Morpho-Physiological Responses and Arbuscular Mycorrhizal Amelioration of *Telfairia Occidentalis* Hook F. to Combinational Stress

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Abstract:

Crops are faced with several definite environmental stresses either concurrently or at different periods during their entire growth and productivity season. Combinational occurrence of stresses and pollution such as salinity stress and crude oil pollution is destructive to crop growth and productivity than these stresses occurring separately at different crop growth. This research was aimed at showing different stress combinations (salinity stress and crude oil pollution) and their impact on *Telfairia occidentalis* and the potential of Arbuscular mycorrhizal fungi (AMF) *Glomus deserticola* in ameliorating these deleterious combinational effects. Results from this study showed distinct characteristics of salinity and crude oil pollution in a single and combined stress effect showing remarked reduction in growth parameters (vine length, leaf area, petiole length and internode length), minerals composition (K, Ca, Fe, Mg, P, and Mn) and chlorophyll contents (a, b and carotenoids) of *Telfairia occidentalis* in the non-mycorrhizal treatments. However, there was an increase in Na^+ content in *T. occidentalis*. Heavy metal analysis revealed high accumulation of Cr, Cd, Cu and Pb in *T. occidentalis*. The AMF *Glomus deserticola* induced reduction in heavy metals contents, and showed remarkable promotion of growth, increased mineral contents, chlorophyll contents and salt tolerance in *Telfairia occidentalis* and as reported by this research to be a latent bioremediating medium against salinity and in sites polluted with organic contaminants such as petroleum hydrocarbons. This research thus supports the continued curiosity surrounding the use of AMF symbiosis as a boost to plants faced with stress as a well-rationaled means.

Keywords:

Crude Oil, *Glomus deserticola*, Heavy Metals, Salinity, Stress, *Telfairia occidentalis*

1. Introduction

In normal field situations, plants are faced with several definite environmental stresses either concurrently or at different periods during their entire growth and productivity season [1]. Combinational occurrence of stresses and pollution such as salinity stress and crude oil pollution is destructive to crop growth and productivity than these stresses occurring separately at different crop growth. Globally, soil salinity has become a rising problem that affects approximately 20 % of all irrigated farmlands and drastically reduces crop productivity significantly [2]. EC (electrical conductivity) with values of about 4 dS/m or more, which is the same as 40 mM of NaCl, are major characteristics of saline soils [3]. Excessive assemblage of certain salt ions, example; Na^{+2}, Na^{+}/K^{+}, Mg^{+2}/Ca^{+2} and Cl^{-}/NO^{-3} is the characteristics of saline soils [3]. The major cause of primary soil salinization which is a natural process is water deficit which occurs in regions with very low soil water. More so, scanty rainfall and high rate of evaporation, which leads to a gradual increase and accumulation in the concentration of salts released during weathering of rocks or by sea spray and tide that may reach the water bodies as a result of storms and winds [4, 5]. Leaching away of the accumulated salt via irrigation of agricultural crops results in secondary salinization of bodies of water may occur which may wash irrigated farm soils into rivers and lakes downstream [5, 6] and, specifically in cases of coastal lagoons, salinization may occur via opening sand fences between the sea and the coastal lagoons [4, 5]. Soil salinity negatively impacts plant physiological processes by reducing and antagonizing nutrient and water uptake [7] and also via toxic negative ion effects on plant cell organelles and enzyme activities [8]. Plants accumulate compatible osmolytes/solutes, employ ion homeostasis, regulates water uptake via aquaporins, and increases production of antioxidants as a physiological mechanisms to cope with salinity stress [9].

Due to the global demand for petroleum as a steady energy source, Crude oil pollution and crude oil impacted soils are widespread across the world. Petroleum (crude oil) and its associated products enter the environments and soils through oil pipeline leakages, pipeline vandalization, oil tankers accidents and rampant incessant disposal of petroleum refinery byproducts leading to alterations in soil physicochemical properties [10]. Agricultural fertile soils soiled with petroleum hydrocarbon (PHC) exhibits very low fertility thus, do not provide adequate growth medium for crop growth, productivity and development [11].

Arbuscular mycorrhizal fungi (AMF) is a Member of the phylum Glomeromycota [12], and are ubiquitous in the soil, they are root symbionts, low host specific and are widely distributed geographically that are associated with almost 80% of terrestrial plants species [13]. AMF are very important in the balancing the ecosystems via contribution towards growth, adaptation and nutrition of plants under severe detrimental environmental conditions [14]. AMF encourages salt tolerance in crops by employing numerous mechanisms, such as promoting nutrient acquisition and uptake [15], in the production plant growth regulatory substances (hormones), enhancing rhizospheric and ailing soil conditions [16], alteration of the host plant's biochemical and physiological constituents [17] and protecting plant roots against (soil-borne) pathogens [18]. Also, AMF enhances plants water uptake capacity which is a physiological process via increase in the root hydraulic conductivity and comfortably adjusting osmotic balance and carbohydrates contents [19]. This may result in increase of plant growth and subsequent reduction of toxic ion effect [20]. They also enhance plant survival on crude oil polluted soil.

Salinity stress is a major problem in South-South coastal region of Nigeria where farmlands are in close proximity and bordered by the sea. This salt water via tidal waves sweeps through farmlands leaving deposits of salts via evaporation which have adverse effects on crops. Also, various activities in crude oil exploration and exploitation in these coastal regions also lead to spillage of oil into these environments. Considering these stresses are mostly studied singly in Nigeria, there has been no study on the combined effect of salinity stress and crude oil pollution in south-south Nigeria where these incidences is observed. Therefore, this research was aimed at showing different stress combinations (salinity and crude oil pollution) and their impact on *Telfairia occidentalis* and the potential of Arbuscular mycorrhizal fungi (AMF) *Glomus deserticola* in ameliorating these deleterious combinational effects.

2. Materials and Methods

2.1 Study Area

Experimental soil samples were collected from surface horizon (0–60 cm). The saline soil collection site is located in Ibeno, a coastal community in Akwa Ibom State, Nigeria. Garden Soil was collected from a farmland in Uyo, Akwa Ibom State, Nigeria.

2.2 Experimental Design

The Randomized Complete Block Design (RCBD) setup using seven (7) different and unique treatments (Table 1) was used for this research which include sterilized garden soil, saline soil, crude oil polluted soil (garden soil mixed with 50 ml crude oil) and saline soil + crude oil (saline soil + garden soil mixed with 50 ml crude oil), mycorrhiza (*Glomus deserticola*) inoculums. *Telfairia occidentalis* seeds were planted after setting up the experiment. Pots of about 12 cm in depth containing about 8 kg of experimental soils were used. About 25 g of mycorrhizal fungus soil containing about 65 spores per 5g of *Glomus deserticola* was placed in form of a layer into a hole made in the middle of the soil in the experimental pots before *Telfairia occidentalis* seeds were planted. Seeds were left for a week to germinate and two weeks for mycorrhizal establish before irrigating with saline water to avoid salinity shock.

Table1. *Outline of the different treatments used in the study*

Treatments	Garden soil + T. occidentalis seedlings
S-C-M-	Saline soil + T. occidentalis seedlings
S+C-M-	Saline soil + Crude oil soil + T. occidentalis seedlings
S+C+M-	Saline soil + T. occidentalis seedlings
S+C+M+	Saline soil + Crude oil soil + Glomus deserticola + T. occidentalis seedlings
S-C+M+	Crude oil soil + Glomus deserticola + T. occidentalis seedlings
S-C-M+	Garden soil + Glomus deserticola + T. occidentalis seedlings
S-C+M-	Crude oil soil + T. occidentalis seedlings

2.3 Physicochemical Properties of the Soil

The physicochemical properties of the experimental soils were carried out according to the standard methods of [21]

2.4 Determination of Growth Parameters

Measurement of the growth parameters of *T. occidentalis* such as percentage germination, Internode length, shoot length, number of nodes, petiole length and leaf area were taken after nine (9) weeks and recorded.

2.5 Heavy Metal Analysis

The powdered samples of *T. occidentalis* were digested with very strong acids, perchloric acid ($HCLO_4$) and nitric acid (HNO_3) and this is called Wet digestion method. The digested samples were read using Unicam 939 Atomic Absorption Spectrophotometer to determine the concentration of Chromium (Cr), Copper (Cu), Cadmium (Cd) and Lead (Pb).

2.6 Determination of Mineral Content

Mineral contents: iron (Fe), manganese (Mn), calcium (Ca), sodium (Na), magnesium (Mg), potassium (K) and phosphorus (P) of *T. occidentalis* samples were carried out using atomic absorption spectrophotometer (AAS), flame photometry and spectrophotometry using standard methods of [21].

2.7 Chlorophyll contents

atLeaf Chlorophyll meter was used to determine chlorophyll a, b and carotenoids contents of *T. occidentalis*.

2.8 Mycorrhizal Spores in Soil

The spores were isolated through wet sieving methods [22]. Using a beaker 100g of each soil samples was weighed and suspended in 500ml of distilled water. The suspension was stirred and allowed to settle for 30secs. Decanting of the suspension over a series of specialized sieves with spore sizes of 45, 53, 60, 65 micrometers was carried out. The decanting procedure was repeated four (4) times and contents of the last three specialized sieves were collected and suspended in 40% w/v sucrose gradient solution, then the sucrose gradient solution was centrifuged at 3000 rpm for 5mins [23]. Using a 38 micrometer sieve, the supernatant was decanted in order to wash away using distilled water the sucrose solution. A grid line plate was used to collect the remaining contents which was then poured for examination and counting of mycorrhizal spores under using a field dissecting microscope.

2.9 Statistical Analysis

All data collected was subjected to one-way analysis of variance (ANOVA). Means were separated using Duncan's multiple range test. Student's t-test was used to determine the significant difference between means of the soil parameters analyzed using Statistical package for social science (SPSS) version 20.0. However, a probability level of $p=0.05$ was considered statistically significant.

3. Results and Discussion

T-test analysis of the physicochemical properties of the experimental soil indicated that there was some significant ($p=0.05$) difference between the garden soil and the polluted soil (crude oil polluted soil and saline soil) (Table2). For the physicochemical properties of the analyzed soils, there was significant difference ($p=0.05$) in parameters such as available phosphorus, clay, sand, base saturation and electrical conductivity (Table2). Heavy metal analysis of the experimental soils also revealed there was significant difference (p=0.05) between the garden soil and polluted soil with high concentration of chromium, copper, cadmium and lead (Table2). Saline soils and soils polluted with crude oil are different from unpolluted soils as a result of alterations in their biological and physico-chemical parameters [24]. This observation is in line with the results of a research by [25] who also observed steady increase in total nitrogen on crude oil impacted soil. [26] Corroborates the observation of this study which indicates reduction in phosphorus contents of the soil impacted by PHC.

Results of growth parameters of *T. occidentalis* taken after nine (9) weeks revealed that the percentage germination, shoot length, petiole length, leaf area, internode length and leaf number of the plant was significantly reduced by combinational stress of salinity + crude oil (S+ C+ M-), non-mycorrhizal inoculated crude oil (S- C+ M-) treatment and salinity treatment (S+ C- M-) also exerted severe adverse effects on the growth morphology of *T. occidentalis* when compared to the control (S- C- M-) (Table 3; Figure 1). Under saline stress conditions, setbacks are observed in growth inhibition and biomass yield. This could be as a result of non-availability of essential nutrients and the expenditure of energy to counter the toxic effects of NaCl [27]. This could also be as a result of the toxic nature of crude oil which may have damaging effect on plant tissues leading to ineffective absorption and distribution of nutrients. The results agree with the study of [28] who observed reduction in shoot length and general growth of soybean (*Glycine max*) as a result of unsatisfactory soil conditions associated with reduction in soil air pore space and high demand of oxygen by hydrocarbonoclastic microorganisms.

Table2. *Physiochemical Properties of the Experimental Soils*

Parameters	(Garden Soil)	(Saline + Crude oil polluted Soil)	t-values
pH	6.75	7.35	0.002
Total Nitrogen (%)	0.47	0.99	0.001
Available P. (mg/kg)	37.34	22.42	16.47*
Silt (%)	4.00	5.60	0.0018
Clay (%)	4.20	12.00	-2.364*
Sand (%)	92.04	82.40	5.165*
Ex. Ca (cmol./kg)	3.75	4.97	-0.042
Ex. Mg (cmol./kg)	4.46	2.80	0.547
Ex. N. (cmol./kg)	0.45	0.20	0.412
Ex. K. (cmol./kg)	0.96	0.38	0.052
Organic Carbon	1.51	3.61	-0.147
Exchange acidity	3.71	3.10	1.054
ECEC (cmol./kg)	6.44	8.86	-1.528
Base saturation	61.72	73.84	-3.257*
EC. (dS/m)	0.06	6.97	-2.004*
Pb (mg/kg)	0.05	5.04	-2.568*
Cu (mg/kg)	2.31	13.13	-2.600*
Cd (mg/kg)	0.82	6.114	-1.850*
Cr (mg/kg)	1.94	33.47	-3.581*

*Significant at p=0.05, Ex – Exchange, ECEC – Effective cation exchange capacity, C/N – Carbon/Nitrogen ratio, EC – Electrical conductivity.

Mycorrhizal inoculated unpolluted treatment (S- C- M+) showed improved growth when compared to the control. Shoot length, petiole length, leaf area, internode length and leaf number of unpolluted mycorrhizal inoculated treatment showed growth stimulated above the control (Table 3; Figure 1). However, inoculation of *T. occidentalis* with *G. deserticola* showed remarkable improvements in crude oil, saline and crude oil/saline polluted treatments when compared to the uninoculated polluted treatments (Table 3). AMF symbiosis association often results in plants resilience to environmental stresses, such as salinity stress, organic contamination such as hydrocarbons [29], temperature extremes [30], phytoxicity [31], heavy metals [32], soil compaction [33] and drought [34]. Numerous researches indicate that several AMF species enhances tolerance to salinity [7].

Table 3. *Growth parameters of T. occidentalis grown for nine (9) weeks*

Treatments	Percentage germination (%)	Shoot length(cm)	Petiole length(cm)	Leaf number	Internode length (cm)	Leaf area(cm^2)
S-C-M-	100 ±0.00[a]	106.13±1.41[a]	9.94±0.43[a]	41.14±1.21[b]	7.59±0.55[a]	121.68±3.45[b]
S+C-M-	55.55±0.58[c]	16.54±0.14[c]	2.34±0.11[c]	8.25±0.53[d]	2.01±0.33[b]	25.15±2.11[d]
S+C+M-	33.33±0.33[d]	10.21±0.52[c]	1.02±0.23[c]	2.35±0.22[e]	0.99±0.72[c]	12.51±0.37[e]
S+C+M+	80.00±0.00[b]	81.42±0.92[b]	5.48±1.48[b]	22.25±0.28[c]	5.17±0.22[a]	88.85±2.48[c]
S-C+M+	100 ±0.00[a]	87.65±2.18[b]	6.14±0.18[b]	27.85±1.95[c]	6.67±0.15[a]	90.17±2.26[c]
S-C-M+	100 ±0.00[a]	119.23±2.53[a]	9.98±0.82[a]	50.25±2.45[a]	8.02±0.87[a]	134.62±3.65[a]
S-C+M-	55.55±0.55[c]	15.62±0.12[c]	2.02±0.67[c]	8.15±1.25[d]	2.07±0.81[b]	22.18±1.37[d]

Data is presented as mean ± SEM of three replicates. [a]Values with different superscript is statistically significant.

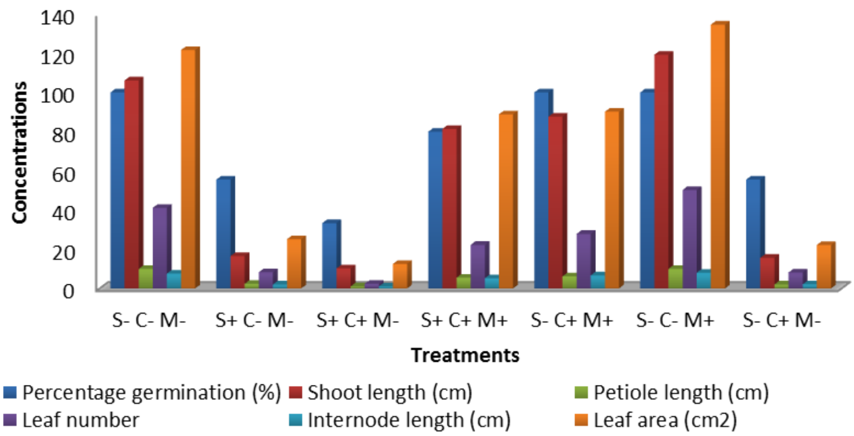

Figure 1. *Growth Parameters of Telfairia occidentalis*

In this research, heavy metal analysis revealed increased concentration of heavy metals in non-mycorrhizal combinational saline + crude oil pollution treatment (S+ C+ M-) and crude pollution treatment (S- C+ M-). Cadmium and chromium recorded highest accumulation in *T. occidentalis*, while lead and copper recorded low accumulation in *T. occidentalis*. *Glomus deserticola* innoculation reduced heavy metal uptake and accumulation in *T. occidentalis* significantly (p=0.05) (Figure 2).

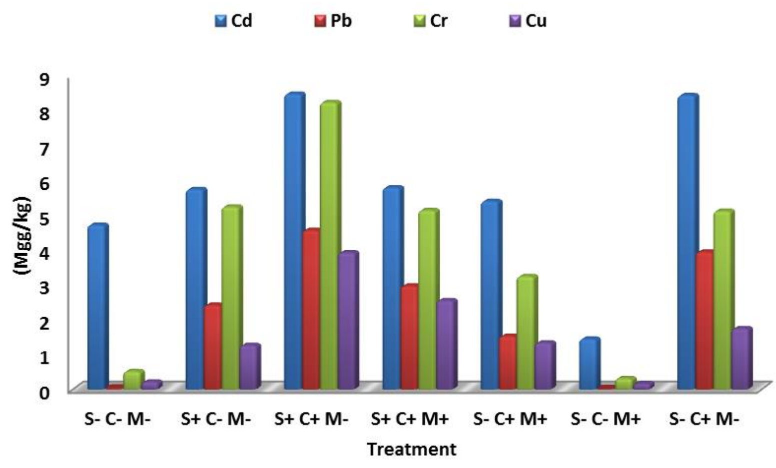

Figure 2. *Heavy Metal uptake of Telfairia occidentalis*

The observations of this research agrees with the observations of [35] who showed the accumulation of zinc, iron, cadmium, lead, manganese and cadmium contents in maize (*Zea mays* L.) grown on soils impacted with crude oil which led to reduced growth and poor yield. Lead and cadmium inhibits mineral uptake via synergistic or antagonistic reactions resulting in reduced growth [36]. Similarly, [37] reported the accumulation of zinc, lead and nickel on seeds and leaves of groundnut *(Arachis hypogea)* grown on spent engine oil polluted soil. Also, [38] reported that certain plants including cassava can extract heavy metals from crude oil polluted soils. Furthermore, [39] stated that the toxic influence of heavy metals on mineral acquisition and uptake results in poor growth of crops in higher levels of crude oil polluted treatments. AMF association with plants often results in increase in plant resilience to organic contaminants such as petroleum hydrocarbons [29].

Mineral elements screening of *T. occidentalis* revealed significant (p=0.05) reduction in potassium, magnesium, calcium and phosphorus across non-mycorrhizal inoculated saline and crude oil pollution treatments, highest reduction in mineral contents of *T. occidentalis* was observed in saline + crude oil combined treatment (S+ C+ M-). However, there was an increase in Na^+ contents in saline treatments (S+ C- M-) (Figure 3).

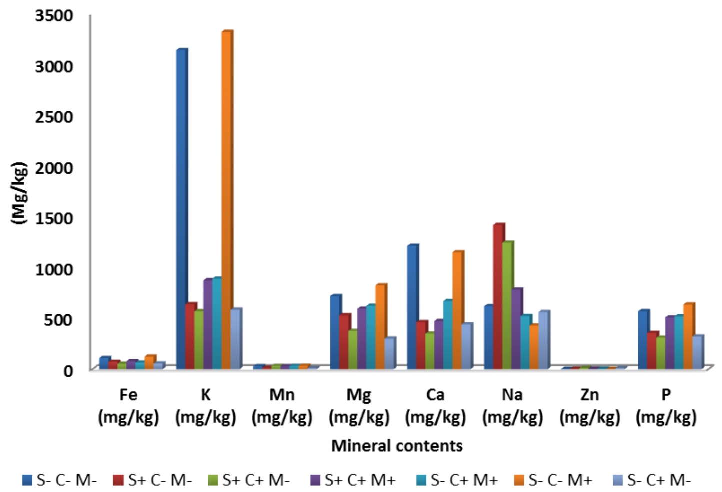

Figure 3. *Mineral contents of Telfairia occidentalis*

Na^+ antagonizes K^+ for binding sites and thus inhibits a number of key physiological functions that's dependent on K^+ availability [27], such as the operation of stomata, transcriptional and enzyme functions [8]. This agrees with the study of [40] who reported that plant mineral elements (P, N, K, Ca and Mg) of *Treculia africana* was inhibited by oil in soil, with all the elements showing significant (p=0.05) reductions with increase in crude oil pollution levels. Similarly, [40] also reported that soil nutrient status as well as plant nutrient uptake is significantly affected by crude oil in soil.

Treatments inoculated with *G. deserticola* showed increase in potassium and phosphorus contents of *T. occidentalis* and significant (p=0.05) reduction in Na^+ content across mycorrhizal inoculated treatments (Figure 3). AMF have been shown to enhance soil mineral nutrients (especially poor mobility nutrients; phosphorus) of plants grown under salt-stress conditions by enhancing and/or selective uptake of nutrients shown to have a positive influence on the composition plants [27]. [29] reported that AMF association with plants promotes mineral uptake under organic contaminants such as petroleum hydrocarbons.

In this study, chlorophyll a, b and carotenoids contents of *T. occidentalis* significantly (p=0.05) decreased in non-mycorrhizal inoculated treatments, with lowest values recorded in S+ C+ M- (combined treatment of salinity + crude oil pollution) (Figure 4).

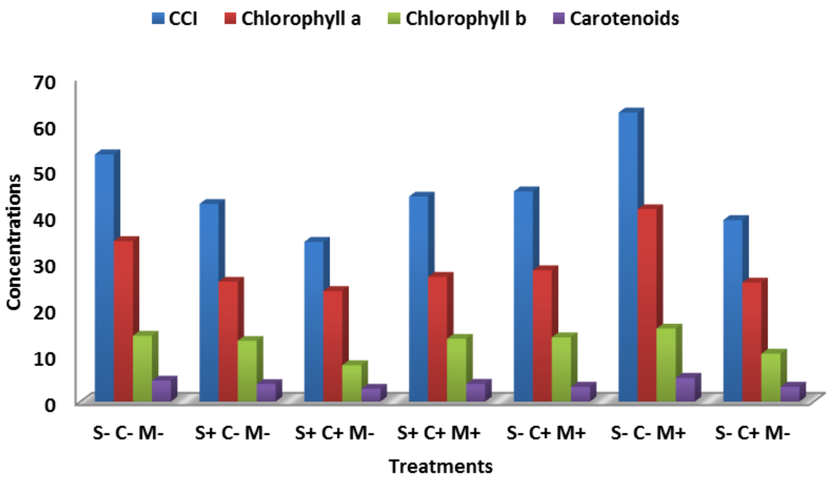

Figure 4. *Chlorophyll contents of Telfairia occidentalis*

AMF inoculation significantly showed improved chlorophyll contents as compared to the non-AMF inoculated treatments. The accumulation of ions and functional disorders observed during opening and closing of stoma under salinity stress conditions may be responsible for the decrease in total chlorophyll content [41, 42, and 43]. Rapid senescence of plant leaves of plants under salt stress may also result in the decrease of chlorophyll content under salt conditions [43]. Salinity sensitive plants show rapid decrease in chlorophyll content under salinity stress is observed more than cultivars with low tolerance [44]. [43] observed statistically significant decrease occurs in the composition of chlorophyll a and chlorophyll b content of plants depending on varied salt concentrations in comparison to the control application.

4. Conclusions

The set of growth parameters, biochemical and physiological analysis carried out in this study showed distinct characteristics of salinity and crude oil pollution in a single and combined stress effect showed remarked reduction in growth, mineral contents and chlorophyll contents of *Telfairia occidentalis* suggesting the severe nature of salinity and crude oil contamination as observed in the study area. In spite the short-term period of stress regime obseverd in this research, severe insights was given on the effect of combined effect of salinity and crude oil contamination as it is obtained in the natural environment of study area. The AMF *Glomus deserticola* induced promotion of growth and salt tolerance in *Telfairia occidentalis* and has been revealed according to this study to be a latent bioremediating medium against salinity and in sites polluted with organic contaminants such as petroleum hydrocarbons. Continued curiosity surrounding the use of AMF symbiosis as a boost to plants faced with stress as a well-rationaled means.

Conflict of Interest

The authors declare that there is no conflict of interest.

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