STRIGA (STRIGA ASIATICA), A PARASITIC WEED INHIBITION BY ARBASCULAR MYCORRHIZAL FUNGI IN SUGARCANE (SACCHARUM OFFICINARUM)

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Abstract

Three Arbuscular Mycorrhizal Fungal (AMF) species Gigaspora margarita, Glomus macrocarpum and Acaulospora laevis along with AMF consortium (above mentioned three species) were compared against a check [without Arbuscular Mycorrhizal (AM) fungi] for the biological control of Striga asiatica in sugarcane (Saccharum officinarum) crop. The experiment was carried out in 23-cm-diameter pots under the controlled growth chamber. The experimental results have clearly revealed that the application of AMF consortium suppressed the striga emergence to a greater extent compared to rest of the treatments. The highest number of striga emerged was observed in the pots which received striga alone (0.35). With respect to the mycorrhizal parameters viz., root colonization and spore count was highest in the treatment received AMF consortium (75.75 percent and 510.25 spore count per 50g of soil respectively). Similarly, plants received AMF consortium recorded highest plant height and total dry matter. 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.

Keywords: Arbuscular mycorrhizal fungi, Striga asiatica, Sugarcane.

I. INTRODUCTION

Sugarcane is an important commercial crop of India occupying around 3.8 million hectares of land with an annual cane production of around 270 million tonnes. According to DES IVth Adv Estimate for 2012-13, the statistical analysis for year wise productivity (tonnes/ha) of sugarcane crop in Karnataka reduced during 2012-13 (84.07 tonnes/ha) compared to 2010-11 (93.76 tonnes/ha). The reduction in productivity was caused due to the deficiency of soil fertility, lack of nutrition supply, disease incidence (rust, leaf spot), insect incidence (Shoot borer etc.) and parasitic weed infestation (Striga etc).

Striga, is a root parasite of cereals and legumes, has attracted much attention of late, as a causative agent for serious lose in crop production in the semi-arid tropics. The life cycle of Striga is mainly dependent on that of its host. Approximately 75% of the overall Striga damage to the host is made during its subterranean stage of development (Bebawi et al., 1984; Parker and Riches, 1993). Rank et al. (2004) demonstrated that Striga exerts a potent phytotoxicity effect on the host. Managing Striga below ground is therefore a crucial task for successful Striga management.

The control of striga is difficult to achieve because of its high fecundity. In addition, seed germination is asynchronous (Worsham and Egley, 1990). Therefore, management of striga infestation needs an integrated approach including host plant resistance, cultural practices, and chemical and biological treatments. Among all the components of this integrated Striga management, biological control could be used in Africa by resource-poor farmers if it gives a demonstrable crop – yield benefit within one growing season (Ahonsi et al., 2002). However, these Striga control methods have given no conclusive and consistent feasible results for the peasant farmer.
Thus, in order to prevent the weed menace as well as to prevent the environmental pollution by herbicides, the biotic interaction is required for effective and sustainable management of weed infestation and which will be a boon to sorghum and sugarcane growing farming community of northern Karnataka wherein devastating losses of yield due to Striga infestations are recorded in recent times. In this regard an attempt has made to use the beneficial microbial community for the control of Striga weed in the sugar cane crop.

Strigaseeds will not germinate unless they are exposed to chemical stimuli, ‘germination stimulants’ produced by and released from plant roots. Most of the germination stimulants identified so far are strigolactones (SLs), which also function as host recognition signals for arbuscular mycorrhizal fungi and a novel class of plant hormones inhibiting shoot branching. Sugarcane is the C-4 Crop which intern produces the strigolactone as its secondary metabolite from roots. 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.

Arbuscular Mycorrhizal Fungi are characterized by the production of intracellular absorptive structures (vesicles, arbuscules) and are the most widespread of soil fungi followed by ectomycorrhizal fungi (Schüßler et al., 2001). The external mycelium of arbuscular mycorrhizal fungi (AMF) acts as an extension of host plant roots and serves as a direct link between roots and soil nutrient reserves. The mutualistic symbiosis between AMF and crop plants results in increased uptake of phosphorus, potassium, nitrogen, and other nutrients, increased growth at high soil temperatures, better growth in low moisture soils, more efficient water utilization, increased levels of cytokinins, and also increased photosynthetic rates and stomatal conductance (Lendzemo, 2004).

Several species of mycorrhizal fungi have also been shown to increase plant biomass and compensate for damage by Striga asiatica, and their metabolites either stimulate or inhibit weed germination in sugarcane variety CO86032. This could become a useful biotic interaction for effective and sustainable integrated management of S. asiatica infestation for the resource-poor farming situations. This study investigated the effectiveness of AMF inoculation on growth and development of sugarcane under S. asiatica infestation.

II. MATERIALS AND METHODS

2.1 Sources of biological materials

Pure isolates of AMF, Gigaspora margarita, Acaulospora laevis, Glomus macrocarpum and AMF consortium of all three were maintained in the weed control scheme, University of Agricultural Science, Dharwad, Karnataka, India and used for the proposed work. Further, striga infected soil were collected from the sugarcane farmers field at yeragatti taluk, Belgavi, Karnataka located at 16° 00’ 46.7” N latitude ; 75° 00’ 09.9” E longitude, and at an altitude of 674 m above mean sea level.
2.2 Experimental details:

Hence, a pot experiment study was undertaken at Main Agricultural Research Station during the period 2013 and 2014 at UAS, Dharwad to assess the effectiveness of AMF inoculation on the suppression of *striga* in sugarcane.

![Influence of AMF on sugarcane plant height (cm)](attachment://influence_of_amf_on_sugarcane_plant_height.png)

Fig.2 Influence of AMF spore load on sugarcane plant height (cm)

An experiment was laid out in Completely Randomised Design (CRD) during 2013-2014 with four replications and five treatments comprising of the standard AMF strains viz., T₁: *Gigaspora margarita*; T₂: *Acaulospora laevis*; T₃: *Glomus macrocarpum*; and T₅: AMF consortium comprising of all three AM fungal strains. The pots were filled with *Striga* infested soil followed by mixing of AMF inoculum as per the treatment schedule before planting of sugarcane. The plants were grown under conducive environment for four months and were watered twice a week with distilled water and Hoagland’s nutrient solution.

![Influence of AMF on chlorophyll content and plant dry matter(gm)](attachment://influence_of_amf_on_chlorophyll_content_and_plant_dry_matter.png)

Fig.3 Influence of AMF spore load on sugarcane chlorophyll content and dry matter

The data on the emergence of *striga*, AM fungal spore count, percent root infection, plant height, dry matter, chlorophyll content, Dehydrogenase enzyme assay, per cent of N and P were recorded in sugarcane.

2.3 Growth parameter

The numbers of *striga* emerged per pots were counted and the plant height was measured from ground level to the tip of the main stem on 90 and 120 days after sowing in five pots per treatment. The chlorophyll content was measured using SPAD meter and the harvested plant samples were air dried for 2 Days followed by the hot air drying at 55°C till samples get dried using hot air oven. Further, plant dry matters per plant were weighed using weighing balance.

2.4 Estimation of dehydrogenase activity:

Dehydrogenase activity in the soil samples was determined as per the procedure described by Casida *et al.* (1964). The intensity of red colored product was measured at 485 nm against methanol as blank using UV-VIS spectrophotometer. (Thermo Scientific, USA).
2.5 Arbuscular Mycorrhizal spore count
The chlamydospores in sugarcane rhizosphere soil were determined by using wet sieving and decantation method as outlined by Gerdemann and Nicholson (1963). Fifty gram of soil sample was taken and the soil suspension was passed through a series of different size sieves (250µm, 106µm, 75µm, 45µm, 37µm) arranged in the descending order of their mesh size.

![Influence of AMF spore load on dehydrogenase activity of sugarcane rhizosphere.](image)

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 stereo zoom microscope (LABOMED).

2.6 Per cent root colonization:
Mycorrhizal root colonization was determined as per the procedure proposed by Philips and Hayman (1970). 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:

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

2.7 Mineral content
2.7.1 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 H₂SO₄ 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 H₂SO₄. 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.

2.7.2 Estimation of phosphorus
2.7.2.1 Pre digestion
Five hundred mg of plant sample was taken in a 250 ml conical flask and 2.5 ml of concentrated HNO₃ 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.

2.7.2.2 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 up to 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.

2.7.2.3 Standard curve:
For obtaining the standard curve, 0.439g of KH$_2$PO$_4$ was dissolved in distilled water and the volume was made up to 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 flask including blank. The volume was made up to 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.

III. RESULTS AND DISCUSSION

The present investigation encompasses screening of 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.

3.1 AMF root colonization and AMF spore count in sugarcane Rhizosphere.
Typical AMF structures, arbuscules, vesicles and hyphae were regularly found in stained roots. Thus, establishment of AMF was effective within roots of Sugarcane. In the treatment receiving AMF consortium per cent AMF root colonization and number of AMF spore per 50 gm of soil were recorded highest i.e., 510.25 and 75.75 per cent respectively (Table 1), followed by the treatment receiving Glomus Macrocarpum (462.50 spore load/ 50gm soil and 67.50 per cent root colonization).

**Table 1.** Influence of arbuscular mycorrhizal fungi on Striga emergence, AMF root colonization and AMF spore count in sugarcane crop.

| Treatment                      | No. of striga/pot (cm) | Percent of AMF root infection (%) | No.of AMF Spore load/50g of soil |
|--------------------------------|------------------------|----------------------------------|---------------------------------|
| T1: without AMF culture + Striga | 1.75 3.25              | 37.50                            | 358.50                          |
| T2: Gigaspora margarita + Striga | 1.25 0.75              | 57.50                            | 363.25                          |
| T3: Acaulospora laevis + Striga  | 1.25 1.25              | 61.25                            | 374.00                          |
| T4: Glomus macrocarpum + Striga  | 0.75 0.75              | 67.50                            | 462.50                          |
| T5: AMF consortium + Striga     | 0.00 0.00              | 75.75                            | 510.25                          |
| Sem±                            | 0.32 0.36              | 3.81                             | 11.99                           |
| CD                              | 0.99 1.11              | 11.73                            | 36.95                           |

In the single AMF spp.Glomus spp. has the ability to infect and colonize plant roots faster than Aculospora and Gigaspora spp., making it highly competitive (Kurle and Pfleger, 1994). The
higher mycorrhizal colonization in *Striga*-susceptible sugarcane could be due to strigolactones root exuded by host plant roots and taken up by AMF since strigolactones stimulate fungal metabolism and branching (Parniske, 2008). Strigolactones, a novel class of phytohormones involved in the regulation of shoot branching in plants are secreted by plant roots and stimulates presymbiotic growth of AMF.

Fig. 5 Influence of Mychorhizha on minerals (Nitrogen and phosphorous) content of sugarcane (cm)

Strigolactones are also plant signal molecules initially characterized as seed germination stimulants for the parasitic weeds *Orobanche* and *Striga*. (Cook C E et al., 1966). Thus in the presence of *S. asiatica* infestation (T1- Absence of AMF), percent mycorrhizal colonization reduced to 37.50% and the number of AMF spore count reduced to 358.50.

### 3.2 Influence of AMF on Number of *Striga* emergence.

The results revealed that the number of *striga* emergence was completely vanished in the treatment receiving AMF consortium (i.e., 0.00) which contains 510.25 AMF spore load per 50 gm of soil and 75.75 per cent of root colonization (Table 1). In the treatment receiving *Glomus macrocarpum*, *striga* emergence was recorded 0.75 which contain 462.50 spore load per 50 gm of soil and 67.50 per cent of AMF root colonization. The highest number of *striga* was emerged in the treatment T1: without AMF culture (1.75 and 3.25 in 90 and 120 DAS respectively) where the spore load and AMF root colonization was lowest compared to other treatment (358.50 and 37.50 per cent respectively). In the treatment receiving *Gigaspora margarita* and *Acaulospora Laevis* the striga emergence was recorded on par with each other.

The ability of AMF to reduce *striga* count can be explained in three ways: (i) The formation of metabolites, especially strigolactones that are responsible for the induction of *striga* germination is down regulated upon mycorrhizal colonization; (ii) Plant metabolites, such as cyclohexenones which arise through carotenoid degradation, that are up-regulated upon mycorrhizal colonization inhibit *striga* germination and; (iii) Mycorrhizal colonization induces mycorrhizosphere effects that negatively impact on *striga* germination (Lendzemo et al., 2007). These results clearly indicated that the bio-control of *stiga* using AMF spp. is a promising strategy to the farmers.

### 3.3 Influence of AMF inoculation on mineral content of sugarcane crop.

In absence of *striga* infestation, colonization with *AMF consortium* increased nitrogen and phosphorous content in sugarcane were recorded 0.91 and 0.78 percent (Table 2).

#### Table 2. Influence of Arbuscular Mycorrhizal Fungi on Dehydrogenase activity, nitrogen percent and phosphorous content of sugarcane crop.

| Treatment       | Dehydrogenase activity (µg TPF formed g⁻¹ soil d⁻¹) | Nitrogen content (%) | Phosphorous content (%) |
|-----------------|---------------------------------------------------|----------------------|-------------------------|
| T1: without AMF culture | 1.99                                               | 0.35                 | 0.37                    |
The N content was recorded in the treatment receiving *G. macrocarpum*, *A. Leavis* and *G. margirita* were on par with each other (0.71, 0.50 and 0.45 per cent). Further, the absence of AMF spp. reduced the percentage of N and P in the sugarcane crop i.e., 0.35 and 0.37 per cent respectively. Earlier studies by Bethlenfalvay (1993) have similarly shown that species and strains of AMF differ in their effectiveness in increasing nutrient uptake and plant growth. The function of extra- and intra-radical forms of AMF hyphae could also explain differences in phosphorus acquisition among the AMF isolates. Hence, the status of the extra-radical mycelium development in the soil appears to be a major determinant of the efficiency of AMF to phosphorus uptake (Rakshit and Bhandoria, 2009). Similar results have been found on soybean cultivars indicating that phosphorus uptake by mycorrhizal plants fluctuate with fungal isolates and genetic variability within cultivars (Diop et al., 2003).

The nitrogen and phosphorus transfer to the sugarcane plants may also be as a consequence of the fungal demand for the nutrients, with both host plant and fungus evolving transporters to take advantage of localized increases in nutrients.

### 3.4 Influence of AMF inoculation on dehydrogenase enzyme activity of sugarcane rhizosphere soil.

Dehydrogenase activity in soil has been used as a measure for overall microbial activity (Chendrayan et al 1979). Thus, the dehydrogenase activity was reduced in the treatment absence of AMF spp. (1.99 μg TPF formed g⁻¹ soil d⁻¹) due to the high number of *striga* emergence. While, in the AMF Consortium highest dehydrogenase activity was recorded followed by *G. macrocarpum* (6.19 and 5.58 μg TPF formed g⁻¹ soil d⁻¹ respectively). These results indicate that the enzyme activity increased in the presence of AMF spp., and reduced in the absence (Table 2).

### 3.5 Influence of AMF inoculation on Sugarcane growth.

The AMF inoculation had significant positive effect on plant growth parameters in absence of *striga* infestation. The sugarcane plant height was benefited by the AMF consortium followed by the *G. macrocarpum* compared to other treatment (Table 3).

**Table 3.** Influence of Arbuscular Mycorrhizal fungi on plant height, plant dry matter and chlorophyll content of sugarcane crop.

| Treatment          | Crop plant height (cm) | Dry Matter (gm) | Chlorophyll content (SPAD) |
|--------------------|------------------------|-----------------|----------------------------|
|                    | 90 DAS                 | 120 DAS         |                            |
| T1: without AMF culture | 107.50                | 134.50          | 8.58                       | 39.75                     |
| T2: *Gigaspora margarita* | 120.00                | 144.75          | 18.68                      | 42.78                     |
| T3: *Acaulospora laevis* | 128.50                | 146.50          | 18.89                      | 42.05                     |
| T4: *Glomus*         | 136.50                | 149.00          | 28.10                      | 45.55                     |
The crop plant height (144.75 cm and 152cm @ 90 and 120 DAS respectively), dry matter and chlorophyll content was observed high (36.66 gm @ harvesting and 48.75 @ flowering Stage respectively) in T5 (AMF Consortium) treatment. This was followed by the treatment received *G. macrocarpum* where the plant height was 136.50 and 149.00 cm at 90 and 120 DAS respectively. The dry matter and the chlorophyll content were recorded 28.10gm and 45.55 respectively. The lowest plant height, dry matter and chlorophyll content were observed in the treatment T1 without AMF culture (107.50cm, 134.50 cm, 8.58 gm and 39.75 respectively).

The present investigation revealed that the phytotoxic effect of *striga* on sugarcane was significantly controlled by the AMF consortium as well as by *G. macrocarpum* alone treatment. AMF consortium influenced great increase in the plant growth parameters compared to other treatments. In the single AMF cultures *G. macrocarpum* has shown more influence on the *striga* inhibition as well as in the plant growth promotion activities. Compared to other AMF species Glomus species will multiply faster in soil. Thus the spore load and the AMF root infection percent was high in this treatment compared to other AMF species. In general were the spore load was high there the number *striga* emergence was totally absent. The overall results revealed that the AMF will inhibit the *striga* emergence @ 450 to 550 spore load per 50gm of soil. Finally, these results conclude the AMF consortium inhibit the *striga* meticulously and the high spore load will inhibit the *striga* and promote the plant growth.

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.

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