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Alleviation of cadmium stress by arbuscular mycorrhizal symbiosis

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ABSTRACT
Owing to the realization of the harmful effect of cadmium on the environment and plants and as the plants are sessile organisms, they need to increase the protective mechanisms to cope with Cd stress. Inoculation the plant with soil microbes at the place of their growing is an important strategy to support the plants against stresses. In this study, trigonella plants were inoculated with arbuscular mycorrhizal (AM) fungi under different CdCl₂ concentrations (0, 2.25, and 6.25 mM). AM inoculation increased growth parameters, chlorophyll, and protein contents. Root colonization was significantly increased at low Cd concentration (2.25 mM) and decreased at high one (6.25 mM). Also, with AM fungal inoculation, the translocation factor of trigonella plants significantly decreased as compared to non-AM ones at both low and high Cd concentrations. In addition, it was clearly that malondialdehyde content of trigonella plants increased significantly at both Cd concentrations and with AM fungal inoculation its content decreased compared to those of non-AM ones. AM inoculation significantly increased antioxidant enzymes activities compared to non-AM ones. Consequently, this study showed a tolerance strategy of AM trigonella plants against Cd stress, thus mycorrhizal symbiosis becomes a promising and suitable as phytostabilizers of Cd stressed soil.

KEYWORDS
Antioxidant enzymes; arbuscular mycorrhizal fungi; cadmium; phytoremediation; trigonella

Introduction
The use of arbuscular mycorrhizal (AM) fungi is one of the promising strategies that form symbiotic relations with the vast majority of horticultural and agricultural important crops (Plouznikoff et al. 2016). Although these fungi produce a high number of spores and are able to grow faster, they have some physiological and morphological potential to grow under different stress conditions. The noteworthy point is that AM fungi can establish a symbiotic association with most terrestrial plants in highly polluted soils (Cabral et al. 2015). The effects of AM fungi on plant growth under stress are mostly due to the superb abilities of these fungi in enhancing the physiological and morphological mechanisms, increasing plant biomass and uptake of immobile nutrients such as P, Zn, and Cu and decreasing metal toxicity to plants (Kanwal et al. 2015; Miransari 2017).

Biological methods of plants-microbes interactions can be used for the bioremediation of contaminated areas with heavy metals. The most important and applicable techniques used for the removal of heavy metals from the environment are bioremediation and phytoremediation (Lebeau et al. 2008). Phytoextraction and phytostabilization are the most researched processes of phytoremediation. In the process of phytoextraction, plants concentrate the heavy metals in their aerial parts by removing them from the soil (Pajević et al. 2016), while the second process does not eliminate the heavy metals from the environment, but immobilizes them in plant roots.

Heavy-metal stress is among the most serious stresses adversely affecting plant growth and the environment. Cadmium (Cd) is a non-essential metal for both plants and animals and has toxic effects when its concentration has exceeded a limit (Zafarzadeh et al. 2018). It occurs naturally at a low concentration in the soil, but its concentration is continuously increased due to the addition of Cd rich phosphate fertilizers, dispersal of sewage sludge, mining and smelting (Liu et al. 2007). It is regarded as an extreme pollutant due to its high toxicity, high solubility in water and easily taken up by plant roots from the soil (Pinto et al. 2004). Cd has been shown to induce many physiological, morphological, structural, and biochemical changes in plants, which is dependent upon plant species, organ/tissue, concentration of metal and exposure period (Benavides et al. 2005).

Plants have developed a series of mechanisms to cope with Cd contamination, among which mycorrhizal association is considered an effective strategy to alleviate Cd phytotoxicity (Hashem et al. 2016). The objectives of the present study were to investigate the role of AM fungal symbiosis on alleviation of Cd stress on growth of trigonella plants in Cd-polluted soils.

Materials and methods
Plant material. Seeds of fenugreek (Trigonella foenum-graecum L.) var. Giza 30 were purchased from Ministry of
Agriculture, Zagazig branch. Seeds with uniform size, color, and weight were chosen for experimental purpose.

**Soil.** The experimental soil, used throughout the present investigation, was collected from the top layer (0–20 cm) from Sharkia Governate, Egypt. The physicochemical properties and nutritional status of the experimental soil are listed in Table 1. Soil was sterilized by 2% formaldehyde to eliminate native AM fungi, covered for 7 days then left 25 days for aeration (Islam and Ayanaba 1981; Thakur and Sharma 2018).

### Table 1. Physicochemical properties and nutritional status of the experimental soil before planting.

<table>
<thead>
<tr>
<th>Property</th>
<th>Sand (%)</th>
<th>Silt (%)</th>
<th>Clay (%)</th>
<th>Soil texture</th>
<th>Saturation percent (SP)</th>
<th>Electric conductivity (EC)</th>
<th>pH</th>
<th>CaCO₃ (%)</th>
<th>Organic matter (%)</th>
<th>Total P (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Value</td>
<td>13.9</td>
<td>27.4</td>
<td>58.7</td>
<td>Clay</td>
<td>69.00</td>
<td>3.45</td>
<td>8.24</td>
<td>4.98</td>
<td>0.37</td>
<td>6.34</td>
</tr>
<tr>
<td>Minerals content (mg/kg soil)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Preparation of AM fungal inoculum

The inoculum of AM fungal species including *Glomus monosporum*, *G. clarum*, *Gigaspora nigra*, and *Acaulospora laevis* were originally isolated from soils of Bahr El-Baqar, Abo-Hamad, Sharkia Governorate, Egypt using the wet sieving and decanting method (Gerdemann and Nicolson 1963). The identified AM fungal spores were left to multiply for 1 month on maize (*Zea mays*) roots as a suitable trap plants.

**Pot experiments.** Experiments were conducted in the greenhouse of Botany Department, Faculty of Science, Zagazig University during Winter 2016. Fenugreek seeds were surface sterilized with 5% sodium hypochlorite for 10 min (Evelin et al. 2013), then subsequently rinsed with sterilized distilled water. Ten sterilized seeds were sown in sterilized plastic pots (25 cm diameter) filled with 2 kg sterilized soil. Pots were placed in the greenhouse under natural photoperiods of 12–13 h and temperatures of 25 ± 4°C in two groups; the first was inoculated with AM fungal propagules at the rate of 100 g (12 spore/1 g soil) per pot at sowing date, the other was non-inoculated. AM fungal inoculums consisted of AM fungal spores, hyphae, and colonized root fragments. After two week of germination, plants were thinned to five per pot. CdCl₂ was added to the soil at the concentrations of 2.25 and 6.25 mM. Cd solutions were applied after 40 days from sowing. Control treatments were applied with tap water. Each treatment was replicated three times.

**Sample collection.** The plant samples were collected after 40 days from Cd treatment, kept in labeled bags and stored at –20°C for further biochemical investigation.

### Plant measurements and analysis

**Growth measurement.** Shoot height, root length, leaves number, fresh, and dry weights of shoot and root were determined. Also, root: shoot (R/S) ratio through using root: shoot dry weight ratios were calculated. All the weights were expressed as grams (g). Shoot and root dry weights were recorded after drying the samples at 80°C until constant weight.

**Chlorophyll content.** Chlorophyll content index (CCI) in leaves was estimated by the CCM-200 plus Chlorophyll Content Meter (Opti-Sciences, Inc.).

### Determination of the total soluble protein content

Total soluble protein in trigonella fresh shoots was extracted with 10 mL of a 25 mM borate buffer (pH 8.5). 0.5 mL of supernatant was added to 5 mL of protein reagent (Coomassie brilliant blue G250) and left for 5 min. The absorbance was read at 595 nm using a UV–visible Spectrophotometer, RIGOL (Model Ultra-3660) and its content was assessed as mg/g FW using bovine serum albumin as standard (Bradford 1976).

### Detection the levels of mycorrhizal colonization

After 40 days of Cd application, 40 root segments of 0.5–1 cm were cut from three plants for treatments. Root samples were cleared with 10% KOH solution and then stained with 0.05% trypan blue in lactophenol (Phillips and Hayman 1970) for 15 min at 90°C. Forty randomly selected stained root pieces were examined microscopically (Carl Zeiss, Italy) at 40X magnification. Mycorrhizal colonization levels of the stained roots were assessed by the method of Trouvelot et al. (1986) according to the frequency of mycorrhizal colonization (F%), the intensity of colonization (M%) and the rate of arbuscular development (A%) using the Mycocalc software (http://www.dijon-inra.fr/mycocalcprg) (Metwally and Abdelhameed 2018).

### Estimation of acid and alkaline phosphatases activity

A known root fresh weight was macerated in 0.1 M borate buffer (pH 8.5), and the homogenate was centrifuged at 6000 rpm for 10 min. Soluble phosphatas activity were estimated quantitively in the supernatant according to the technique of Gianinazzi-Pearson and Gianinazzi (1976).

**Estimation of cadmium and translocation factor.** Cadmium in trigonella shoot and root was estimated using Inductively Coupled Plasma Spectrometry (Ultima 2 JY Plasma) at Central Lab of Agricultural Research, Faculty of Agriculture, Zagazig University, and their contents were expressed as μg/g DW (Karaca 2004). The transfer of Cd from roots to shoots was evaluated in term of translocation factor (TF) (Gupta et al. 2008), which termed as ratio of heavy metals in plant shoot to that in plant root.
Table 2. Effect of Cd concentrations on growth parameters of AM and non-AM trigonella plants.

<table>
<thead>
<tr>
<th>Cd (mM)</th>
<th>AM Status</th>
<th>Shoot height (cm)</th>
<th>Root length (cm)</th>
<th>Shoot FW (g)</th>
<th>Root FW (g)</th>
<th>Leaves number</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Non-AM</td>
<td>29.50 ± 0.78a</td>
<td>18.00 ± 0.48b</td>
<td>1.54 ± 0.04b</td>
<td>0.41 ± 0.01cd</td>
<td>9.67 ± 0.26b</td>
</tr>
<tr>
<td></td>
<td>AM</td>
<td>30.60 ± 0.81a</td>
<td>20.33 ± 0.54a</td>
<td>1.89 ± 0.05a</td>
<td>0.75 ± 0.02a</td>
<td>11.33 ± 0.29a</td>
</tr>
<tr>
<td>2.25</td>
<td>Non-AM</td>
<td>23.33 ± 0.62c</td>
<td>15.33 ± 0.41c</td>
<td>1.09 ± 0.03d</td>
<td>0.27 ± 0.01e</td>
<td>9.33 ± 0.25b</td>
</tr>
<tr>
<td></td>
<td>AM</td>
<td>26.00 ± 0.67b</td>
<td>17.00 ± 0.45b</td>
<td>1.37 ± 0.04c</td>
<td>0.50 ± 0.01b</td>
<td>10.00 ± 0.26b</td>
</tr>
<tr>
<td>6.25</td>
<td>Non-AM</td>
<td>20.67 ± 0.55d</td>
<td>13.67 ± 0.36d</td>
<td>0.75 ± 0.02e</td>
<td>0.38 ± 0.01d</td>
<td>8.33 ± 0.22c</td>
</tr>
<tr>
<td></td>
<td>AM</td>
<td>25.0 ± 0.66bc</td>
<td>15.0 ± 0.39cd</td>
<td>1.15 ± 0.03d</td>
<td>0.44 ± 0.02c</td>
<td>9.33 ± 0.25b</td>
</tr>
</tbody>
</table>

Cadmium

<table>
<thead>
<tr>
<th>Treatments</th>
<th>AM status</th>
<th>Shoot DW (g)</th>
<th>Root DW (g)</th>
<th>R/S ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Non-AM</td>
<td>0.197 ± 0.01b</td>
<td>0.34 ± 0.01c</td>
<td>0.171 ± 0.006c</td>
</tr>
<tr>
<td></td>
<td>AM</td>
<td>0.234 ± 0.01a</td>
<td>0.056 ± 0.01a</td>
<td>0.240 ± 0.006a</td>
</tr>
<tr>
<td>2.25</td>
<td>Non-AM</td>
<td>0.130 ± 0.01e</td>
<td>0.20 ± 0.01d</td>
<td>0.154 ± 0.004d</td>
</tr>
<tr>
<td></td>
<td>AM</td>
<td>0.162 ± 0.01d</td>
<td>0.039 ± 0.01b</td>
<td>0.242 ± 0.006a</td>
</tr>
<tr>
<td>6.25</td>
<td>Non-AM</td>
<td>0.115 ± 0.01f</td>
<td>0.20 ± 0.01d</td>
<td>0.172 ± 0.005c</td>
</tr>
<tr>
<td></td>
<td>AM</td>
<td>0.177 ± 0.01c</td>
<td>0.036 ± 0.01bc</td>
<td>0.205 ± 0.005b</td>
</tr>
</tbody>
</table>

The data are mean of three replicates ± standard error. Value within each column marked with different letters means difference significant at p < 0.05. * Indicates statistical significance; ns, non-significant.

Results and discussion

Growth parameters

Cadmium stress as shown in Table 2 affected the growth of trigonella plants significantly in a negative way where shoot height, root lengths, and leaves number decreased with increasing Cd concentration over the control. Shoot, root fresh, and dry weights behave in a similar way to their corresponding lengths, those negative effects were more apparent in non-AM plants than AM one. The inhibitory effects of Cd stress are in agreement with the results of Jamali et al. (2014) and Sheikh-Assadi et al. (2015) who reported that the retardation in plant growth could be attributed to high uptake and accumulation of Cd in plant parts.

However, AM fungal inoculation mostly enhanced these growth parameters in AM trigonella plants versus non-AM ones regardless of the Cd concentration; these results were in harmony with those of Rabie (2005) who stated that AM fungi caused a decrease in the inhibiting effects of heavy-metal pollutants on red kidney and wheat plants, as AM fungi helps the host plant to acquire nutrients especially P by means of extraradical mycorrhizal hyphae and transporting them to the root tissues (Abdel-Fattah and Asrar 2012; Metwally and Abdelhameed 2018). Also, the increase in growth of AM trigonella plants can be related to reduced Cd uptake and upgrading in ROS detoxification mechanism (Table 3 and Figure 4). Our data are in accordance with previous studies by Sharma et al. (2016) on wheat genotypes inoculated with AM fungi under Cd stress which revealed that Cd tolerance is ascribed to augmented accumulation of stress metabolites such as sugar, proteins, proline, and glycine betaine eventually leading to high growth.

Another noteworthy result was that root/shoot ratios (R/S) of AM trigonella plants were higher than non-AM ones grown in both control and Cd-polluted soil, this was in line with the results of El-Sherbeny et al. (2012) who stated that inoculation of plants with AM fungi caused an increasing in root/shoot ratios than non-AM one.

Chlorophyll and total soluble protein content

Chlorophyll is essential for photosynthesis and very susceptible to environmental stress such as heavy metals (Chaturvedi et al. 2018). Exposures to Cd lead a statistically significant
decrease in CCI of trigonella leaves as shown in Figure 1a. The reduction corroborates well with the finding of Andrade et al. (2008). Where during photosynthesis protochlorophyllide reductase binds NADH which contains sulfhydryl groups, in which Cd interferes with this groups involved in biosynthesis of chlorophyll which leads to decrease in chlorophyll content and diminishing the supply of Mg$^{2+}$, Fe$^{2+}$, and Zn$^{2+}$ during stress (Van Assche and Clijsters 1990).

The worth mentioning result was that the CCI of AM trigonella leaves also showed decrease in chlorophyll upon exposure to Cd stress, but this reduction was lesser than their non-AM counterparts. This is in accordance with the results of Chaturvedi et al. (2018), that may be attributed to the increase in the uptake of P which is one of the important components required for the photosynthetic process (Abdel-Fattah and Asrar 2012).

Figure 1b shows that Cd concentrations caused a significant increase in total soluble protein content either in AM or non-AM trigonella shoots compared to control ones. These results are in coherence with Abdul Qados (2011), which may be attributed to de novo synthesis of stress proteins provoked by metal exposure. The worth mentioned result is that with AM fungal symbiosis further augmentation in protein content was observed in trigonella that grown either in control or Cd contaminated soils but in Cd contaminated soil, this enhancement was significantly greater relative to the control. This result is a good conformity with the results of Hashem et al. (2016) which attributed to AM mediated activation of certain plant genes and accumulation of nutrients which fundamental of several metabolically active compounds. Conflicting to our results Rabie (2005) reported that heavy metals in polluted soil significantly diminished protein content in red kidney and wheat plants.

![Figure 1. Effect of Cd concentrations on (a) chlorophyll content index (CCI) and (b) protein content of AM and non-AM trigonella shoots. Values represent the mean of three replicates. Different letters indicate statistical differences at $p < 0.05$. Error bars are standard error of the mean. * Indicates statistical significance.](image)

<table>
<thead>
<tr>
<th>Cd (mM)</th>
<th>AM status</th>
<th>F (%)</th>
<th>M (%)</th>
<th>A (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Non-AM</td>
<td>0.0c</td>
<td>0.0c</td>
<td>0.0c</td>
</tr>
<tr>
<td></td>
<td>AM</td>
<td>70b</td>
<td>66.5b</td>
<td>28.5b</td>
</tr>
<tr>
<td>2.25</td>
<td>Non-AM</td>
<td>0.0c</td>
<td>0.0c</td>
<td>0.0c</td>
</tr>
<tr>
<td></td>
<td>AM</td>
<td>100a</td>
<td>95a</td>
<td>57a</td>
</tr>
<tr>
<td>6.25</td>
<td>Non-AM</td>
<td>0.0c</td>
<td>0.0c</td>
<td>0.0c</td>
</tr>
<tr>
<td></td>
<td>AM</td>
<td>63.6b</td>
<td>60.45b</td>
<td>25.91b</td>
</tr>
</tbody>
</table>

The data are mean of three replicates. Value within each column marked with different letters means difference significant at $p < 0.05$. * Indicates statistical significance.

### Mycorrhizal colonization levels

Table 4 shows the effect of different Cd concentrations amended to soil on frequency of mycorrhizal colonization (F%), intensity of cortical infection (M%) and arbuscular frequency (A%) of AMF trigonella plant roots grown under different concentrations of Cd.

![Table 4. Frequency of mycorrhizal colonization (F%), intensity of cortical infection (M%), and arbuscular frequency (A%) of AMF trigonella plant roots grown under different concentrations of Cd.](image)
Concerning to M%, the percent of increase was about 42.85 at low Cd concentration and a decrease was about 9% at high concentration compared to AM plants grown in control soil. The reason behind the increase of colonization at low Cd concentrations and decrease at high concentration may be that AM fungal species have varying tolerance to heavy metals.

Figure 2. Photomicrographs of structural colonization of AM fungi in the stained roots of fenugreek. (a) and (b) Arbuscules (Ar) in cortical cells of AM fenugreek root tissues. (c) and (d) Inter and intracellular vesicles (V), intraradical hypha (IH) and coiled hypha (CH) in AM fenugreek root tissues. CW: cell wall of trigonella root cortical cell.

Figure 3. Effect of Cd concentrations on phosphatases enzymes: (a) acid and (b) alkaline phosphatases (nmol PNP/min) of AM and non-AM trigonella roots. Values represent the mean of three replicates. Different letters indicate statistical differences at \( p < 0.05 \). Error bars are standard error of the mean. * Indicates statistical significance.
also been conflicting results. For instance, our results are in harmony with results. Our results are conflicting with those of Burleigh et al. (2003) and Rabie (2005). Also, the reduction in AM colonization as Cd concentrations increased may be connected to reduced growth of their root in Cd treated plants (Sharma et al. 2016).

Also, A% has the same manners, this finding corroborates well with that of Sheikh-Assadi et al. (2015) who reported that sensitivity of AM fungi to heavy metal expressed as a decline in spore germination, root colonization, and hyphal growth. Additionally, the internal Cd concentration may well suppress AM fungal colonization (de Souza et al. 2012). In general, our results of mycorrhiza plant interactions proved tremendously that AM fungi still function in Cd-contaminated soil indicating that the tested Cd concentrations were not detrimental to AM fungi.

**Acid and alkaline phosphatases activity**

Phosphatases are important hydrolytic enzymes which are extensively distributed in plants. Figure 3a and b show that soluble acid phosphatase activity was much higher than alkaline phosphatase, and their activities were greatly affected by AM fungal colonization. These results agree with Goicoechea et al. (1996) and Subramanian et al. (2011) in alfalfa and maize plants, respectively. These extrapolations emphasized the prime role of AM fungal inoculation in increasing phosphatases activity of trigonella plants. The close link between the activity of mycorrhizal phosphatases and both the amplitude of the mycorrhizal growth responses and arbuscular frequency (A%) supports the suggestion that this phosphatase enzyme is involved in P assimilation by AM fungi (Abdel-Fattah and Asrar 2012).

On the other hand, phosphatase activities of the AM and non-AM root extracts of trigonella plants were greatly reduced with increasing Cd concentration. Where heavy-metal ions considered being inhibitors for enzyme activity, this was in harmony with results. Our results are conflicting to results of Tsekov and Galabova (2003) who reported that acid phosphatase activity increased under the stress of high levels of heavy metals, and is one possible process of detoxification and resistance.

**Cadmium uptake and translocation**

Table 5 demonstrates the nonexistence of Cd in both shoot and root of AM and non-AM inoculated trigonella plants grown in control soil. While with Cd addition, it was apparent that AM trigonella plants accumulated high concentrations of Cd in their roots and translocated it to their shoots but in lesser concentrations. In contrast to AM plants, non-AM plants had the different pattern. This could be explained by the fact that AM colonization increased plant uptake of metals by mechanisms such as sequestration of metals by polyphosphate granules in fungal vacuoles (Turnau et al. 1993) and enlargement of the absorbing area, volume of accessible soil, and efficient hyphal translocation (Yu et al. 2004). Contrary to our results, Andrade et al. (2008) reported that sunflower plants inoculated with AM fungi had a great capacity to gather Cd in their shoots more greater than in their roots.

The TF (Table 5), the ratio of the shoot to root metals, indicates internal metal translocation. There was a decrease in its value in AM trigonella plants compared to non-AM plants grown at both low and high Cd concentrations. Where at low concentration the decrease was 25.38% while at high concentration was 67.39%. Furthermore, these results suggested that the AM fungi act with trigonella plants as a heavy-metal filter to maintain low heavy-metal concentrations in aboveground plant tissues. In contrast to our results, Andrade et al. (2008) reported that AM colonization increased Cd tolerance of AM sunflower plants had a greater TF than non-AM ones. In this connection, Deng et al. (2004) reported that plant species which have strong ability to reduce metal translocation from roots to shoots are suitable as phytostabilizers for metal contaminated lands.

Also, the effect of Cd stress on trigonella plants was indicated by tolerance index (Ti), as shown in Table 5. It was obvious that Ti decreased with Cd applications and the percentage of decreasing was greater in plants grown at higher Cd concentrations compared to lower one. Also, the results indicated that trigonella plants showed a higher Ti in the presence of AM fungi than those of non-AM plants. The increase varied from 4.5% at low Cd concentration to 31% at the high one. Therefore, it was proposed that AM fungal colonization had a potentiality of increasing Cd tolerance of trigonella plants. This finding is in good agreement with that of Burleigh et al. (2003) and Rabie (2005).

**Mycorrhizal dependency**

Table 5 shows that Cd application increase MD of trigonella plants compared with those grown under control conditions.

### Table 5. Cd concentrations (μg/g DW) in shoot and root, translocation factor (TF), tolerance index (Ti), and mycorrhizal dependency (MD) of AM and non-AM trigonella grown under different Cd concentrations.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Cd (mM)</th>
<th>AM status</th>
<th>Shoot</th>
<th>Root</th>
<th>TF</th>
<th>Ti</th>
<th>MD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Non-AM</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>AM</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>20.7 ± 0.55c</td>
</tr>
<tr>
<td>2.25</td>
<td>Non-AM</td>
<td>147.98 ± 3.92c</td>
<td>190.70 ± 5.05d</td>
<td>0.776 ± 0.02c</td>
<td>66 ± 1.03bc</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AM</td>
<td>130.70 ± 2.88d</td>
<td>225.72 ± 6.05c</td>
<td>0.579 ± 0.01b</td>
<td>69 ± 1.05b</td>
<td>25.5 ± 0.67b</td>
<td></td>
</tr>
<tr>
<td>6.25</td>
<td>Non-AM</td>
<td>226.35 ± 6.91a</td>
<td>246.28 ± 6.52b</td>
<td>0.919 ± 0.02a</td>
<td>58 ± 0.91c</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AM</td>
<td>200.83 ± 5.98b</td>
<td>266.05 ± 6.88a</td>
<td>0.549 ± 0.01b</td>
<td>76 ± 1.07a</td>
<td>36.9 ± 0.98a</td>
<td></td>
</tr>
</tbody>
</table>

The data are mean of three replicates ± standard error. Value within each column marked with different letters means difference significant at p < 0.05. * Indicates statistical significance.
Although AM fungal colonization was reduced at high Cd concentration, plants grown at this concentration had the highest MD values followed by plants grown at low and control ones, respectively. Where at low concentration, MD was 25.5 but at the high one, MD was 36.9. This finding is in good agreement with that of Rabie (2004) who suggested that AM fungal application to the soil may play an intelligible main role in the synergistic interactions. This result emphasizes that AM fungal symbiosis could be potentially effective in protecting plants exposed to high levels of Cd. Thus, under heavy-metal stress, improved growth of trigonella plants highly depends on AM colonization to overcome the detrimental effect of Cd stress. This may be a sign of the ecological importance of AM fungal association for plant growth and survival under stress (Rabie and Almadini 2005).

**Lipid peroxidation (malondialdehyde (MDA) content)**

Figure 4a shows that MDA content of trigonella leaves was significantly increased with increasing Cd concentrations in both AM and non-AM plants. This finding is in good agreement with that of Hashem et al. (2016). Cd causes oxidative stress through indirect mechanisms, for example, decrement of the antioxidative defense, electron transport chain disruption, and lipid peroxidation motivation (Smeets et al. 2005). But it was noticeably that with AM fungal inoculation, MDA content was significantly lower than those of non-AM plants. This may be due to the extensive increase in antioxidant activities in AM plants (see Figure 4b–d) leading less lipid peroxidation where antioxidants scavenges the radical production before reacting with the membrane lipids as Hashem et al. (2016) and Sharma et al. (2016) observed in Solanum lycopersicum and wheat genotypes inoculated with AM fungi, respectively.

**Antioxidant enzymes**

Antioxidant enzymes play an important role in scavenging ROS and averting the oxidative stress that prompted damaging effects on numerous sensitive molecules. Results showed...
an increase in SOD activity of trigonella shoots with Cd application and with AM fungal inoculation further increase in its activity was noticed (Figure 4b). Also, POD and CAT activities (Figure 4c and d) behave in a similar pattern as in SOD activity. These results are in good agreement with those of Rabie (2005) and Chaturvedi et al. (2018) that can be attributed to the over production of ROS or over expression of genes coding for antioxidant enzymes. Figure 4 shows further stimulation of their activities with AM fungal symbiosis in trigonella plants greater than those in non-AM one. This may be attributed to the contribution of hyphal transport of slowly diffusing micronutrient ions such as Zn and Cu which serve as co-factor for these enzymes (Subramanian et al. 2011). This result explains our previous mentioned result of reduction in MDA content in AM trigonella plants (Figure 4a).

Based on these data, it is conceivable to conclude that the AM fungi play a prime role for stimulation and activation of the antioxidative system in trigonella plants grown in Cd-polluted soil and also suggested that AM plant symbiosis can prevent unfavorable conditions such as heavy-metal stress.

**Conclusion**

Cd, as one of the most toxic elements in the soil and environment, can affect plant and AM fungal growth and function. From aforementioned results, we consolidate evidence for the potential of AM to protect trigonella plants against Cd stress. AM trigonella plants accumulate high concentrations of Cd in their root system and translocate it to their shoot system but in lesser concentrations. Thus, TF decreased in its value in AM trigonella plants compared to non-AM ones. Furthermore, AM fungal colonization lessens the damaging effects of Cd on plant development by increasing antioxidant enzymes activity (SOD, POD, and CAT) and total soluble protein content, decreasing MDA content and diminishing the influences of Cd on plant physiology. If so, mycorrhizal symbiosis could be good candidates for phytostabilization and revegetation of Cd-polluted soil.

**References**


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