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Response of *Telfairia occidentalis* (Hook) to Arbuscular Mycorrhizal Fungi And *Gliricidia sepium* Leaves Manure in Spent Engine Oil Contaminated Soil

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Abstract: This experiment was conducted to investigate the response of *Telfairia occidentalis* (Hook) to arbuscular mycorrhizal fungus, *Glomus mosseae*, inoculation in spent engine oil contaminated soil ameliorated with *Gliricidia sepium* leaves manure. Spent engine oil contamination added vanadium to the soil while increasing aluminium, nickel, barium and lead concentrations by 281.8%; 623%; 3,700% and 1,400% respectively. Contamination with spent engine oil completely prevented germination or survival of *T. occidentalis* seedlings in the absence of mycorrhizal fungus inoculation and significantly ($P < 0.05$) reduced growth in the absence of both *G. mosseae* inoculation and *G. sepium* leaves manure application. Inoculation with *G. mosseae* increased essential elements uptake and reduced the concentration of heavy metals in *T. occidentalis* leaves. In conjunction with *G. sepium* leaves manure, the essential elements' uptake was higher in inoculated plants than in its absence. Mycorrhizal fungus colonization was also reduced by spent engine oil contamination but increased by *G. sepium* manure application. The positive synergistic effect of *G. mosseae* inoculation in conjunction with *G. sepium* leaves manure application on *T. occidentalis* growth and reduction of heavy metal uptake can be of great benefit in the production of this crop even in spent engine oil contaminated soil.

Key words: Soil · Spent oil · Manure · Mycorrhiza · *Telfairia occidentalis*

INTRODUCTION

Spent engine oil (SEO) is expired, used or waste lubricating oil drained from automobile or generating set engines during servicing in order to refill the crankcase with fresh oil. Disposal of this spent oil into any available space, gutters or uncultivated plots of land has led to serious soil contamination. Environmental pollution caused by spent engine oil (SEO) is becoming more wide spread than crude oil pollution in all parts of Nigeria [1]. Studies have shown that spent engine oil contains large quantities of hydrocarbons [2, 3], amines, phenols and benzenes as well as heavy metals such as vanadium, barium, lead, aluminium, nickel and iron [4]. Most of these heavy metals and hydrocarbons eventually get absorbed into plants tissues [5, 6] and at certain levels of intake constitute serious health hazards to humans [7]. The hydrocarbons create hydrophobic environment in the soil which limits water absorption to plants roots [8]. Spent engine oil's presence in the soil is also known to reduce soil aeration, immobilize soil nutrients and lower soil pH [9], while decreasing catalase activity and increasing dehydrogenase activity of the soil [10]. SEO contaminated soils like any other petroleum contaminated soil should be of great concern not only because they are unsuitable for agricultural and recreational use but are also potential sources of surface and ground water contamination [11]. The presence of SEO in the soil also adversely affect germination, growth and yield of crops [12].

One of the major problems facing most developing urban cities in Nigeria is how to satisfy the vegetable needs of their ever increasing population. Fertile plots for home gardens are fast disappearing. Peasant gardeners have resorted to roadside cultivation despite its consequent erosion risks. Large amounts of inorganic fertilizers are needed to sustain reasonable growth for subsequent yield.

Fluted pumpkin (*Telfairia occidentalis* Hook) is a popular leafy vegetable highly priced in many parts of Nigeria. Its high nutritional value and palatability make this vegetable a desirable one in most families' pots. Nearly all parts of this plant are useful [13]. One of the

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constraints to *T. occidentalis* production in Nigeria is lack of alternative land and inorganic fertilizer [13]. This vegetable is cultivated by resource poor gardeners who just manage any available plot of land including plots contaminated with spent engine oil beside auto-mechanic workshops. They use poultry droppings and/or inorganic fertilizers where they can afford to boost their vegetative yield.

Some studies have demonstrated that inoculation with arbuscular mycorrhizal fungi (AMF) can enhance growth of many plants [14, 15, 16]. They are also known to enhance plant growth and establishment in contaminated soils while reducing toxic effects of pollutants [17], as well as to help in ameliorating the effect of petroleum oil contaminated soil in some plants [18, 19]. Apart from the use of mycorrhiza, organic matter or manure has been demonstrated to be effective in bioremediation of contaminated soils [20]. The use of *Gliricidia sepium* leaves as mulch has been shown to increase the yield of several crops including taro and rice [21, 22]. *G. sepium* leaves have been shown to accumulate nitrogen, phosphorus and potassium [23]. Its manure can be a very good substitute for NPK inorganic fertilizers. With the emphasis now on organic farming in most parts of the world, *G. sepium* leaves can be adopted to increase soil fertility for increased crop production without the risk of surface or underground water contamination. There is however no research work, so far documented, done to assess the effect of AMF inoculation and/or *Gliricidia sepium* leaves manure on the germination and growth of *Telfairia occidentalis* in spent engine oil contaminated soil.

This study was therefore conducted to investigate the effect of arbuscular mycorrhizal fungus, *Glomus mosseae* and *Gliricidia sepium* leaves manure on the germination, growth and nutrient uptake of *T. occidentalis* grown in spent engine oil contaminated soil.

MATERIALS AND METHODS

The experiment was laid out in a completely randomized design with three treatments namely: *Glomus mosseae* inoculation, spent engine oil contamination and *Gliricidia sepium* leaves manure application. The investigation was carried out using perforated 45cm × 40cm black polythene planting bags. Fifteen such planting bags were each filled with 10kg of sieved soil which had previously been oven sterilized at 100°C for 48 hours. Those designated for spent engine oil (SEO) treatment were each mixed thoroughly with 3litres of SEO and allowed to drain for two weeks after which soil samples were taken along with samples from non-SEO treated soil for heavy metals (Pb, Al, Ni, Ba, V) analysis according to the procedure described in [24]. The soil in each bag was then watered to field capacity and allowed to drain overnight before planting. 2kg of chopped *Gliricidia sepium* green leaf manure was added to manure designated bags and incorporated into the soil.

Three seeds of *Telfairia occidentalis* were planted in each bag. Mycorrhizal inoculation was done at the time of planting by placing 1kg crude inoculum consisting of spores, hyphae and root fragments of maize infected with *Glomus mosseae* in planting holes before sowing the seeds. The non-mycorrhizal treatments were given autoclaved equivalents. Each treatment was replicated three times.

Growth Measurements and Data Analysis: Number of seeds germinating, number of leaves and length of vines were taken at weekly intervals for the first 5 weeks after planting (WAP). Five weeks after planting, the first nipping was done. Subsequent nipping was done at two weeks intervals until the eleventh week after planting when the final harvesting was done. At each nipping, plants were separated into stems and leaves and oven dried at 70°C to constant dry weight. At the end of the experiment, whole plants were carefully uprooted after thoroughly loosening the soil with water. Each plant according to its treatment and replicate was then separated into roots, stems and leaves and put in their respective appropriately labeled envelopes after representative sub-samples of feeder roots have been taken for mycorrhizal fungus colonization assessment. All the envelopes were oven dried at 70°C to constant dry weight to determine their biomass yield. Representative sub-samples of dried leaves were taken from different treatments in three replicates for nutrient analysis.

Plant Samples Analysis: Dried samples of leaves were ground using a laboratory grinder. 3.0g of each sample was placed in a clean platinum crucible and ashed at 450-500°C and allowed to cool to room temperature in a desiccator. The ash from each sample was later dissolved in 5ml of 20% hydrochloric acid and transferred into 100ml volumetric flask and made up to the mark with distilled water. This was thoroughly shaken to mix well before being taken for heavy metal concentration analysis. The determination of heavy metal (Pb, Al, Ni, Ba, V) concentration of the sample solution was carried out using atomic absorption spectrophotometer (Pye Unicam

2900) following the method of AOAC [24], dry sample procedure. Samples' analysis for nitrogen content was done using the micro-Kjeldahl method and the digest was determined using a Technicon auto-analyzer while those for phosphorus, potassium, calcium and magnesium contents were carried out using the dry ashing procedure and determined using atomic absorption spectrophotometer [25].

AMF colonization was assessed by clearing and staining feeder roots using the procedure of Koske and Gemma [26] for clearing and detecting mycorrhizae. Percentage root colonization was determined by scanning vertical and horizontal gridlines at 15- 45× magnification with a dissection microscope after the method of Giovanetti and Mosse [27].

Cumulative dry weights of leaves and vines were calculated. All data were subjected to a combined analysis of variance (ANOVA) technique using the Windows version of Statistical Analysis System [28] and Duncan's multiple range test was used to compare the means at 0.05 level of probability when the F- ratio was significant.

RESULTS

Effect of Spent Engine Oil (SEO) Contamination on the Heavy Metals Content of the Soil: Soil contamination with spent engine oil added to and significantly increased the concentration of heavy metals in the soil (Table 1). Vanadium which was initially altogether absent was added to the soil by SEO contamination while aluminium concentration was increased by 281.78%; nickel by 623%; barium by 3,700% and lead by 1,400%.

Effect of SEO Contamination on *T. occidentalis* Seed Germination: Spent engine oil in the absence of manure and *G. mosseae* inoculation completely prevented germination of telfairia seeds as at 2 weeks after planting. In the presence of *Glomus mosseae* inoculation or both *Gliricidia sepium* leaves manure and *Glomus mosseae* inoculation, the seeds germinated and growth was sustained.

Effect of SEO Contamination on the Growth, Biomass Yield and AMF Colonization of *T. occidentalis*: Inoculation with *G. mosseae* and *G. sepium* leaves manure application significantly enhanced production of leaves (Table 2) and growth of vines with the longest ones occurring in the absence of spent engine oil after five weeks (Table 3). Soil contamination with SEO reduced vegetative growth with the worst effect recorded in the absence of manure (Tables 4&5). The root biomass yield as determined by the final harvest followed the same trend as shoot (Table 5). Colonization of roots by *G. mosseae* was adversely affected by SEO contamination (Table 5). The lowest percentage of AMF colonization occurred in the absence of manure while the highest was found in the mycorrhiza with manure treatment. There was a significant positive relationship between biomass yield and AMF colonization.

Effect of SEO Contamination on Plant Nutrient Uptake: Spent engine oil contamination significantly reduced nutrient uptake for essential elements while building up the heavy metals' concentration in *T. occidentalis* (Table 6). Inoculation with *G. mosseae* increased nutrient

Table 1: Concentration of heavy metals in the soil before and after contamination (mg kg^{-1})

Element	Uncontaminated soil	Contaminated soil
Lead (Pb)	0.01	0.15
Aluminium (Al)	25.21	96.25
Nickel (Ni)	1.25	7.48
Barium (Ba)	0.01	0.38
Vanadium (V)	0.00	0.19

Table 2: Effects SEO contamination on the number of *T. occidentalis* leaves (leaf $plant^{-1}$)

Treatment	2WAP	3WAP	4WAP	5WAP
$M^-O^+m^+$	-	-	-	-
$M^+O^+m^-$	-	*3±1c	5±1d	7±1d
$M^+O^+m^+$	3±1b	5±1b	8±1c	12±1c
$M^+O^-m^+$	6±2a	9±2a	14±1a	17±1a
$M^-O^-m^-$	4±1b	6±1b	10±1b	14±1b

M^+: mycorrhiza inoculated; M^-: mycorrhiza uninoculated; O^+: SEO contaminated; O^-: SEO uncontaminated; m^+: manure incorporated; m^-: manure not incorporated; WAP: weeks after planting; *Means of three replicates ± s.e.m. Means in the same column followed by different letters are significantly different at P < 0.05 according to Duncan's multiple range test

Table 3: Effects of SEO contamination on the length of vine (cm)

Treatment	2WAP	3WAP	4WAP	5WAP
§$M^-O^+m^-$	-	-	-	-
$M^+O^+m^-$	-	*3.8±2.45d	6.8±2.45d	10.2±1.28d
$M^+O^+m^+$	4.3±2.69c	8.9±1.92c	12.0±0.96c	16.5±1.49c
$M^+O^-m^+$	17.1±4.55a	36.8±8.35a	77.5±10.46a	101.6±10.77a
$M^-O^-m^-$	10.6±2.14b	25.5±6.04ab	45.9±5.44b	61.8±8.90b

§See foot note under table 2

Table 4: Effects of SEO contamination on *shoot dry weight (gplant^{-1})

Treatment	5 WAP	7 WAP	9 WAP	11 WAP
§$M^-O^+m^-$	-	-	-	-
$M^+O^+m^-$	-	*2.4±0.79d	4.6±1.30d	5.1±1.80d
$M^+O^+m^+$	2.8±0.17c	4.0±0.65c	6.5±1.17c	9.5±1.93c
$M^+O^-m^+$	8.2±2.94a	15.1±3.02a	20.5±3.18a	30.5±3.38a
$M^-O^-m^-$	4.7±1.90b	9.8±3.80b	13.9±4.80b	20.0±3.80b

§See foot note under table 2. *Nipped shoots with edible leaves

Table 5: Effects of SEO contamination on plant dry weight accumulation (gplant^{-1}) and AMF colonization at 11WAP

Treatment	Root	δShoot	Amf Colonization (%)
§$M^-O^+m^-$	-	-	-
$M^+O^+m^-$	1.1±0.25d	7.1±1.6d	41.0±12.3c
$M^+O^+m^+$	1.8±0.12c	13.7±1.5c	76.1±8.2b
$M^+O^-m^+$	3.1±0.50a	39.7±4.8a	98.0±1.8a
$M^-O^-m^-$	2.4±0.21b	26.0±3.1b	-

§See foot note under table 2. δCumulative total of nipped shoots with edible leaves and final harvested left over shoots with aged non-edible leaves

Table 6: Effects of SEO on nutrient and heavy metal uptake in *T. occidentals* (mgkg^{-1})

Treatment	N	P	K	Ca	Mg	Pb	Al	Ni	Ba	V
§$M^+O^+m^-$	*2.870d	1999.80c	148.50d	87.20d	64.90c	0.33a	86.32a	6.34a	0.24a	0.08a
$M^+O^+m^+$	4.130b	2666.40b	253.20b	133.80b	58.70b	0.26b	81.36b	4.47b	0.22a	0.08a
$M^+O^-m^+$	5.810a	2999.70a	280.20a	145.70a	69.40a	0.05c	78.52bc	1.42d	0.13bc	0.03b
$M^-O^-m^-$	3.810c	1666.50d	216.40c	100.50c	46.30d	0.05c	69.47d	3.47c	0.16b	0.08a

§M^+: mycorrhiza inoculated; M^-: mycorrhiza uninoculated; O^+: SEO contaminated; O^-: SEO uncontaminated; m^+: manure incorporated; m^-: manure not incorporated. *Means in the same column followed by different letters are significantly different at P < 0.05 according to Duncan's multiple range test

uptake while reducing the concentration of heavy metals. In conjunction with *G. sepium* leaves manure, the essential elements' uptake was higher than in its absence while the heavy metals concentration was significantly reduced except for barium and vanadium (Table 6).

DISCUSSION

Some previous studies have reported the presence of heavy metals in spent engine oil [4]. The increase in the concentration of heavy metals in SEO contaminated soil confirms their presence in the SEO which according to ATSDR [29], come from the wear and tear of engine parts. The total prevention of germination by SEO in the absence of *G. mosseae* and *G. sepium* leaves manure application was possibly caused by the inhibitory effects of some volatile fractions with less than three rings which could have been present in SEO. According to Henner *et al.* [30], these volatile fractions have been found to inhibit germination of some seeds. Also the effect of SEO on membrane permeability must have inhibited the initial imbibition of water by the *T. occidentalis* seeds thereby delaying or totally preventing their germination.

The enhanced growth of *T. occidentalis* in inoculated and manured treatment can be attributed to increased mineralization and nutrient extraction by the *G. mosseae* as well as additional nutrients from the gliricidia leaves manure. Also the amelioration effect of the manure on the SEO contaminated soil must have provided a conducive rhizosphere for both root growth and mycorrhizal fungus colonization which would have led to a wider soil exploration for nutrients and water extraction. The reduction in AMF colonization by SEO contamination in this study is similar to an earlier result by Kirk *et al.* [31] using diesel fuel. This reduction according to them could have resulted from an alteration of plant exudation, an important medium of signals from the host plant roots to the AMF, caused by the contaminant. Also the unsatisfactory conditions for soil microbial life that usually accompany such pollution [32] could have adversely affected the AMF spore germination, hyphal growth and subsequent infection of host roots.

The high nutrient concentration in inoculated plants could have resulted from the enhanced nutrient uptake by *G. mosseae* while the reduction by SEO contamination can be attributed to nutrient immobilization by its constituent hydrocarbons [9]. The low concentration of heavy metals in inoculated plants is an indication that the *G. mosseae* could have absorbed them and sequestered some portions of these heavy metals in their mycelia retained in the roots.

CONCLUSION

This investigation has demonstrated that mycorrhizal fungus, *Glomus mosseae* in conjunction with *G. sepium* leaves manure can be used to increase the vegetative growth of *T. occidentalis* even in spent engine oil contaminated soil. Thus at low input cost the production of *T. occidentalis* can be boosted.

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