-	-	-	+
102	+	-	-	+	-	-	-	+	-	-	+	-	-	+	-	-	-	-	-
103	-	-	-	-	-	-	+	+	-	-	-	-	+	-	-	+	-	-	-
104	-	-	-	+	-	+	-	-	-	+	+	-	-	-	-	-	-	-	+
105	+	-	-	-	-	-	+	+	-	-	-	-	-	+	-	-	-	-	-
106	-	-	+	+	-	-	+	-	-	-	-	-	+	-	-	+	-	-	-
107	-	-	-	+	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-
108	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
109	-	-	-	+	-	-	+	+	-	-	-	-	+	-	-	-	-	-	-
110	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
111	-	-	-	-	-	+	+	+	-	+	-	-	-	-	-	-	-	-	+
112	+	-	-	-	-	+	+	-	-	+	-	-	-	+	-	-	-	-	+
113	+	-	+	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-
114	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-
115	-	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
116	-	-	-	-	-	+	+	-	-	-	-	-	+	-	-	-	-	-	+
117	+	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-
118	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	+
119	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
120	-	-	+	+	-	+	-	+	-	-	-	-	+	-	-	+	-	-	-



60 DAP



90 DAP



120 DAP

Plate 9c Emergence of *Striga* in uninoculated controlled pots at 60, 90 and 120 DAP



Plate 10 Parasitization of Striga in sugarcane

4.2.2 Number of *Striga* per pot:

The number of *Striga* per pot were recorded and presented in Table 3.

The number of *Striga* emerged was maximum with UIC (51) followed by the UASDAMF13 (47) and UASDAMF16 (45), UASD AMF11 (44). The lower number of *Striga* was observed in the treatment received UASDAMF1 , UASDAMF3 and UASDAMF15 (44, 39, 41 respectively). However zero number of *Striga* emergence was observed in treatments received AMF consortium(STD), AMF consortium(native) and native AMF isolates UASDAMF2, UASDAMF5, UASDAMF9 and UASDAMF12.

4.2.3 The impact of AMF on biomass of *Striga* plants at 120 DAP

The biomass of *Striga* plants was recorded and presented in Table 4 and Fig 1

4.2.3.1 Shoot *Striga* dry matter (g/plant)

The shoot dry weight of *Striga* was found to be higher in UIC compared to all other isolates (18.02). Among the native AMF isolates UASDAMF16 , UASDAMF13 and UASDAMF11 (17.58, 15.34, 15.90 g respectively) recorded highest *Striga* biomass compared to rest of the native isolates. The treatment received AMF consortium (STD), AMF consortium (native) and native isolates like UASDAMF2, UASDAMF5, UASDAMF9 and UASDAMF12 are recorded zero emergence of *Striga*.

4.2.3.2 Root dry matter (g/plant)

The root dry matter in UIC was recorded highest shoot biomass (2.60 g). The treatment which received UASDAMF13 and UASD AMF11 recorded significantly higher root dry matter (1.92 g) and (1.72 g) as compared to other treatments.

4.2.3.3 Total dry matter (g/plant)

Significantly highest total dry matter (20.62 g/plant) was recorded higher in treatment received UIC over of the treatments. The second highest total dry matter was recorded with UASDAMF16 (19.11 g) followed by UASDAMF13 (17.52 g) and

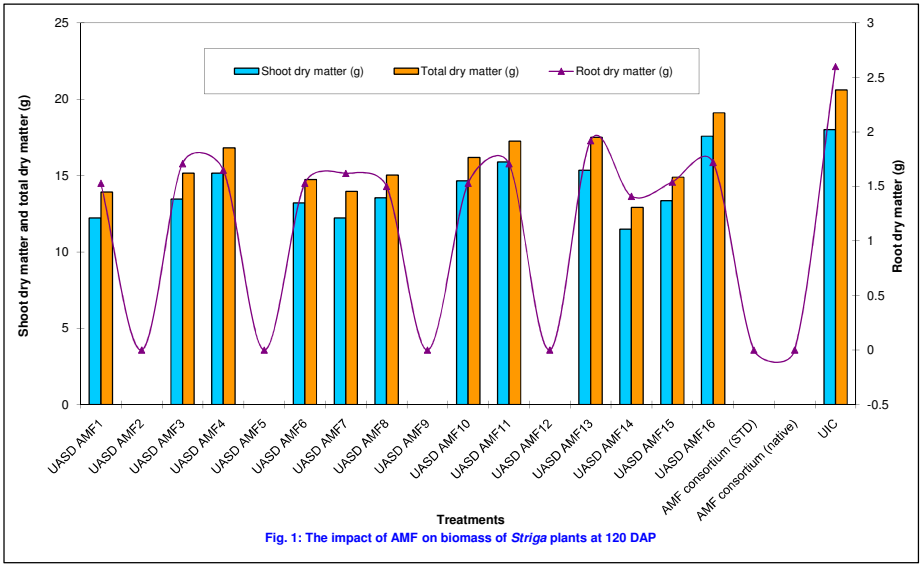
Table 3: Screening of arbuscular mycorrhizal fungi against *Striga* (no./pot)

Treatments	UASD AMF1	UASD AMF2	UASD AMF3	UASD AMF4	UASD AMF5	UASD AMF6	UASD AMF7	UASD AMF8	UASD AMF9	UASD AMF10	UASD AMF11	UASD AMF12	UASD AMF13	UASD AMF14	UASD AMF15	UASD AMF16	Std. AMF consortium	native AMF consortium	UIC
Number of days after sugarcane planting																			
60 DAP	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
61	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
62	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
63	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
64	2	0	0	1	0	1	0	0	0	0	0	0	0	0	0	1	0	0	1
65	1	0	1	3	0	2	0	1	0	2	0	0	0	1	0	0	0	0	2
67	2	0	0	0	0	3	0	0	0	3	0	0	0	2	0	1	0	0	3
68	3	0	2	3	0	3	1	1	0	3	0	0	0	3	0	2	0	0	3
69	4	0	1	0	0	0	0	2	0	0	0	0	0	3	0	3	0	0	0
70	0	0	2	3	0	3	1	0	0	3	0	0	1	0	0	3	0	0	3
71	3	0	3	1	0	0	2	1	0	0	1	0	2	3	2	0	0	0	0
72	0	0	3	0	0	3	0	1	0	3	2	0	3	0	3	3	0	0	3
73	3	0	0	1	0	1	2	0	0	1	3	0	4	3	3	0	0	0	1
74	1	0	3	2	0	0	2	0	0	0	3	0	0	0	0	3	0	0	0
75	0	0	0	0	0	1	0	1	0	1	0	0	3	0	3	1	0	0	1
76	1	0	3	1	0	2	1	2	0	2	3	0	0	1	0	0	0	0	2
77	2	0	1	1	0	0	0	0	0	0	0	0	3	2	3	1	0	0	0
78	0	0	0	0	0	1	1	0	0	1	3	0	1	0	1	2	0	0	1
79	1	0	1	1	0	1	0	2	0	1	1	0	0	1	0	0	0	0	1
80	1	0	2	1	0	0	2	0	0	0	0	0	1	1	1	1	0	0	0
81	1	0	0	2	0	0	0	1	0	0	1	0	2	0	2	1	0	0	0
82	0	0	1	0	0	1	0	2	0	1	2	0	0	0	0	0	0	0	2
83	1	0	1	0	0	2	2	0	0	2	0	0	1	1	1	0	0	0	1
84	2	0	0	1	0	0	0	2	0	0	1	0	1	2	1	1	0	0	0
85	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	2	0	0	0
86	0	0	1	1	0	1	2	1	0	1	0	0	0	0	0	0	0	0	1
87	1	0	2	2	0	0	0	1	0	0	0	0	1	1	1	0	0	0	0
88	0	0	0	0	0	1	0	0	0	1	2	0	2	0	2	1	0	0	1
89	0	0	0	2	0	2	0	1	0	2	1	0	0	1	0	0	0	0	2
90	2	0	1	0	0	0	0	2	0	0	0	0	0	2	0	1	0	0	0
91	0	0	0	0	0	2	0	0	0	2	0	0	1	0	1	2	0	0	2

92	2	0	1	0	0	0	0	1	0	0	1	0	0	2	0	0	0	0	0
93	0	0	2	0	0	0	2	1	0	0	0	0	1	0	1	2	0	0	0
94	0	0	0	0	0	2	2	0	0	0	1	0	2	0	2	0	0	0	0
95	0	0	2	1	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0
96	0	0	0	1	0	1	0	1	0	1	0	0	2	0	2	0	0	0	1
97	1	0	0	0	0	0	1	2	0	0	2	0	0	1	0	0	0	0	0
98	0	0	0	0	0	0	1	0	0	2	0	0	2	0	0	3	0	0	3
99	2	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0
100	0	0	1	0	0	1	0	1	0	0	0	0	0	0	0	2	0	0	0
101	0	0	0	0	0	1	3	0	0	2	0	0	1	0	1	0	0	0	2
102	0	0	0	2	0	0	0	1	0	0	1	0	0	2	0	0	0	0	1
103	0	0	0	0	0	0	3	2	0	0	0	0	2	0	2	2	0	0	0
104	0	0	0	1	0	0	0	0	0	2	2	0	0	0	0	0	0	0	2
105	0	0	0	0	0	0	3	2	0	0	0	0	0	2	0	0	0	0	0
106	0	0	1	1	0	0	1	0	0	0	0	0	2	0	2	2	0	0	0
107	0	0	0	2	0	0	0	2	0	0	2	0	0	0	0	1	0	0	1
108	0	0	0	0	0	2	1	0	0	0	0	0	0	0	0	0	0	0	0
109	0	0	0	2	0	0	2	1	0	0	0	0	2	0	2	0	0	0	0
110	0	0	0	0	0	0	0	0	0	0	2	0	0	4	0	0	0	0	0
111	0	0	0	0	0	2	1	1	0	2	0	0	0	0	0	0	0	0	2
112	2	0	0	0	0	2	1	0	0	2	0	0	0	2	0	0	0	0	2
113	2	0	1	0	0	0	0	0	0	0	0	0	0	2	0	2	0	0	1
114	0	0	3	1	0	0	0	1	0	0	0	0	0	0	0	2	0	0	0
115	0	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0
116	0	0	0	0	0	1	2	0	0	0	0	0	2	0	2	0	0	0	1
117	1	0	0	0	0	0	0	0	0	0	2	0	2	0	2	0	0	0	0
118	0	0	0	0	0	1	0	0	0	0	2	0	0	0	0	0	0	0	0
119	0	0	0	0	0	0	1	0	0	0	0	0	0	2	0	0	0	0	4
120	0	0	1	1	0	1	0	0	0	0	2	0	1	0	0	1	0	0	0
Total no. of Striga plants	40	0	41	39	0	42	42	43	0	40	44	0	47	41	41	45	0	0	51

Table 4: The impact of AMF on biomass of *Striga* plants at 120 DAP

Treatments	Shoot dry matter (g)	Root dry matter (g)	Total dry matter (g)
UASD AMF1	12.22	1.53	13.934
UASD AMF2	0	0	0
UASD AMF3	13.46	1.71	15.17
UASD AMF4	15.17	1.65	16.83
UASD AMF5	0	0	0
UASD AMF6	13.21	1.53	14.74
UASD AMF7	12.23	1.62	13.96
UASD AMF8	13.54	1.50	15.04
UASD AMF9	0	0	0
UASD AMF10	14.66	1.53	16.19
UASD AMF11	15.90	1.71	17.26
UASD AMF12	0	0	0
UASD AMF13	15.34	1.92	17.52
UASD AMF14	11.50	1.41	12.91
UASD AMF15	13.36	1.54	14.90
UASD AMF16	17.58	1.72	19.11
AMF consortium (STD)	0	0	0
AMF consortium (native).	0	0	0
UIC	18.02	2.60	20.62
S.Em±	0.22	0.03	0.23
CD(0.05)	0.62	0.07	0.65



UASDAMF11 (17.26 g). AMF consortium (STD) and AMF consortium (native) followed by native isolates like UASDAMF2, UASDAMF5, UASDAMF9 and UASDAMF12 are recorded zero emergence of *Striga* results in zero total dry matter production.

4.3 Influence of the AM fungal isolates on growth parameters of Sugarcane plants

4.3.1 Plant height (cm).

The plant height of sugarcane was recorded at 30 days intervals and presented in Table 5, Fig 2 and Plate 11 to 13.

The plant height was found to increase steadily with number of days after inoculation viz., 30, 60, 90, and 120 DAP. The plants inoculated with the AM fungal isolates were found to be always superior over uninoculated control plant. However the plant height differed significantly among the plants inoculated with efficient AMF consortium (STD) and AMF consortium (native) of native isolates with over UIC.

At 30 DAP AMF consortium (STD) recorded the maximum plant height followed by AMF consortium (native) (30.8 cm and 28 cm respectively). Among the treatments received native isolates, UASDAMF9 (27.2 cm), UASDAMF5 (27 cm), UASDAMF12 (26.4 cm) and UASDAMF2 (26.2 cm) recorded highest height significantly compared to rest of native isolates and the UIC. The lowest plant height recorded in treatments received UIC (15.6 cm) followed by UASDAMF16 (16.80 cm), UASDAMF13 (18.10cm), UASDAMF11 (18.20 cm).

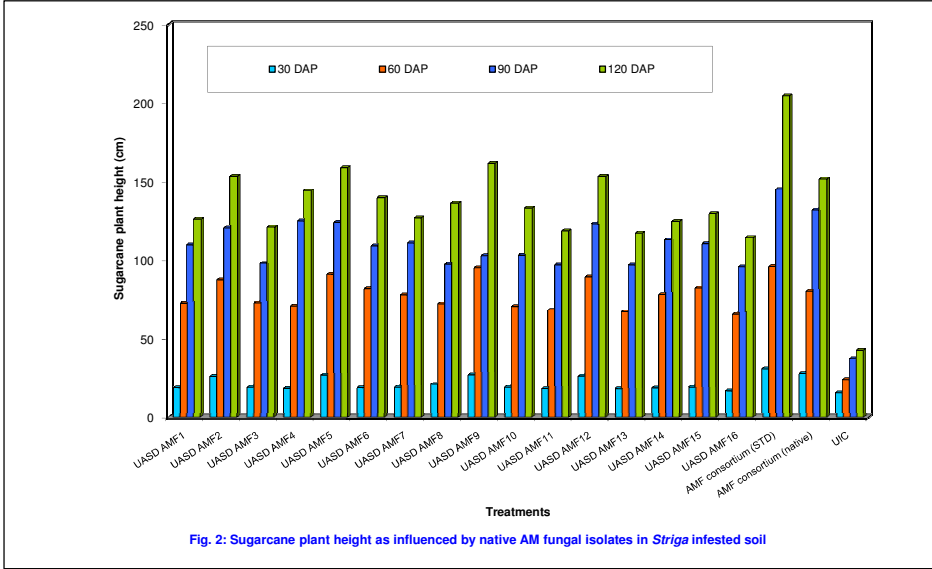
At 60 DAP the plant height was also significantly higher in AMF consortium (STD) and AMF consortium (native) over UIC. Similar trend was observed at 90 and 120 DAP.

4.3.2 Stem girth (cm)

The data on stem girth of sugarcane plants were recorded and presented in Table 6 and Fig 3.

Table 5: Sugarcane plant height as influenced by native AM fungal isolates in *Striga* infested soil

Treatments	Sugarcane plant height (cm)			
	30 DAP	60 DAP	90 DAP	120 DAP
UASD AMF1	18.80	72.20	109.20	125.60
UASD AMF2	26.20	87.20	120.20	153.00
UASD AMF3	19.00	72.40	97.60	120.80
UASD AMF4	18.30	70.40	124.50	144.00
UASD AMF5	27.00	90.60	123.60	158.40
UASD AMF6	18.80	81.20	108.60	139.40
UASD AMF7	19.00	77.40	110.40	126.40
UASD AMF8	21.00	71.80	96.80	136.00
UASD AMF9	27.20	94.60	102.60	161.60
UASD AMF10	19.00	70.20	102.80	133.00
UASD AMF11	18.20	67.80	96.40	118.40
UASD AMF12	26.40	89.00	122.60	153.00
UASD AMF13	18.10	66.60	96.40	117.00
UASD AMF14	18.60	77.60	112.60	124.20
UASD AMF15	19.00	81.60	110.00	129.60
UASD AMF16	16.80	65.00	95.20	114.20
AMF consortium (STD)	30.80	95.40	144.80	204.20
AMF consortium (native).	28.00	79.60	131.80	151.40
UIC	15.60	24.20	37.60	42.80
S.Em±	0.47	1.45	2.39	3
CD(0.05)	1.33	4.08	6.73	8.45





**AMF CONSORTIUM
(STD)**

UIC

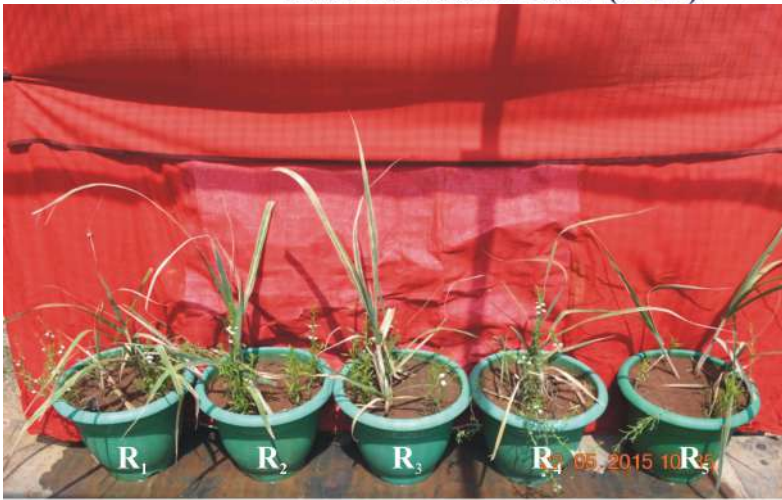
**AMF CONSORTIUM
(Native isolates)**



Plate 11 Sugarcane plant height as influenced by AMF isolates in presence of Striga



AMF CONSORTIUM (STD)



UIC



AMF CONSORTIUM (Native isolates)

Plate 12 Sugarcane plant height as influenced by AMF consortium



UASDAME2



UASDAME5



UASDAME9



UASDAME12



UIC

Plate 13 Sugarcane plant height as influenced by single inoculation of native AMF isolates

Table 6: Sugarcane stem girth as influenced by native AM fungal isolates in *Striga* infested soil

Treatments	The stem girth of sugarcane plants (cm)		
	60 DAS	90 DAS	120 DAS
UASD AMF1	4.22	5.18	6.30
UASD AMF2	5.41	6.18	7.18
UASD AMF3	4.02	4.88	6.28
UASD AMF4	3.8	5.44	6.14
UASD AMF5	5.60	7.14	8.26
UASD AMF6	3.86	5.12	6.12
UASD AMF7	4.18	5.26	6.28
UASD AMF8	3.64	5.12	5.64
UASD AMF9	5.94	7.17	8.28
UASD AMF10	3.66	5.4	5.44
UASD AMF11	4.00	4.16	5.30
UASD AMF12	5.50	7.04	7.58
UASD AMF13	4.36	4.14	5.24
UASD AMF14	4.08	4.74	6.02
UASD AMF15	4.54	5.28	6.22
UASD AMF16	3.58	4.06	5.22
AMF consortium (STD)	6.24	7.46	8.88
AMF consortium (native)	6.16	7.22	8.38
UIC	2.18	2.73	3.30
S.Em±	0.09	0.06	0.08
CD(0.05)	0.25	0.17	0.24

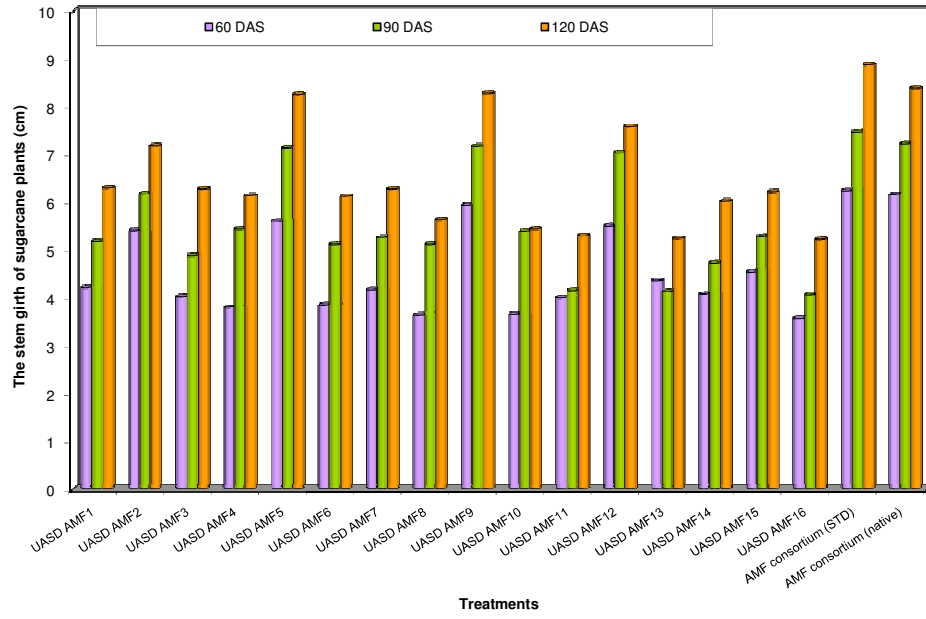


Fig. 3: Sugarcane stem girth as influenced by native AM fungal isolates in *Striga* infested soil

At 60 DAP the sugarcane plants which received the AMF consortium(STD) recorded greater stem girth (6.24cm) of Sugarcane plants followed by plants received AMF Consortium(native) (6.16 cm) as compared to UIC (2.18 cm).Among the treatments received native isolates, UASDAMF9 (5.94), UASDAMF5 (5.60), UASDAMF12 (5.50) and UASDAMF 2 (5.41) significantly increased the stem girth at all growth stages of sugarcane plants with compared with UIC.

At 90 DAP also the plant received the AMF consortium (STD) recorded maximum stem girth (7.46 cm) of followed by plants mycorrhized with AMF consortium (native) (7.22 cm) as compared to uninoculated control (UIC) viz., *Striga* alone (2.73 cm).

Similar trend was observed at 120 DAP, stem girth was significantly influenced by AMF consortium (STD) followed by AMF consortium (native) and native isolates UASDAMF9 (8.28 cm), UASDAMF5 (8.26 cm), UASDAMF12 (7.58 cm), and UASDAMF2 (7.15).

4.3.3 Chlorophyll content (SPAD reading)

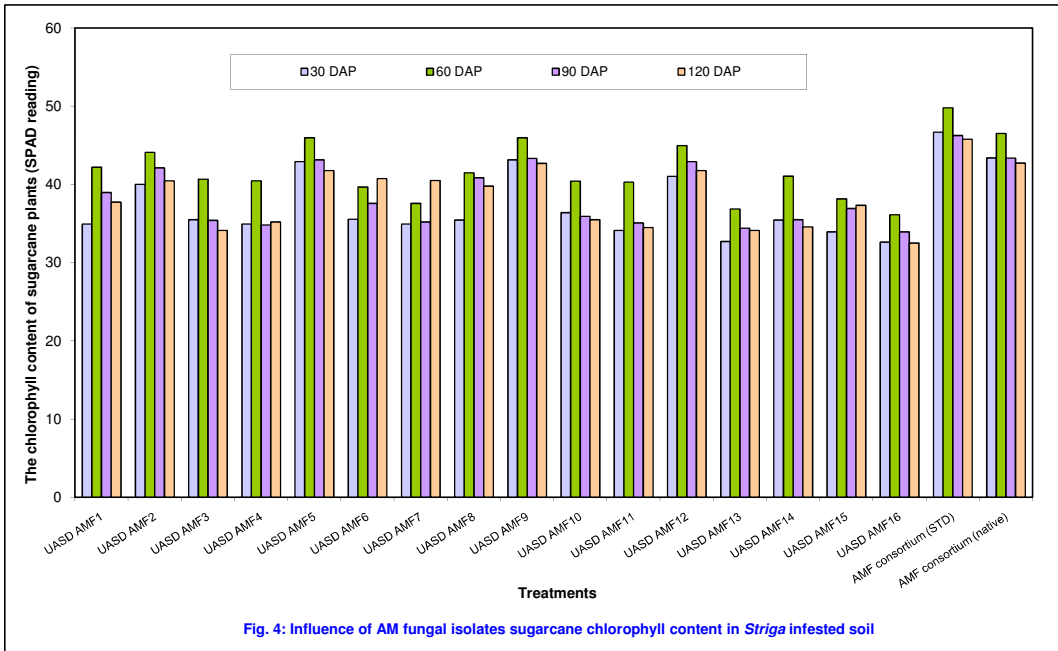
The results of the chlorophyll content of the sugarcane phyllosphere are presented in Table 7, Fig 4 and Plate 14.

At 30 DAP, inoculation of standard AMF to Sugarcane significantly improved the chlorophyll content (46.68) followed by consortium of all native isolates (43.38). Among native isolates, UASDAMF 12(43.14), UASD AMF 9 (42.9), UASDAMF 2 (41.02), and UASDAMF5 (40.00) recorded the highest chlorophyll content. The lowest chlorophyll content was recorded on UIC (29.8) followed by few native isolates UASD AMF16 (32.62), UASDAMF13 (32.68), UASDAMF11 (34.18).

At 60 DAP, the chlorophyll content was significantly influenced in sugarcane plants due to the inoculation of mycorrhiza compared to UIC. The sugarcane plants received AMF consortium (STD) (49.8) followed by AMF consortium (native) (46.52) showed a significant increase in chlorophyll content compared over UIC (36.12). Plants received single inoculation native AMF isolates UASDAMF2 (45.95), UASDAMF5 (44.97), UASDAMF9 (45.52), UASDAMF12 (44.10) recorded highest

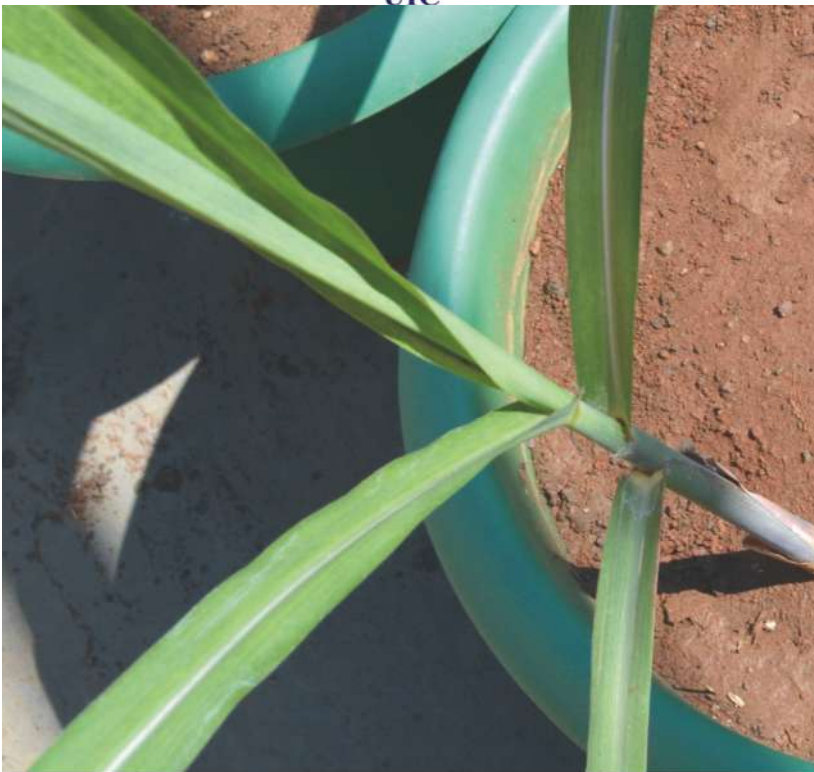
Table 7: Sugarcane chlorophyll content as influenced by native AM fungal isolates in *Striga* infested soil. (SPAD readings)

Treatments	The chlorophyll content of sugarcane plants.			
	30 DAP	60 DAP	90 DAP	120 DAP
UASD AMF1	34.94	42.20	38.96	37.72
UASD AMF2	40.00	44.10	42.10	40.46
UASD AMF3	35.50	40.67	35.42	34.12
UASD AMF4	34.94	40.46	34.80	35.20
UASD AMF5	42.90	45.95	43.14	41.78
UASD AMF6	35.54	39.65	37.58	40.74
UASD AMF7	34.94	37.56	35.20	40.50
UASD AMF8	35.44	41.47	40.84	39.80
UASD AMF9	43.14	45.97	43.32	42.68
UASD AMF10	36.38	40.42	35.92	35.50
UASD AMF11	34.12	40.30	35.06	34.48
UASD AMF12	41.02	44.95	42.90	41.78
UASD AMF13	32.68	36.87	34.40	34.12
UASD AMF14	35.44	41.05	35.50	34.56
UASD AMF15	33.94	38.15	36.92	37.34
UASD AMF16	32.62	36.12	33.94	32.50
AMF consortium (STD)	46.68	49.80	46.26	45.78
AMF consortium (native).	43.38	46.52	43.36	42.72
UIC	29.80	35.45	31.96	25.28
S.Em±	0.48	0.83	0.81	0.82
CD(0.05)	1.34	2.34	2.29	2.30





UIC



AMF CONSORTIUM (STD)

Plate 14 Sugarcane chlorophyll content as influenced by native AM consortium

chlorophyll content over UIC. The similar trend was observed at 90 DAP and 120 DAP.

4.3.4 The influence of AMF on biophysical parameters of sugarcane plants.

The data on biophysical parameters of sugarcane plants as influenced by different AM fungal isolates is presented in Table 8 and Fig 5.

4.3.4.1 Rate of photosynthesis (μ mol of $\text{CO}_2/\text{m}^2/\text{sec}$)

In general, rate of photosynthesis was significantly higher in treatments receiving AM fungal isolates. AMF consortium (STD) recorded highest photosynthetic rate followed by AMF consortium (native) recorded higher rate of photosynthesis (18.91 and 18.16 respectively) as compared to other treatments. Among the native isolates, higher photosynthetic rate was recorded in treatments received UASDAMF9 (17.91), followed by UASDAMF5 (17.29), UASDAMF12 (17.10) and UASDAMF2 (16.75) and the treatments received UIC recorded lower rate of photosynthesis.

4.3.4.2 Stomatal conductance (μ mol / m^2/sec)

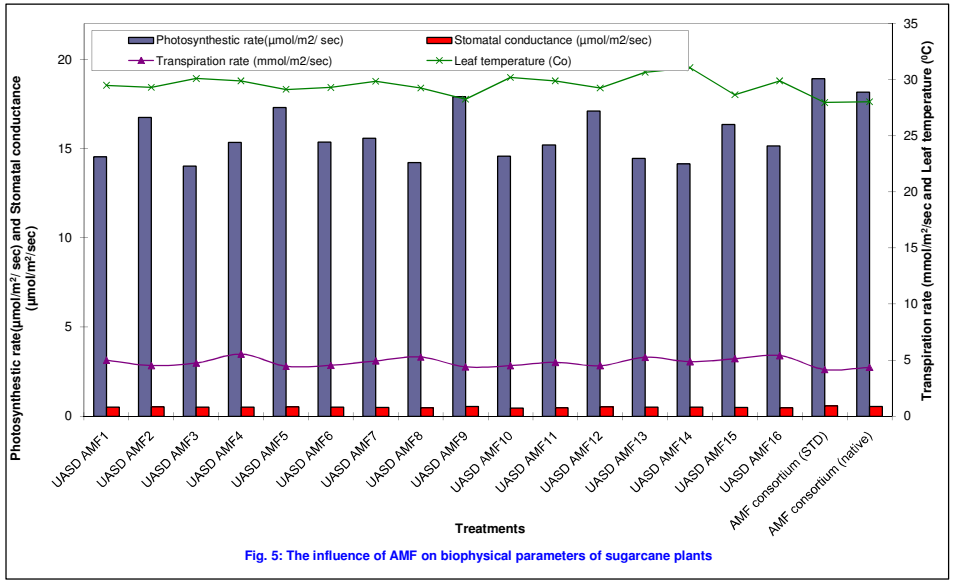
The data with respect to stomatal conductance was significantly higher with AMF consortium (STD) (0.58) followed by AMF consortium (native) (0.55) as compared to other treatments. Among the native isolates, highest stomatal conductance was recorded with native isolates UASDAMF9 (0.54), followed by UASDAMF5 (0.53), UASDAMF12 (0.52) and UASDAMF2 (0.52) and lower stomatal conductance was recorded treatments received UIC (0.45).

4.3.4.3 Rate of transpiration (μ mol of $\text{H}_2\text{O} / \text{m}^2/\text{sec}$)

The lower rate of transpiration was measured with AMF consortium (STD) (4.16) followed by AMF consortium (native) (4.36) as compared to other treatments. Among the native isolates UASDAMF9 (4.40), followed by UASDAMF5 (4.46), UASDAMF12 (4.50) and UASDAMF2 (4.51). The data clearly indicated that the UIC recorded higher rate of transpiration (5.54).

Table 8: The influence of AMF on biophysical parameters of sugarcane plants

Treatments	Photosynthetic rate($\mu\text{mol}/\text{m}^2/\text{sec}$)	Stomatal conductance ($\mu\text{mol}/\text{m}^2/\text{sec}$)	Transpiration rate ($\text{mmol}/\text{m}^2/\text{sec}$)	Leaf temperature ($^{\circ}\text{C}$)
UASD AMF1	14.54	0.51	4.99	29.50
UASD AMF2	16.75	0.52	4.51	29.33
UASD AMF3	14.02	0.51	4.74	30.10
UASD AMF4	15.35	0.50	5.53	29.90
UASD AMF5	17.29	0.53	4.46	29.13
UASD AMF6	15.36	0.51	4.54	29.30
UASD AMF7	15.58	0.49	4.94	29.86
UASD AMF8	14.21	0.47	5.28	29.26
UASD AMF9	17.91	0.54	4.40	28.26
UASD AMF10	14.57	0.45	4.50	30.20
UASD AMF11	15.20	0.48	4.80	29.90
UASD AMF12	17.10	0.52	4.50	29.26
UASD AMF13	14.46	0.50	5.26	30.66
UASD AMF14	14.15	0.50	4.86	31.07
UASD AMF15	16.35	0.49	5.12	28.66
UASD AMF16	15.15	0.47	5.40	29.90
AMF consortium (STD)	18.91	0.58	4.16	27.96
AMF consortium (native).	18.16	0.55	4.36	28.03
UIC	13.59	0.45	5.54	31.75
S.Em \pm	0.35	0.01	0.14	0.85
CD(0.05)	0.99	0.04	0.39	2.44



4.3.4.4 Leaf temperature (°C)

The application of AMF reduced the leaf temperature and the data depicts the leaf temperature was recorded lower in AMF consortium (STD) followed by AMF consortium (native) (27.96 and 28.03 °C respectively) as compared to other treatments. Further the sugarcane plants received single native isolates UASDAMF9 (28.26 °C), followed by UASDAMF5 (29.13 °C), UASDAMF12 (29.26 °C) and UASDAMF2 (29.33 °C) were recorded lowest leaf temperature, compared to UIC (31.75 °C).

4.3.5 Sugarcane biomass

Sugarcane dry matter was recorded and presented in Table 9, Fig 6. and Plate 15)

4.3.5.1 Shoot dry matter (g/plant)

The highest shoot dry matter was recorded in the plants received AMF consortium (STD) (125.50 g/plant) followed by AMF consortium (native) (117 g/plant). Among individual native AMF isolates UASDAMF9 (100 g/plant) recorded highest shoot dry matter followed by UASDAMF5 (99.5 g/plant), UASDAMF2 (99 g/plant) and UASD AMF 12 (99 g/plant). The lowest shoot dry matter was observed in the treatment, which received UIC (47.50 g/plant).

4.3.5.2 Root dry matter (g/plant)

During the present investigation the plants received AMF consortium (STD) (52 g/plant) followed by AMF consortium (native) (50 g/plant) recorded highest root dry matter. Among individual native AMF isolates UASDAMF9 (45.50 g/plant) recorded highest root dry matter followed by UASDAMF5 (44.5 g/plant), UASDAMF2 (43 g/plant) and UASDAMF12 (42 g/plant). However, the lowest root dry matter was observed in non mycorrhized plants, (17.5 g/plant)

4.3.5.3 Total dry matter (g/plant)

The total dry matter was recorded in the plants received AMF consortium (STD) (178 g/plant) followed by AMF consortium (native) (178 and 167 g

Table 9: Influence of AM fungal isolates on shoot and root biomass of Sugarcane plants at 120 DAP

Treatments	Shoot dry matter (g)	Root dry matter (g)	Total dry matter (g)
UASD AMF1	88.00	34.50	122.50
UASD AMF2	99.00	42.00	142.50
UASD AMF3	85.50	37.00	122.50
UASD AMF4	77.00	39.50	116.50
UASD AMF5	99.50	44.50	143.50
UASD AMF6	85.50	30.00	115.50
UASD AMF7	89.00	33.00	122.00
UASD AMF8	74.50	36.50	111.00
UASD AMF9	100.50	45.50	145.00
UASD AMF10	88.00	38.50	126.50
UASD AMF11	74.00	33.00	107.00
UASD AMF12	99.00	43.50	142.00
UASD AMF13	73.50	35.50	106.00
UASD AMF14	77.00	36.25	113.25
UASD AMF15	86.50	36.75	123.25
UASD AMF16	70.50	34.00	100.50
AMF consortium (STD)	125.50	52.50	178.00
AMF consortium (native)	117.00	50.00	167.00
UIC	47.50	17.50	65.00
S.Em±	2.93	1.18	2.45
CD(0.05)	8.68	3.50	7.25

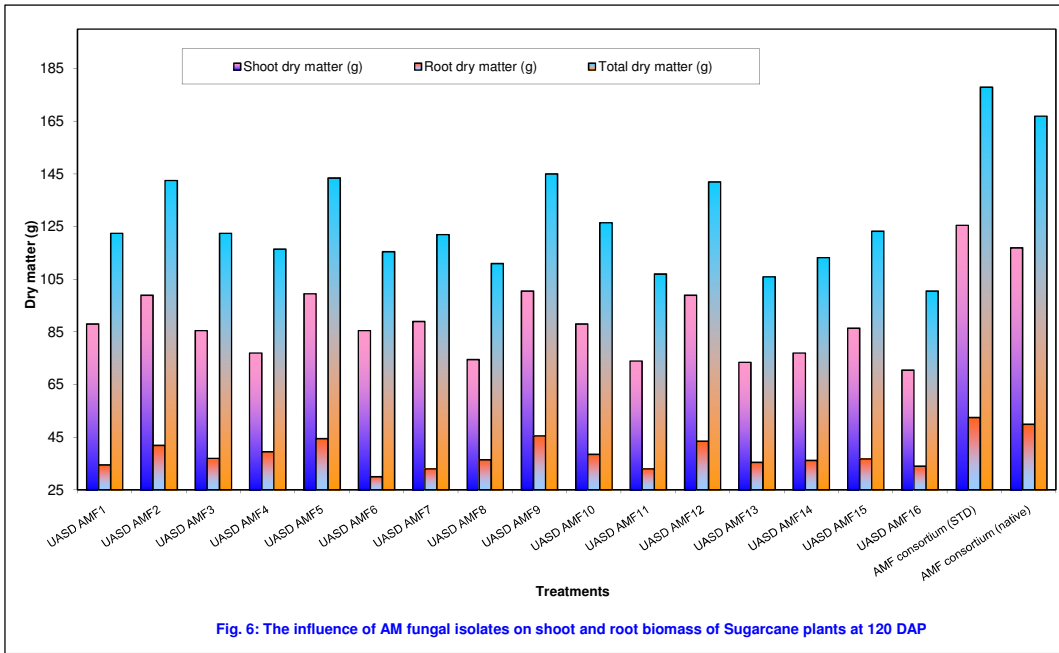




Plate 15 Root growth and biomass of sugarcane as influenced by AMF consortium

respectively). Among individual native AMF isolates UASDAMF9 (145 g) significantly influence the total dry matter followed by UASDAMF5 (143 g), UASDAMF2 (142 g) and UASDAMF12 (142 g). The lowest total dry matter was observed with the treatment, which received UIC (65 g/plant).

4.4 Dehydrogenase Influence of AM fungal isolates on mycorrhizal parameters

4.4.1 Mycorrhizal parameters

4.4.1.1 Spore count

The data on mycorrhizal spore load was enumerated at 30, 60, 90, and 120 DAP and the values are presented in Table 10 and Fig 7.

The highest mycorrhizal spore load was recorded with AMF consortium (STD) followed by AMF consortium (native) at 30, 60, 90 and 120 DAP.

At 120 DAP the spore load was highest in the treatment having AMF standard consortium (687.5/50 g soil) followed by AMF consortium (native) (668/50 g soil). Among individual native isolates UASDAMF9 (641.5/50 g soil) significantly influenced spore load followed by UASDAMF5 (625.5/50 g soil), UASDAMF12 (623.5/50 g soil) and UASDAMF2 (615/50 g soil). Least number of spore load was recorded with non mycorrhized sugarcane plants, UIC (211/50 g soil).

4.4.1.2 Per cent root colonization

The per cent root colonization ranged from 40.5 to 72.5 per cent and are depicted in Table 11, Fig 8 and Plate 16.

The maximum mycorrhizal colonization was recorded in the plants inoculated with AMF consortium (STD) (72.5%) followed by AMF consortium (native) (70%). Among individual native isolates UASDAMF12 (68 %) showed highest root colonization followed by UASDAMF9 (67.5%), UASDAMF2 (67.5%) and UASDAMF5 (67%). The lowest root colonization was observed in the plants which received UIC (25%). However, among the native isolates UASDAMF16 (40.50%) recorded lowest per cent root colonization.

Table 10: Mycorrhizal spore count as influenced by AM fungal isolates

Treatments	Mycorrhizal spore count (Number of spore 50 g ⁻¹)			
	30 DAP	60 DAP	90 DAP	120 DAP
UASD AMF1	156.50	210.00	281.50	540.00
UASD AMF2	220.50	271.50	310.50	615.00
UASD AMF3	162.50	220.50	273.50	564.50
UASD AMF4	183.00	227.50	288.50	594.00
UASD AMF5	230.00	282.50	366.50	625.50
UASD AMF6	193.50	238.00	283.50	572.00
UASD AMF7	219.50	233.00	271.50	584.50
UASD AMF8	164.50	195.00	268.00	528.50
UASD AMF9	238.50	292.00	373.00	641.50
UASD AMF10	150.50	209.00	288.50	592.50
UASD AMF11	154.00	194.00	267.50	527.00
UASD AMF12	226.00	277.50	349.50	623.50
UASD AMF13	141.50	191.50	254.00	497.50
UASD AMF14	155.00	199.00	274.50	556.00
UASD AMF15	158.50	206.50	285.00	551.50
UASD AMF16	139.50	191.50	234.00	454.50
AMF consortium (STD)	255.00	305.00	384.50	687.50
AMF consortium (native)	227.00	300.00	379.00	668.00
UIC	137.00	146.50	167.00	211.00
S.Em±	6.08	6.35	4.47	10.25
CD(0.05)	17.18	18.81	13.24	30.33

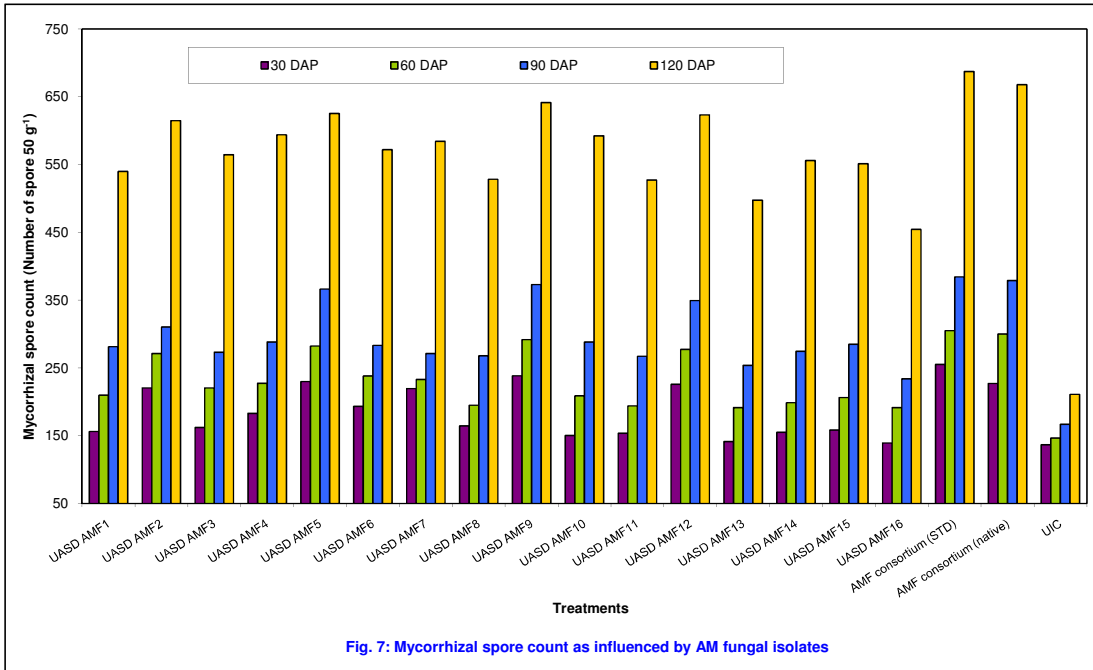


Table 11: Influence of AM fungi on Mycorrhizal root colonization in Sugarcane plants

Treatments	Per cent root colonization(%)
UASD AMF1	51.00
UASD AMF2	66.00
UASD AMF3	47.00
UASD AMF4	51.50
UASD AMF5	67.50
UASD AMF6	47.50
UASD AMF7	53.50
UASD AMF8	52.00
UASD AMF9	68.00
UASD AMF10	58.00
UASD AMF11	51.00
UASD AMF12	67.50
UASD AMF13	50.50
UASD AMF14	51.00
UASD AMF15	52.50
UASD AMF16	40.50
AMF consortium (STD)	72.50
AMF consortium (native).	70.00
UIC	25.50
S.Em±	1.23
CD(0.05)	3.64

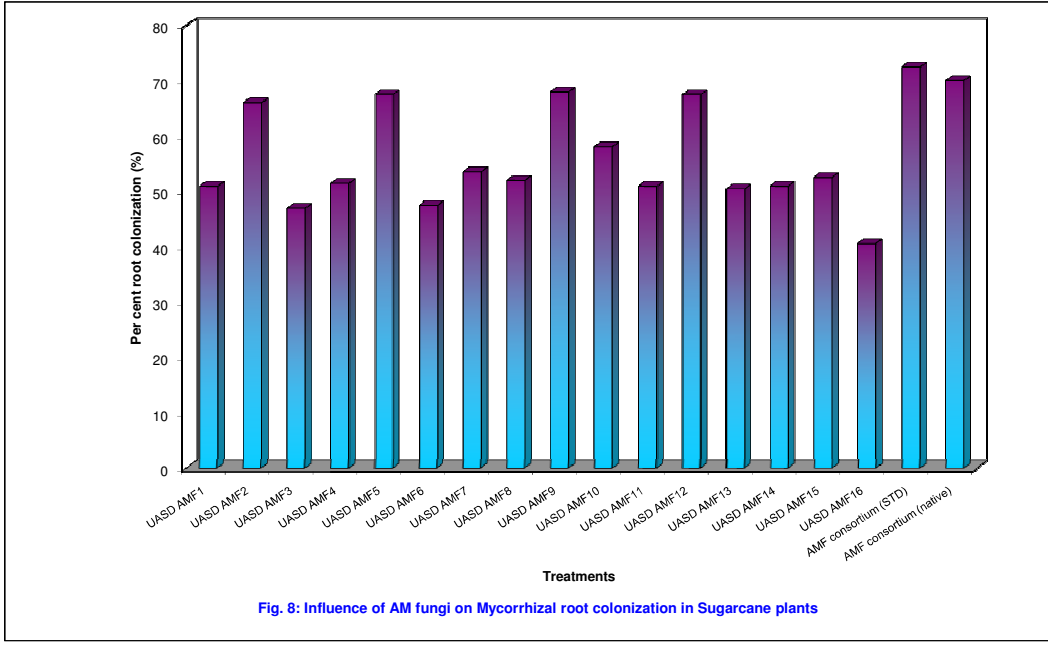


Fig. 8: Influence of AM fungi on Mycorrhizal root colonization in Sugarcane plants

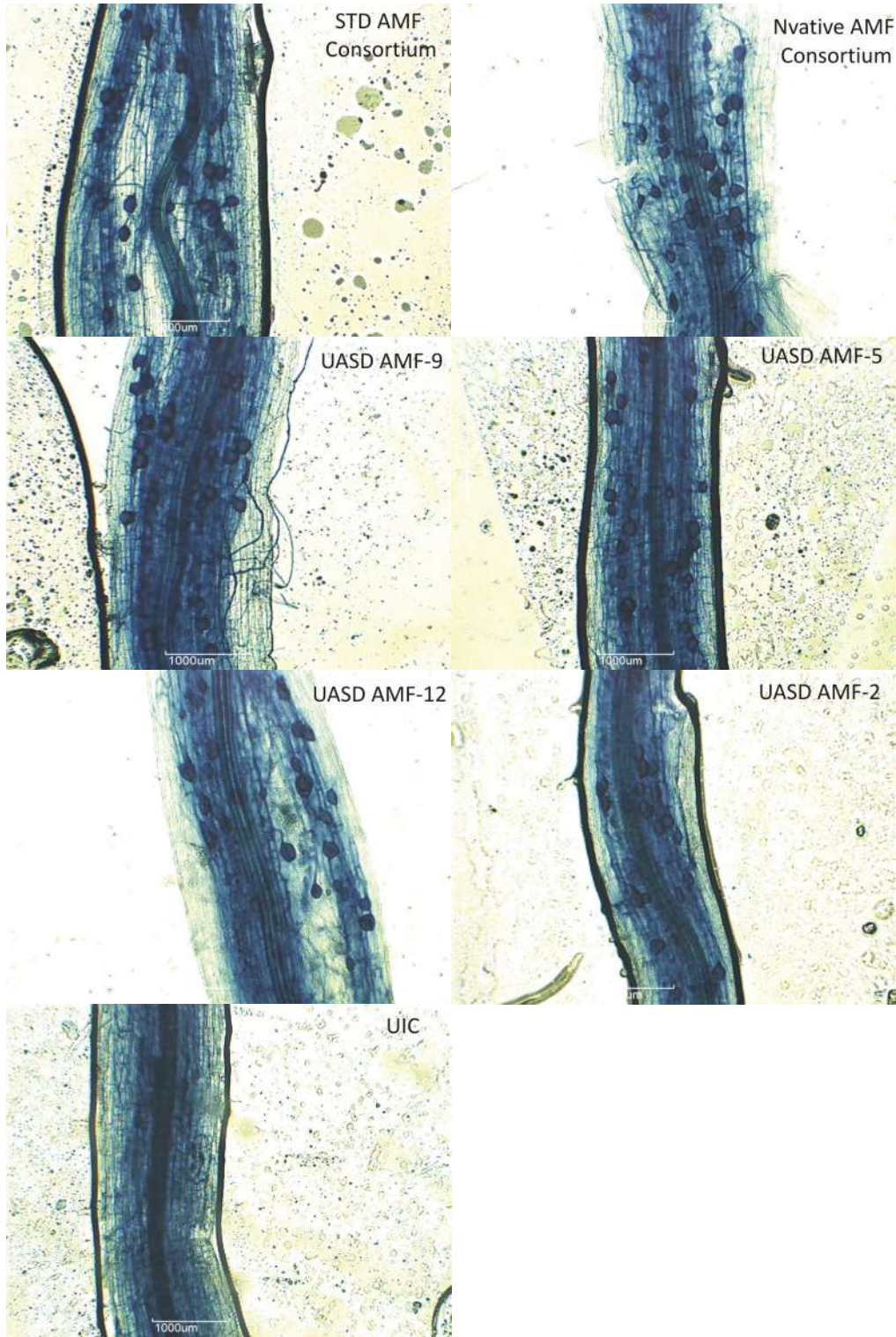


Plate 16 Sugar cane root colonization by native AMF isolates in presence of Striga

4.5 Influence of AM fungal isolates on soil enzyme activity

4.5.1 dehydrogenase activity ($\mu\text{g TPF formed g}^{-1} \text{ soil d}^{-1}$)

The dehydrogenase activity was recorded at different growth stages and presented in Table 12 and Fig 9.

The dehydrogenase activity was observed highest in treatments received AMF consortium (STD) and AMF consortium (native) compared to UIC at 30, 60, and 90 DAP.

At 120 DAP highest dehydrogenase activity was recorded in the plants received AMF consortium (STD) (14.29) followed by AMF consortium (native) (12.59 μg). Among individual native AMF isolates UASDAMF2 (12.44 μg) exhibited highest dehydrogenase activity followed by UASDAMF12 (11.99 μg), UASDAMF9 (11.87 μg) and UASDAMF5 (11.15 μg). The lowest dehydrogenase activity was observed in the treatment, which received UIC (4.15 μg).

4.5.2 Phosphatase activity ($\mu\text{g pnp released g}^{-1} \text{ soil h}^{-1}$).

The phosphatase activity was recorded at different growth stages and presented in Table13 and Fig 10.

At 120 DAP highest activity was recorded in the plants received AMF consortium (STD) (46.42 μg) followed by AMF consortium (native) (45.08 μg). Among individual native AMF isolates UASDAMF9 (12.44 μg) showed highest dehydrogenase activity followed by UASDAMF5 (11.99 μg), UASDAMF12 (11.87 μg) and UASDAMF 2 (11.15 μg). The lowest dehydrogenase activity was observed in the treatment, which received UIC (4.15 μg) followed by UASDAMF16 (4.80 μg) and UASD AMF11 (5.60 μg).

4.6 Chemical analysis of Sugarcane plants

The data on N and P content of sugarcane plants are presented in Table.14 and Fig. 11.

4.6.1 Total N content (%)

Table 12: The influence of AM fungi on dehydrogenase activity in sugarcane plants rhizosphere

Treatments	Dehydrogenase activity($\mu\text{g TPF formed g}^{-1} \text{ soil d}^{-1}$)			
	30 DAP	60 DAP	90 DAP	120 DAP
UASD AMF1	1.62	7.52	7.61	7.92
UASD AMF2	2.14	9.05	10.94	12.44
UASD AMF3	1.75	6.23	7.13	8.26
UASD AMF4	1.62	6.80	6.12	7.84
UASD AMF5	2.29	10.38	12.13	11.15
UASD AMF6	1.35	6.47	8.84	9.27
UASD AMF7	1.85	5.37	7.56	8.69
UASD AMF8	1.56	7.62	8.31	9.10
UASD AMF9	2.39	11.41	12.16	12.44
UASD AMF10	1.69	7.29	7.64	9.22
UASD AMF11	1.32	5.33	5.32	5.64
UASD AMF12	2.27	9.74	11.17	11.87
UASD AMF13	1.26	5.28	6.51	7.77
UASD AMF14	1.75	5.57	8.97	10.51
UASD AMF15	1.66	6.06	7.63	8.14
UASD AMF16	1.74	5.14	3.81	4.80
AMF consortium (STD)	2.93	13.2	14.16	14.29
AMF consortium (native)	2.41	11.79	12.30	12.59
UIC	1.16	4.61	4.67	4.15
S.Em \pm	0.11	0.15	0.21	0.29
CD(0.05)	0.31	0.46	0.61	0.87

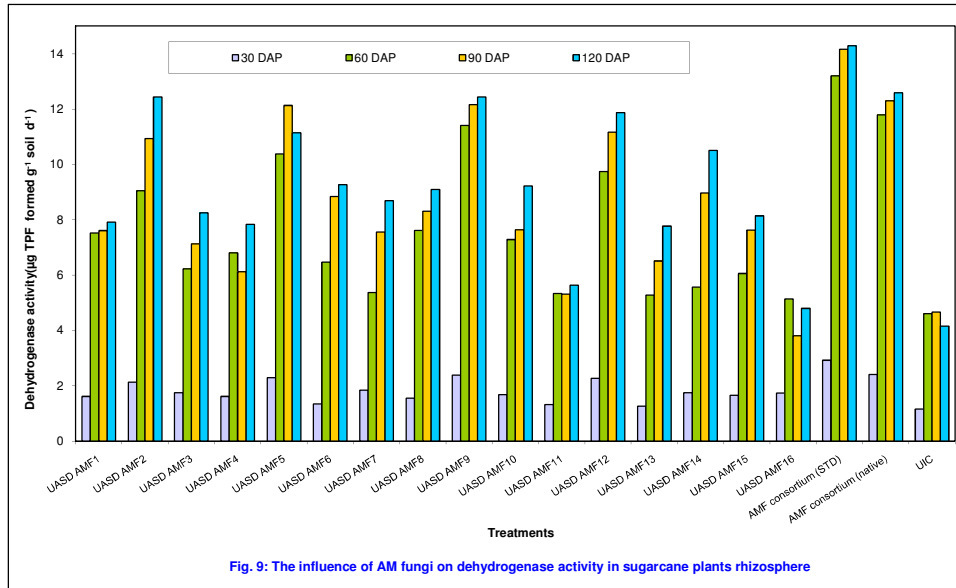
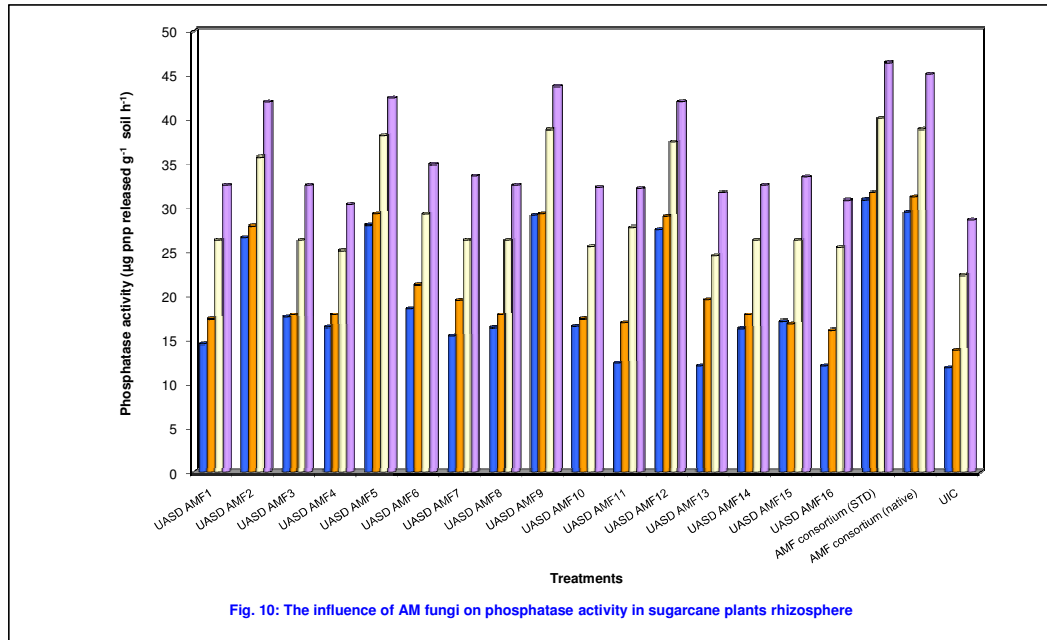


Table 13: The influence of AM fungi on phosphatase activity in sugarcane plants rhizosphere

Treatments	Phosphatase activity ($\mu\text{g pnp released g}^{-1} \text{ soil h}^{-1}$)			
	30 DAP	60 DAP	90 DAP	120 DAP
UASD AMF1	14.52	17.35	26.33	32.58
UASD AMF2	26.64	27.88	35.71	41.92
UASD AMF3	17.55	17.85	26.33	32.58
UASD AMF4	16.46	17.85	25.10	30.35
UASD AMF5	27.98	29.33	38.16	42.41
UASD AMF6	18.55	21.27	29.29	34.92
UASD AMF7	15.46	19.45	26.33	33.58
UASD AMF8	16.40	17.85	26.33	32.58
UASD AMF9	29.15	29.33	38.83	43.75
UASD AMF10	16.52	17.37	25.55	32.30
UASD AMF11	12.40	16.96	27.73	32.18
UASD AMF12	27.50	29.01	37.50	41.96
UASD AMF13	12.04	19.53	24.55	31.69
UASD AMF14	16.31	17.85	26.33	32.58
UASD AMF15	17.10	16.81	26.33	33.48
UASD AMF16	12.04	16.12	25.44	30.80
AMF consortium (STD)	30.85	31.69	40.17	46.42
AMF consortium (native).	29.51	31.15	38.86	45.08
UIC	11.81	13.83	22.32	28.57
S.Em\pm	0.54	0.75	1.01	1.19
CD(0.05)	1.55	2.24	2.99	3.54



The N content in the sugarcane plant was significantly increased due to the inoculation of native AM fungal isolates over uninoculated control.

Maximum N concentration was observed in the plants inoculated with AMF consortium (STD) (0.73 %) and AMF consortium (native) (0.69%). Among native isolates UASDAMF9 (0.62%) showed higher N content. The least N concentration was observed with UIC (0.24 %).

4.6.2 Total P content (%)

The P concentration in the plants inoculated with AMF consortium (STD) and AMF consortium (native) was found to increase significantly over UIC. The P concentration was highest in treatments received AMF consortium (STD) (0.60 %) and AMF consortium (native) (0.58%). Among native isolates UASDAMF9 (0.57 %) and UASDAMF5 (0.56 %) were found to influenced with more P content compared to UIC (0.31%).

Table 14: Influence of AM fungi on nitrogen and phosphorus content of sugarcane plants at 120 DAP

Treatments	N content (%)	P content (%)
UASD AMF1	0.46	0.42
UASD AMF2	0.53	0.55
UASD AMF3	0.41	0.44
UASD AMF4	0.29	0.37
UASD AMF5	0.54	0.56
UASD AMF6	0.29	0.35
UASD AMF7	0.33	0.36
UASD AMF8	0.35	0.36
UASD AMF9	0.62	0.57
UASD AMF10	0.36	0.35
UASD AMF11	0.35	0.36
UASD AMF12	0.59	0.55
UASD AMF13	0.28	0.36
UASD AMF14	0.37	0.45
UASD AMF15	0.29	0.40
UASD AMF16	0.27	0.38
AMF consortium (STD)	0.73	0.60
AMF consortium (native)	0.69	0.58
UIC	0.24	0.31
S.Em±	0.01	0.01
CD(0.05)	0.03	0.04

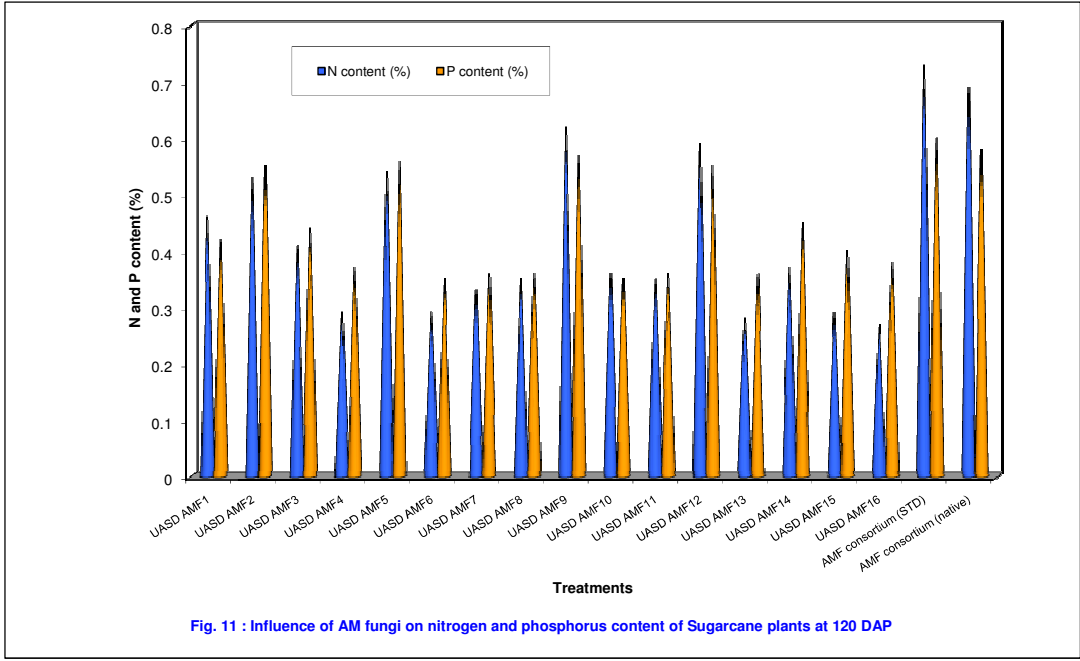


Fig. 11 : Influence of AM fungi on nitrogen and phosphorus content of Sugarcane plants at 120 DAP

5. DISCUSSION

Sugarcane is an important commercial crop of India, having 50.64 lakh hectare area with a productivity of 66.94 t/ha during 2012-13. Karnataka stands third position in area (4.25 lakh hectares). Year wise productivity (t/ha) of sugarcane crop showed a reduction during 2012-13 (84.07 t/h) compared to 2010-11 (93.76 t/ha). The reported cane yield losses is due to poor growth of Sugarcane.

The low yield of sugarcane may be attributed to many factors. One of the major causes for yield reduction in sugarcane is due to the significant losses caused by the root parasitic weeds such as *Striga*. *Striga* establishes directly over the vascular system of the host plant, drains out water and nutrients from the host, resulting in yield losses ranging from 15 to 75 per cent depending on the extent of infestation. Control of *Striga* has been very difficult because of its high fecundity. Each *Striga* plant is capable of producing 50,000 to 5, 00,000 minute seeds that may remain viable for more than 10 years in the soil (Bebawi *et al.*, 1984). Even at low levels of infection, damage to cereals can be substantial (Gurney *et al.*, 1999). Crop rotation and other chemical management practices have not been very effective in controlling the extent of *Striga* infestation. But, there are a number of resistance mechanisms of *Striga* which include low stimulant production, mechanical barriers to parasite ingress, chemical defense (antibiosis) in which microorganisms may produce certain chemical compounds to discourage the growth of *Striga* seedlings (Hassan *et al.*, 2009). Of late, there have been many reports on inhibition of germination of *Striga* by AM fungi (Lendzemo *et al.*, 2001), other fungi (Abbasher *et al.*, 1998; Hess *et al.*, 2002), rhizobacteria (Ahonsi *et al.*, 2002) and fluorescent pseudomonads (Berner *et al.*, 1999).

Some soils are known to be suppressive to *Striga*, and their suppressiveness was attributed to microbial population (Ciotola *et al.*, 1995). Report on natural biotic suppressiveness was found in Nigeria. It included soils from farmers' fields in 11 locations which showed a highly significant overall reduction in number of attached parasite (43%) in non-sterilized soils compared to sterilized soils which was attributed to soil suppressiveness of microbial

origin. In spite of this natural suppressiveness, cereals planted in these soils still suffered very low yields following *Striga* infestation.

Hence, AM fungi isolated from such suppressive soils were isolated and screened under pot cultures. The results from this preliminary study showed that mycorrhizal fungi have the potential to reduce damage by *Striga* sp.

The present pot culture study was conducted by using the *Striga* infested soil and native AM spores isolated from the *Striga* suppressive soil of sugarcane. It revealed that AMF consortium (STD), AMF consortium (native) and single native AM fungal isolates like UASDAMF9, UASDAMF5, UASDAMF2, UASDAMF12, completely reduced the *Striga* parameters like number of *Striga* emerged and biomass of *Striga* as compared to UIC. Reduced *Striga* number and biomass was also obtained earlier in maize inoculated with AMF (Othira *et al.*, 2012). Upon colonization by AMF, the quality and quantity of root exudates substantially changed. Maschner *et al.* (1997) observed that mycorrhizal colonization simplified the composition of the rhizosphere soil solution. Such community-wide changes may also affect the germination of *Striga* seeds. The observed decline in *Striga* in mycorrhizal maize could be attributed to increased nitrogen availability.

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 signaling molecules that play a vital role as germination stimulants of the parasitic *Striga*, *Orobanch*e and *Phelipanche* sp. and are exuded into the rhizosphere by the roots of their host 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.*, 2009). When plants are subjected to a shortage in the available phosphate the production and release of strigolactones into the rhizosphere are increased (Lopez-Raez, *et al.*, 2008; Yoneyama *et al.*, 2007). 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*, *Orobanch*e 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. Nitrogen is also traded in the symbiotic interaction and its deprivation also triggers an increase in the production of strigolactones in sorghum (Yoneyama *et al.*, 2001; Yoneyama *et al.*, 2007). Field experiments have shown that AM symbiosis delayed the emergence and reduced the number of *Striga* parasites on sorghum (Lendzemo *et al.*, 2005). Root exudates of sorghum plants colonized by AM fungi had less germination stimulatory activity (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 although uptake of the strigolactones by the AM fungi cannot be excluded as yet.

Walter *et al.* (2010) showed that an intermediate of the strigolactones biosynthetic pathway can also be converted into mycorradicin, an Apo carotenoid that accumulated in mycorrhized roots. Possibly, these two biosynthetic pathways competed with each other, which could result in reduced Strigolactones production upon AM colonization. The development of *Striga* on sorghum plants colonized by AM fungi was also shown to be delayed suggesting also AM fungi induced post-attachment resistance.

The results of our study indicated that AMF consortium (STD), AMF consortium (native) along with native AMF isolates UASDAMF9, UASDAMF5, UASDAMF12, and UASDAMF2 showed increased stimulation in all the growth parameters of sugarcane over uninoculated control at 30, 60, 90 and 120 DAP.

The inoculation of AMF consortium and native isolates *viz.*, UASDAMF9, UASDAMF 5, UASDAMF 12, and UASDAMF2 showed maximum increase in the plant height and stem girth over uninoculated control plants. Reduction in plant height in UIC could be associated with *Striga* damage as earlier reported by Olakojo *et al.* (2001) and Badu-Apraku *et al.* (2008) on maize varieties. The significant increase in plant height of sugarcane could be due to positive

interactions between the plant and AMF. This supported the view of Mohammad *et al.* (2003), Reider, (2003) and Salami *et al.* (2005).

Therefore, AMF should be combined with host-resistance approach in integrated *Striga* management. Improvement in the diameter and height has been observed earlier as well in case of tomato which was inoculated with *Glomus mosseae* (Liasu and Ogundola *et al.*, 2006). Inoculation of AMF isolates showed maximum increase in shoot length over control plants. These results are in confirmity with the findings of Duponnoisa *et al.* (2005). 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. Abdel and Mohamedin (2000); Franco and Garza (2006) also observed increased chlorophyll content. This may be due to the increased balanced mineral nutrients like P and K content in the leaves of mycorrhizal plants (Giri and Mukerjii, 2004). Zuccarini (2007) reported increased concentration of chlorophyll content and total foliar area due to mycorrhization in grapes. Further, they have also noticed an improvement in the chlorophyll contents (chlorophyll a, b and total chlorophyll).

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

Gworgwor and Weber (2003) reported that root dry matter of sorghum was not affected by *Striga* in any treatment, whereas shoot dry matter was significantly reduced, except in the presence of *Glomus mosseae* where the amount of shoot dry matter was restored to that of the control. *Glomus mosseae* was the effective AM fungal species and was effective in improving plant growth and in controlling *Striga*. The high shoot weight produced by sorghum inoculated with *Glomus mosseae* indicated an efficient compensatory effect of the AM in the presence of *Striga*, as well as the significant control of *Striga*.

The present study also revealed that the AMF consortium (STD), AMF consortium (native) and native AMF isolates UASDAMF9, UASDAMF5, UASDAMF12 and UASDAMF2 showed a marked increase in the shoot and root dry matter and total dry matter content of Sugarcane plants over uninoculated control. A significant increase in the growth parameters like plant height, chlorophyll content and stem girth in the inoculated plants, in the present investigation, has led to increased dry matter content of sugarcane. The increase in growth and biomass of inoculated plants strongly depends on their ability to access minerals from the soil. Therefore, positive effects of AM fungi on P content could be related to the ability of the AMF to enhance soil P (Smith *et al.*, 1985; Mali *et al.*, 2009 and Somchit *et al.*, 2008). Diop *et al.* (2003) reported that the indigenous AM fungi (*Glomus aggregatum*) significantly increased the shoot P, shoot N in *Solanum aethiopicum* cultivars, resulting in increased growth and biomass of the uninoculated plants.

In the present study, *Striga* susceptible sugarcane variety CO-86032, showed higher content of N and P content in the treatments which received AMF consortium (STD), AMF consortium (native), and native isolates UASDAMF9, UASDAMF5, UASDAMF12, UASDAMF2 compared to uninoculated control. Earlier studies by Lendzemo (2009) have similarly shown that species and strains of AMF increased nutrient uptake and plant growth. The function of intra-radical forms of AMF hyphae could also explain the status of the extraradical forms of AMF hyphae which could also explain differences in phosphorus acquisition among the AMF isolates. Hence, the status of the extraradical mycelium development in the soil appears to be a major determinant of the efficiency of AMF to phosphorus uptake. Jamal, (2014) found similar observations on soybean cultivars indicating that phosphorus uptake by mycorrhizal plants fluctuated with fungal isolates. In the presence of *Striga* also similar results have been obtained by Othila *et al.*, (2012). They found increased N and P content in both maize cultivars *viz.*, *Striga* susceptible Nyamilambo and *Striga* tolerant KSTP94 with the inoculation of *Glomus spp* over uninoculated control. The nitrogen and phosphorus transfer to the maize plants may also be a consequence of the fungal demand for the nutrients.

The perusal of data on spore count and per cent root colonization varied among the inoculated plants. The sugarcane inoculated with the AMF consortium (STD), AMF consortium (native) and four native isolates UASDAMF9, UASDAMF5, UASDAMF12, UASDAMF2 recorded increased number of spores and root colonization over UIC. 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. Tomato seedlings inoculated with *Glomus leptotichum* recorded higher root colonization percentage, growth and yield parameters. 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. Such increased root colonization levels in plants inoculated with AMF fungi have been reported by several workers (Jansa *et al.*, 2008; Meyer and Wooldridge, 2008 and Mali *et al.*, 2009).

The present experimental data revealed that the treatments which received AMF consortium (STD) and AMF consortium (native) showed maximum dehydrogenase and phosphatase activities compared to UIC. The activity of AM fungus and microorganisms present in the mycorrhizosphere could be a source of different soil enzymes required for biochemical reactions.

Furthermore, microorganisms in the rhizosphere may contribute to P nutrition through the synthesis and release of phosphatases when P is not available. The increase in dehydrogenase activity and bacterial counts during the development of mycorrhizal soil, suggests that the increase in phosphatase activity during development period is mediated by rhizospheric microorganisms. Mycorrhizal colonization clearly led to distinctive increase in the activities of enzymes involved in P dynamics.

Pacovsky *et al.*, (1991) revealed that an increased polyphosphate hydrolase activity in AM fungi associated roots, resulting in enhanced phosphate availability in the mycorrhizosphere. McDonald *et al.* (1978) reported higher dehydrogenase and phosphatase in rhizosphere of *Glomus mossae* treated plants. Tisserant *et al.* (1993) revealed the presence of alkaline phosphatase in *Glomus* infected roots of *Platanus acerifolia*. Selvaraj (1998) reported an increased level of alkaline phosphatase activity in *G. fasciculatum* inoculated roots

of *P. juliflora*. Dubey and Fulekar (2011) conducted an experiment on soil based mycorrhizal inoculum with sorghum as the host plant in green house for a period of 75 days using pot culture technique. The physicochemical characteristics, mycorrhizal status, acid phosphatase activity, alkaline phosphatase activity, dehydrogenase activity were assessed at a frequency of 15 days in the rhizospheric soil samples. Development of mycorrhiza was characterized by AM spore count and colonized root length percentage in Sorghum. Phosphatase and microbial activity (as represented by viable counts and dehydrogenase activity) were also found to be increasing during the process of mycorrhiza development. In the same way, the results of the present investigation have clearly indicated a stimulatory effect of efficient native AMF fungal isolates on soil enzyme activity viz., dehydrogenase, and phosphatase.

6. SUMMARY AND CONCLUSIONS

An attempt was made to isolate and screen AM fungi from the *Striga* suppressive soil samples of Sugarcane field collected from Yergatti village of Belgaum district. Furthermore, the AMF isolates were also screened for their ability to reduce the parasitism of *Striga* on Sugarcane under pot culture studies.

- As many 16 AMF isolates were isolated from *Striga* suppressive soils. Out of these twelve AMF isolates belong to the genus *Glomus* and four were *Acaulospora*.
- The results of the pot experiment have revealed that the native AMF isolates UASDAMF9, UASDAMF5, UASDAMF12, UASDAMF2, AMF consortium (STD) and AMF consortium (native) suppressed the *Striga* parameters viz., *Striga* emergence, *Striga* number, and *Striga* biomass.
- The experiment revealed that the AMF consortium (STD), AMF consortium (native) and native isolates viz., UASDAMF9, UASDAMF5, UASDAMF12, and UASDAMF2 stimulated the plant growth parameters, plant biomass and physiological parameters of sugarcane over uninoculated plants in the presence of *Striga*.
- The N and P content was found to be increased significantly due to the application of AMF consortium (STD), AMF consortium (native) and native AMF isolates, UASDAMF9, UASDAMF5, UASDAMF12, and UASDAMF2 over UIC.
- The shoot and root dry weight of sugarcane was found to increase significantly due to the inoculation of AM fungal isolates as compared to UIC. AMF consortium (STD) (178 g) and AMF consortium (native) (167 g) showed significant increase in total biomass.
- The chlorophyll content and biophysical characters of Sugarcane plants were found maximum in the treatments which received AMF consortium (STD), AMF consortium (native), and native isolates UASDAMF9, UASDAMF5, UASDAMF12 and UASDAMF2.

- Leaf temperature and transpiration rate were maximum in UIC, UASDAMF16, UASDAMF13 isolates while AMF consortium (STD) and AMF consortium (native) recorded lower leaf temperature.
- Inoculation of AMF isolates significantly improved the spore count and per cent root infection over the uninoculated plants. The maximum spore count and per cent root infection were recorded in the treatment which received AMF consortium (STD) (687spores/50g), AMF consortium (native) (668 spores/50 g) and native isolates viz., UASDAMF9 , UASDAMF5, UASDAMF12, and UASDAMF2
- In general, the inoculation of AM fungal isolates significantly influenced the soil enzyme activities. The highest dehydrogenase and phosphatase activities were recorded in the treatment which received AMF consortium (STD) and AMF consortium (native).
- The present study has indicated that UASDAMF9, UASDAMF5, UASDAMF12 and UASDAMF2 isolates are quite promising in inhibiting *Striga* seed germination similar to AMF consortium (STD) and AMF consortium (native) under pot culture studies and also these isolates were found to be efficient in increasing the growth parameters, biomass and nutrient status of sugarcane plants.
- Thus, it can be concluded that some *Striga* suppressive soils harbor efficient native AMF isolates which naturally suppress the *Striga* germination and it is possible to isolate these native AMF strains and used as tool in the *Striga* management.

Future line of work

1. The mechanism of *Striga* suppression by AM fungi need to be elucidated.
2. There is need to develop the integrated technologies for using efficient native mycorrhiza under field conditions for effective control of *Striga*.
3. To standardize the methods of application of AMF for effective suppression of *Striga* under field condition.
4. Molecular characterization of efficient native AMF isolates.

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Appendix I: Hogland nutrient solution

Macronutrients	
Ca(NO ₃)	0.94g
KNO ₃	0.615g
MgSO ₄ .7H ₂ O	0.52g
EDTA	0.0373g
FeSO ₄ .7H ₂ O	0.0278g
Micronutrients	
H ₃ BO ₃	0.028g
MnSO ₄	0.034g
ZnSO ₄ .7H ₂ O	0.022g
(NH ₄) ₆ MO ₇ O ₂₄ .4H ₂ O	0.001g
CuSO ₄ .5H ₂ O	0.001g
H ₂ SO ₄	5ml
Distilled water	1000 ml

ISOLATION AND SCREENING OF ARBUSCULAR MYCORRHIZAL FUNGI FOR SUPPRESSION OF *STRIGA*, A PARASITIC WEED IN SUGARCANE

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ABSTRACT

An investigation was carried out to isolate native AM fungal (AMF) isolates from the *Striga* suppressive soils of sugarcane growing areas of northern Karnataka. Further, sixteen native AMF isolates were screened for their ability to suppress *Striga* as well as plant growth promotional abilities in sugarcane under pot culture studies. The pots were filled with *Striga* infested soil prior to the planting of sugarcane sets of equal bud size. AMF inoculum @ 150 g pot⁻¹ was mixed thoroughly with the top 10 to 15 cm of the soil and pots were treated with Hoagland's nutrient solution once a week. The data on the emergence of *Striga*, *Striga* biomass, plant height, chlorophyll content and soil microbiological activities were recorded. The *striga* emergence was significantly inhibited in the treatment received standard AMF consortium, followed by AMF native consortium, UASDAMF -2, UASDAMF-5, UASDAMF-9 and UASDAMF-12. While, the UIC recorded highest number of *striga* infestation. Furthermore, the plant height, chlorophyll content, soil enzyme activities and spore load/ 50gm of soil were recorded highest with native AMF consortium over uninoculated control. Thus, our preliminary findings are indicative of the effectiveness of AMF in protecting Sugarcane against *Striga* infestation and hence can be a promising strategy to develop a biological tool for *Striga* control.