EFFECT OF ARBUSCULAR MYCORRHIZAL FUNGI (AMF) ON GROWTH AND YIELD OF SUNFLOWER (*Helianthus annuus* L.)

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KEYWORDS

*Helianthus annuus*

AM fungi

Growth parameters

Seed yield

ABSTRACT

The arbuscular mycorrhizal fungi (AMF) are a group of plant growth promoting organisms related to improve the overall growth of various crops. Hence the present study was aimed to investigate the agronomical characteristics induced by AMF in sunflower (*Helianthus annuus* L.). Three different indigenous AM fungi such as *Glomus mosseae*, *Glomus fasciculatum*, *Acalospora scrobiculata* isolated from the sunflower rhizosphere soil were used either alone or in various combinations for the study. The plants were sampled randomly at 40DAI, 60DAI, 85 DAI and used to analyze the number of leaves, root biomass, shoot biomass, root length, shoot length, head diameter and seeds weights. The obtained results clearly indicated that the AM significantly influenced all the morphological parameters when compared to uninoculated control.

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1 Introduction

Sunflower (Helianthus annuus L.), a member of compositae family is the most important edible oilseed crop in the world (Weiss, 2000). Which is mainly grown in Rabi season as a rain fed crop mostly by marginal farmers. Sunflower seed has high concentration of linolic acid, a poly unsaturated fatty acid is essential for human beings and cannot be synthesized by animal body (Seiler, 2007). The rhizosphere, representing the thin layer of soil surrounding plant root zone and soil occupied by the roots, supports large and metabolically active groups of micro organisms such as bacteria, fungi, actinomycetes and mycorrhizae (Balagurunathan et al., 2012). These microorganisms deliver positive impact on growth and yield of crop plants through several mechanisms such as 1) N₂ fixation, 2) induction of IAA, 3) mineralization of phosphates, 4) secreting antimicrobial substances. Micorrhiza is symbiotic association between the soil fungi and roots of higher plants (Smith et al., 2010). These fungi enhance the plant growth through making availability of mineral nutrients such as P, Zn and Cu (Phiri et al., 2003). Colonization of AM fungi in cortical tissues of sunflower increased growth parameters of sunflower (Jalaluddin & Hamid, 2011). It has been recently reported that AMF play a vital role in protecting plant from soil borne pathogen and root-knot causing nematode (Jalaluddin et al., 2008). Hence an attempt was made to reveal the effect AM fungi on the agronomical characteristics of sunflower which is more economic and eco friendly.

2 Materials and Methods

2.1 preparations of AM fungus inoculums

Onion (Allium cepa L.) plants were used as host for AM fungal inoculums preparations. Three dominant indigenous AM fungi such as Glomus mosseae, G. fasiculatum, A. scrobiculata were used for study. The efficient strains of AM fungi were isolated from the rhizospheric soil sample of higher plant. The AMF propagules were obtained from the soil by ‘Wet Sieving and Decanting Method’ (Gerdemann & Nicolson, 1963). The initial inoculum of AM fungus (G. mosseae, G. fasciculatum, A. scrobiculata) was raised by ‘Funnel Technique’ (Menge & Timmer, 1982) using Onion as host. After 40 days, seedling roots were processed to study AM colonization (Phillips & Hayman, 1970) and soil samples were studied for spore quantification.

2.2 Pot and Potting Mixture

Pots of 35×25 cm size were selected for the experiment. Pots were filled with sterilized sand: soil (1: 3). A layer of inoculum consisting of AM colonized root pieces and soil containing spores were spread over the pot mixture. The sunflower seeds were surface sterilized with 0.1% mercuric chloride solution, containing Tween -80 as surfactant, for three minutes and washed with sterile water. Five seeds were sown in each earthen pot separately. Seedlings without mycorrhizal inoculums severed as control. The following treatments were studied in triplicates.

Control (uninoculated plants)

G. mosseae alone
G. fasciculatum alone
A. scrobiculata alone
G. mosseae + G. fasciculatum
G. mosseae + A. scrobiculata
G. fasciculatum + A. scrobiculata
G. mosseae + G. fasciculatum + A. scrobiculata

2.3 Evaluation of agronomical characters

The tested plants were harvested at 40, 60 and 85days after inoculation (DAI) and used for measuring various parameter analyses like the shoot (cm), root length (cm), fresh and dry weight (mg /g), the number of leaves per plant, number of seeds per head, head diameter, hundred seeds weight were determined.

2.4 Statistical analysis

The data were analyzed using Annova version 11.5. The mean values are ranked using Duncan’s Multiple Range Test, (DMRT).

3 Results and Discussion

3.1 Effect of AMF on the number of leaves

It was found that the leaf number was greater in the plant treated with AM fungi than the untreated control and significantly varied among the treatments at 40DAI, 60DAI, 85DAI. The individual inoculation of A. scrobiculata had greater influence on the number of leaves (9.66±0.57) at 40DAI. However the trend changed while sampling at 60 DAI and 85 DAI where G. fasciculatum (12±1.5) and G. mosseae (15.67±0.58) showed more number of leaves, respectively. G. fasciculatum had higher influence when it was combined with A. scrobiculata at 40DAI (11.66±0.57) and at 85DAS (18±2.00) but at 60DAI the combination of G. fasciculatum A. scrobiculata and G. mosseae (16±1.00) gave better result (Table-2). Findings of present study are in conformity with the finding of Boureima et al. (2007) who have revealed that AM fungi inoculation significantly increased the number of leaves in Sesamum indicum in response to Glomus mosseae (86%) and G. fasciculatum (67%).

3.2 Influence of AMF on Shoot biomass

It has been reported that phosphorus is very essential for the synthesis of nucleic acids and other biomolecules and P deficiency suppress the growth and yield of sunflower (Zabillaga et al., 2002). The increase in shoot biomass due to G. epigaem inoculation could be attributed to the fact that
mycorrhizal fungi generally enhance nutrient element uptake especially immobile elements such as P (Motosugi et al., 2002; Riaz, 2007). AM fungi inoculation had significant influence on root and shoot dry weight of red kidney and wheat plants (Rabie, 2005). In this investigation the shoot biomass was greater in the combined inoculation than the single and uninoculated plants. The positive impacts of AMF on growth and biomass yield have been shown by earlier studies (Hemashenpagam & Selvaraj, 2011).

3.3 Influence of AMF on root length

AMF colonization facilitates the nutrient absorption due to increase in root surface area (Khan et al., 2000). The root length was increased when the plant inoculated with \( G. \text{fasciculatum} \) alone (8.23±0.46cm) and the triple inoculation of \( G. \text{fasciculatum} + A. \text{scrobiculata} + G. \text{mosseae} \) (8.76±0.47cm) after 40 days of inoculation. The root length were significantly with in the treatment at all the sampling. The combined inoculation of \( G. \text{mosseae} \) with \( G. \text{fasciculatum} \) and \( A. \text{scrobiculata} \) enhanced the root length at 60DAI sampling. The combined inoculation of \( G. \text{fasciculatum} \) with \( A. \text{scrobiculata} \) increased the root length up to 16±0.5cm at 85DAI (Table- 3), thereby facilitated the better uptake of nutrients. The nutrient uptake by AM fungi inoculated plants has been well documented over control. This results conforms the findings of Moradi et al.(2013), who have observed that either the application of \( G. \text{mosseae} \), \( G. \text{intraaridices} \) alone or combination of these two AM fungus significantly increased the root length by 21.1%, 7% and 12.4% compared to control respectively in chickpea.

3.4 Shoot length

AM fungal inoculation increased the cytokinins content in shoots (Bass & Kuiper, 1989), which intern promote the cell division and cell expansion thus play major role in shoot morphology. The result obtained in this investigation clearly shows that AM fungus has significant influence on shoot length. The shoot length was found to be higher (17.33±1.52 cm) in plant inoculated with \( G. \text{mosseae} + G. \text{fasciculatum} + A. \text{scrobiculata} \) at 40DAI (Table- 1). The same trend was observed at 60DAI and 85DAI (Table-2&3). The individual application of \( G. \text{fasciculatum} \) also had greater influence on shoot length at 85 DAI (60±1.00 cm). These results are in accordance with the findings of Ramakrishnan & Selvakumaran (2012). In their investigation they reported that the shoot length of tomato was significantly greater (28.51±0.855 cm) when the plant was inoculated with \( G. \text{fasciculatum} \) and \( G. \text{intraaridices} \) when compared to control.

3.5 Influence of AMF on root biomass

AMF typically alter root morphology, more specifically they increase root surface area and there by enhance soil nutrient uptake potential (Copetta et al., 2006). Furthermore, Karthikeyan et al. (2008) revealed that the inoculation of \( G. \text{mosseae} \) increased the root fresh and dry weight of \( Catharanthus \text{roseus} \). This suggest that the increase in root fresh weight of AM inoculated plant was due to the formation of external mycelium also found that positive correlation between root fresh weight and AM fungal colonization. In the present experiment it was observed that the root fresh and dry weight was increased in the plant inoculated with \( G. \text{mosseae} \), \( G. \text{fasciculatum} \) and \( A. \text{scrobiculata} \) (5.04±0.74 mg/ plant, 2.34±0.08mg/ plant) respectively, at 40DAI. The same trend was observed at 85DAI, however at 60 DAI fresh and dry weight of root was found to be higher in \( G. \text{fasciculatum} + A. \text{scrobiculata} \) inoculated plants (8.50±0.1 mg/ plant, 6.67±0.15mg/ plant) respectively (Table- 1-3).

Karthikeyan et al. (2009) reported that \( G. \text{fasciculatum} \) increased the root dry weight of four medicinal plants namely \( Ocimum \text{sancum} \) (11.04 g/plant), \( Catharanthus \text{roseus} \) (14.98g /plant), \( Coleus \text{forskholii} \) (10.00g /plant), \( Crynbopogon \text{flexosus} \) (15.20 g / plant). A similar finding was reported by Gupta & Janarthanan (1991) who found that the biomass increased with treatment of \( G. \text{agregatam} \) in \( Palmarosa \text{enchances} \). Result was further conformed by the findings of Gogoi & Singh (2011). They have reported that the inoculation of \( Glomus \text{sp} \), \( G. \text{fasciculatum} \), \( G. \text{clarum} \) significantly increased the root fresh and dry weight of \( Piper \text{longum} \). The root biomass varied within the treatments, which may be possibly due to the host preference of AM species (Sailo & Bagyaraj, 2005).

3.5 Seed yield

Synergistic effects of dual inoculation of \( A. \text{chroococcum} \) strains with \( G. \text{fasciculatum} \) increased boll weight and seed cotton yield (Paul et al., 2011). In present study the number of seeds per head was significantly higher in AM inoculated plants than in uninoculated control plants. It was observed that the number of seeds vary among the treatments and found to be higher in the plants treated with \( G. \text{mosseae} + G. \text{fasciculatum} + A. \text{scrobiculata} \) followed by \( G. \text{mosseae} + A. \text{scrobiculata} \) and \( A. \text{scrobiculata} \) alone(189.33±6.03, 138.33±17.39, 136.66±20.82) respectively. The head diameter and seeds weight were also measured and it was found to significantly vary with the different treatments. The seeds weight was greater in the plant treated with \( G. \text{mosseae} + G. \text{fasciculatum} + A. \text{scrobiculata} \) followed by \( G. \text{mosseae} + A. \text{scrobiculata} \) While individual inoculation with \( A. \text{scrobiculata} \) gave better results (Table 3).
### Table 1 Effect of AM fungal application on the various agronomical changes of *H. annuus* at 40 DAI.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Root length (cm)</th>
<th>Shoot length (cm)</th>
<th>Number of leaves</th>
<th>Root fresh weight (mg)</th>
<th>Leaf fresh weight (mg)</th>
<th>Root dry weight (mg)</th>
<th>Leaf dry weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.27±0.87</td>
<td>8.03±0.45</td>
<td>6.00±1.00</td>
<td>2.26±0.23</td>
<td>0.6±0.1</td>
<td>0.11±0.03</td>
<td>0.28±0.10</td>
</tr>
<tr>
<td><em>G. mosseae</em> alone</td>
<td>6.53±1.15</td>
<td>12.5±0.5</td>
<td>6.66±0.57</td>
<td>2.8±0.60</td>
<td>1.4±0.1</td>
<td>0.43±0.45</td>
<td>0.41±0.08</td>
</tr>
<tr>
<td><em>G. fasciculatum</em> alone</td>
<td>8.23±0.46</td>
<td>14.26±0.46</td>
<td>8.00±1.00</td>
<td>3.47±0.23</td>
<td>1.7±0.1</td>
<td>0.56±0.43</td>
<td>0.6±0.06</td>
</tr>
<tr>
<td><em>A. scrobiculata</em> alone</td>
<td>7.9±0.96</td>
<td>13.83±1.60</td>
<td>9.66±0.57</td>
<td>2.6±0.41</td>
<td>1.42±0.16</td>
<td>0.52±0.03</td>
<td></td>
</tr>
<tr>
<td><em>G. mosseae</em> + <em>G. fasciculatum</em></td>
<td>6.93±2.02</td>
<td>14.33±1.52</td>
<td>11.00±1.00</td>
<td>4.30±0.34</td>
<td>2.46±0.30</td>
<td>1.56±0.12</td>
<td>0.62±0.36</td>
</tr>
<tr>
<td><em>G. mosseae</em> + <em>A. scrobiculata</em></td>
<td>6.76±0.75</td>
<td>16.33±1.44</td>
<td>8.33±1.15</td>
<td>4.7±0.50</td>
<td>3.66±0.76</td>
<td>2.08±0.33</td>
<td>1.19±0.12</td>
</tr>
<tr>
<td><em>G. fasciculatum</em> + <em>A. scrobiculata</em></td>
<td>8.46±0.92</td>
<td>17.33±1.52</td>
<td>11.66±0.57</td>
<td>4.12±0.48</td>
<td>4.96±0.50</td>
<td>2.53±0.12</td>
<td>1.75±0.44</td>
</tr>
<tr>
<td><em>G. mosseae</em> + <em>G. fasciculatum</em> + <em>A. scrobiculata</em></td>
<td>8.76±0.47</td>
<td>17.33±1.52</td>
<td>12.00±2.00</td>
<td>5.04±0.74</td>
<td>3.83±0.49</td>
<td>2.34±0.08</td>
<td>2.47±0.11</td>
</tr>
</tbody>
</table>

Data are mean value of three replicates which was repeated thrice. ±, standard deviation. Mean value followed by different alphabet/s with in a column differ significantly over one another at P≤ 0.05 lead by Duncan’s Multiple Range Test.

### Table 2 Effect of AM fungal application on the various agronomical changes of *H. annuus* at 60 DAI.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Root length (cm)</th>
<th>Shoot length (cm)</th>
<th>Number of leaves</th>
<th>Root fresh weight (mg)</th>
<th>Leaf fresh weight (mg)</th>
<th>Root dry weight (mg)</th>
<th>Leaf dry weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.33±0.15</td>
<td>25.83±1.04</td>
<td>10.00±1.00</td>
<td>4.10±0.45</td>
<td>4.61±0.1</td>
<td>2.63±0.15</td>
<td>0.73±0.02</td>
</tr>
<tr>
<td><em>G. mosseae</em> alone</td>
<td>11.93±0.74</td>
<td>48.33±2.88</td>
<td>12.00±1.00</td>
<td>8.10±0.25</td>
<td>8.53±0.25</td>
<td>6.40±0.11</td>
<td>1.45±0.09</td>
</tr>
<tr>
<td><em>G. fasciculatum</em> alone</td>
<td>10.56±0.15</td>
<td>45.66±2.51</td>
<td>12.00±1.50</td>
<td>6.56±0.20</td>
<td>7.51±0.1</td>
<td>4.66±0.20</td>
<td>1.26±0.27</td>
</tr>
<tr>
<td><em>A. scrobiculata</em> alone</td>
<td>9.33±0.70</td>
<td>46.33±0.57</td>
<td>11.00±1.00</td>
<td>5.73±0.66</td>
<td>6.83±0.67</td>
<td>6.20±0.10</td>
<td>1.32±0.27</td>
</tr>
<tr>
<td><em>G. mosseae</em> + <em>G. fasciculatum</em></td>
<td>9.26±0.46</td>
<td>45.66±1.15</td>
<td>13.33±0.57</td>
<td>5.66±0.47</td>
<td>7.63±1.33</td>
<td>4.37±0.23</td>
<td>1.15±0.34</td>
</tr>
<tr>
<td><em>G. mosseae</em> + <em>A. scrobiculata</em></td>
<td>11.8±0.62</td>
<td>44.66±1.15</td>
<td>13.00±1.00</td>
<td>5.60±0.1</td>
<td>7.13±0.90</td>
<td>4.53±0.35</td>
<td>1.11±0.26</td>
</tr>
<tr>
<td><em>G. fasciculatum</em> + <em>A. scrobiculata</em></td>
<td>12.56±0.40</td>
<td>52.33±2.51</td>
<td>13.66±0.57</td>
<td>8.50±0.1</td>
<td>8.27±0.11</td>
<td>6.67±0.15</td>
<td>2.23±0.02</td>
</tr>
<tr>
<td><em>G. mosseae</em> + <em>G. fasciculatum</em> + <em>A. scrobiculata</em></td>
<td>12.73±0.30</td>
<td>55.02±1.00</td>
<td>16.00±1.00</td>
<td>7.53±0.63</td>
<td>8.36±0.25</td>
<td>5.93±0.40</td>
<td>2.38±0.15</td>
</tr>
</tbody>
</table>

Data are mean value of three replicates which was repeated thrice. ±, standard deviation. Mean value followed by different alphabet/s with in a column differ significantly over one another at P≤ 0.05 lead by Duncan’s Multiple Range Test.
Table 3 Effect of AM fungal application on the various agronomical changes of *H. annuus* 85 DAI.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Root length (cm)</th>
<th>Shoot length (cm)</th>
<th>Number of leaves</th>
<th>Root fresh weight (mg)</th>
<th>Leaf fresh weight (mg)</th>
<th>Root dry weight (mg)</th>
<th>Leaf dry weight (mg)</th>
<th>Number of seeds per head</th>
<th>Head diameter (cm)</th>
<th>100 seeds weight (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10.00±0.50</td>
<td>42.66±2.51</td>
<td>12.33±0.57</td>
<td>7.83±0.42</td>
<td>5.93±0.45</td>
<td>3.8±0.36</td>
<td>0.99±0.18</td>
<td>96.00±1.02</td>
<td>3.17±0.35</td>
<td>3.73±1.12</td>
</tr>
<tr>
<td><em>G. mosseae</em> alone</td>
<td>15.46±1.00</td>
<td>60.00±1.00</td>
<td>15.67±0.58</td>
<td>10.43±0.15</td>
<td>7.7±0.36</td>
<td>1.57±0.11</td>
<td>117.33±2.52</td>
<td>4.36±0.40</td>
<td>4.52±0.48</td>
<td></td>
</tr>
<tr>
<td><em>G. fasciculatum</em> alone</td>
<td>13.07±0.61</td>
<td>56.00±1.00</td>
<td>14.66±0.58</td>
<td>9.86±0.30</td>
<td>7.63±0.23</td>
<td>1.22±0.11</td>
<td>114.33±2.08</td>
<td>4.67±0.29</td>
<td>4.29±1.17</td>
<td></td>
</tr>
<tr>
<td><em>A. scrobiculata</em> alone</td>
<td>12.66±0.76</td>
<td>49.00±1.00</td>
<td>15.00±2.64</td>
<td>8.50±0.1</td>
<td>6.16±0.51</td>
<td>1.47±0.39</td>
<td>136.66±2.82</td>
<td>4.77±0.57</td>
<td>4.91±0.68</td>
<td></td>
</tr>
<tr>
<td><em>G. mosseae</em> + <em>G. fasciculatum</em></td>
<td>13.33±0.30</td>
<td>52.67±1.53</td>
<td>16.33±1.15</td>
<td>8.7±0.45</td>
<td>7.27±1.00</td>
<td>1.46±0.16</td>
<td>115.66±5.50</td>
<td>5.2±0.35</td>
<td>5.09±0.73</td>
<td></td>
</tr>
<tr>
<td><em>G. mosseae</em> + <em>A. scrobiculata</em></td>
<td>12.96±0.47</td>
<td>59.02±5.57</td>
<td>16.33±1.52</td>
<td>9.36±0.25</td>
<td>7.27±0.45</td>
<td>1.55±0.03</td>
<td>138.33±1.39</td>
<td>5.77±0.46</td>
<td>6.14±0.46</td>
<td></td>
</tr>
<tr>
<td><em>G. fasciculatum</em> + <em>A. scrobiculata</em></td>
<td>16.00±0.50</td>
<td>88.33±2.88</td>
<td>18.00±2.00</td>
<td>10.47±0.50</td>
<td>9.5±1.15</td>
<td>2.65±0.19</td>
<td>113.33±4.50</td>
<td>4.40±0.7</td>
<td>4.51±0.35</td>
<td></td>
</tr>
<tr>
<td><em>G. mosseae</em> + <em>G. fasciculatum</em> + <em>A. scrobiculata</em></td>
<td>15.16±0.29</td>
<td>95.00±13.22</td>
<td>18.00±1.00</td>
<td>11.96±0.55</td>
<td>10.5±10.5</td>
<td>9.73±0.32</td>
<td>2.02±0.36</td>
<td>189.33±6.03</td>
<td>6.33±0.29</td>
<td>6.53±0.17</td>
</tr>
</tbody>
</table>

Data are mean value of three replicates which was repeated thrice. ±, standard deviation. Mean value followed by different alphabet/s with in a column differ significantly over one another at P≤ 0.05 lead by Duncan’s Multiple Range Test.
This result confirms the previous report of Balagurunathan et al. (2012) who have reported that the vegetative components (leaf area and height) and reproductive components (head weight, number of seeds and 1000 seeds weight) were maximum when the plant was treated with Azotobacter and Phosphobacter than chemical fertilizer. They also suggested that biological fertilizer play an important role in sunflower generative growth. Soleimanzadeh, (2010) revealed that mycorrhizal plants had about 7% more number of seeds in head when compared to non-mycorrhizal plants, which indicated that mycorrhizae plays an important role in sunflower generative growth and therefore makes a significant increase in the number of seeds per head. Jalaluddin (2005) observed that the 100 seeds weight of Soybean was maximum when the plant was treated with dual inoculation of G. macrocarpum and Bradyrhizobium japonicum.

**References**


Phillips JM, Hayman DS (1970) Improved procedure for clearing roots and staining VAM fungi for rapid assessment of


