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Response of Okra (*Abelmoschus esculentus*) Seedlings to Spent Oil-Contaminated Soil Enhanced with Mycorrhizal Fungi

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Abstract

Okra (Abelmoschus esculentus) is a widely cultivated vegetable known for its nutritional and economic importance. Mycorrhizal fungi form symbiotic associations with plant roots, enhancing nutrient uptake and improving plant tolerance to environmental stresses. This study explored the adaptation of Abelmoschus esculentus (L.) Moench to spent oil-contaminated soil amended with mycorrhizal fungi, focusing on plant height, number of leaves, and leaf surface area as indicators of growth and adaptation. The results indicated significant differences in plant growth across various treatments. The plants grown in uncontaminated soil amended with mycorrhizal fungi exhibited the tallest growth, reaching 18 cm by 22 days after planting, suggesting a positive adaptation to the soil environment. The number of leaves in the contaminated treatments decreased over time, particularly at 17 DAP, indicating that spent oil contamination adversely affected leaf retention. Leaf surface area increased over time for all treatments, with the largest leaf area observed in the uncontaminated soil amended with mycorrhizal fungi, reaching 51.25 cm² at 22 DAP, compared to 49.17 cm² in the control. The observations suggest that mycorrhizal fungi can enhance the adaptation of Abelmoschus esculentus to spent oil-contaminated soils by improving plant growth and mitigating some of the adverse effects of contamination. However, the findings also underscore the complexity of remediating heavily contaminated soils, where the fungi's effectiveness may be limited by the severity of the pollution.

1. INTRODUCTION

Abelmoschus esculentus, commonly known as okra, is a valuable crop widely cultivated for its nutritional and economic benefits. It is a source of vitamins, minerals, and dietary fiber, making it important for food security and nutrition (Gemede *et al*., 2015). Okra is also recognized for its bioactive compounds, which exhibit antioxidant and anti-inflammatory properties, contributing to its medicinal value and potential in promoting human health (Elkhalifa *et al*., 2021). However, okra's sensitivity to soil contaminants poses a significant challenge to its cultivation in polluted environments. The introduction of mycorrhizal fungi as a soil amendment offers a promising approach to improving okra's resilience and productivity in contaminated soils (Fayuan *et al*., 2022).

Soil contamination by spent oil is a growing environmental concern, particularly in regions with extensive industrial activities and improper waste disposal practices. Spent oil, comprising various hydrocarbons and heavy metals, adversely affects soil health, microbial activity, and plant growth (Ossai *et al*., 2020). The contaminants can disrupt soil structure, reduce nutrient availability, and increase toxicity levels, making it challenging for plants to thrive in such environments (Rodriguez-Rodriguez *et al*., 2016). Also, the presence of spent oil in soil can lead to the accumulation of toxic substances in the food chain, posing serious risks to human health and ecosystems (Singh and Haritash, 2019). Over time, these contaminants can persist in the environment, leading to long-term degradation of soil quality and biodiversity loss (Khan *et al*., 2011).

Mycorrhizal fungi form symbiotic associations with plant roots, enhancing nutrient uptake, water absorption, and resistance to environmental stressors. These fungi improve plant growth in contaminated soils by facilitating nutrient acquisition and enhancing soil structure (Chen *et al*., 2023). Additionally, mycorrhizal fungi contribute to the stabilization of soil aggregates, which improves soil porosity and reduces erosion, further aiding in plant establishment and growth in challenging environments (Fall *et al*., 2022). The symbiotic relationship between mycorrhizal fungi and plant roots is crucial for the survival and growth of plants in adverse conditions, as it enhances the plant’s ability to access nutrients and water that are otherwise limited in contaminated soils (Kuyper *et al*., 2021). This relationship is also known to increase the tolerance of plants to heavy metals and other toxic elements in the soil, as mycorrhizal fungi can sequester these harmful substances in their hyphae, reducing their availability to the plant (Li *et al*., 2023).

Adeyemi *et al* (2021), demonstrated that arbuscular mycorrhizal fungi could improve the growth and heavy metal tolerance of plants in polluted soils. This study highlights the potential of mycorrhizal fungi as a sustainable solution for managing soil contamination and promoting plant growth. Like many crops, okra's growth and yield can be significantly affected by adverse soil conditions, including contamination with spent oil (Umoren *et al*., 2024). Investigating the adaptation of *Abelmoschus esculentus* to spent oil-contaminated soils, with the aid of mycorrhizal fungi, could provide valuable insights into alternative cultivation strategies for contaminated lands. Therefore, this study aimed to investigate the adaptation of *Abelmoschus esculentus* to spent oil contaminated soil amended with mycorrhizal fungi.

2. MATERIALS AND METHODS

2.1 Study Area

An outdoor experiment was conducted at the Department of Plant Science and Biotechnology, Rivers State University, Nkpolu-Oroworukwo Port Harcourt, Nigeria. The area lies at approximately latitude 4.797° N and longitude 6.979° E. The region experiences a tropical climate with an annual rainfall ranging from 2,400 mm to 4,000 mm, predominantly occurring between March and October.

2.2 Source of Sample

Soil sample was obtained from the Botanical Garden, Rivers State University. *Abelmoschus esculentus* seeds was obtained from Kuch-99 Agriculture & Seeds Ltd. 7 Mann Street, Off Wetheral Road, Owerri, Imo State, Nigeria. Spent oil was obtained from an Automobile Mechanic Workshop, Ikoku Flyover, Mile 2, Port Harcourt, Nigeria. Rootgrow Mycorrhizae Fungi was obtained from Plant-works Ltd, Unit 930 Comforth drive, Kent Science Park, Sittingbourne, Kent ME9 8px, United Kingdom (UK).

was obtained from

2.3 Sample Description

Soil sample used was loamy soil. *Abelmoschus esculentus* short variety was used for the study and Rootgrow Mycorrhizae Fungi inoculum contains *Funneliformis mosseae*, *F. geosporus*, *Claroideoglomus claroideum*, *Glomus microaggregatum*, and *Rhizophagus irregularis* (Robinson-Boyer *et al*., 2016).

2.4 Sample preparation

Five kilograms of loamy soil was put into a perforated black polyethene bags, contaminated with different concentrations of spent oil and labelled accordingly. A total of 18 bags were used.

2.5 **Methodology**

Soil treatment

Soil samples were contaminated with measured levels of spent oil to simulate polluted condition. The spent oil was properly mixed with the soil, with the spent oil pollutants having their control experiments. The polluted and unpolluted soil samples were allowed to stand for 14 days.

Soil amendment

Mycorrhizal fungus was the material used for the amendment. The experiment consisted of 5kg soil contaminated with 100ml and 200ml spent oil, amended with 15g of Mycorrhizal fungi. Amendment was properly mixed in each polythene bag containing the soil and was left for 14 days before okra seeds were planted.

Planting of Okra

Okra seeds were planted fourteen days after soil amendment. Four seeds were planted per bag at 2cm deep. Each bag was thinned to one plant per bag after two weeks.

Experimental Design, Data collection and Statistical Analysis

The experiment was arranged in a Completely Randomized Design (CRD), replicated three (3) times for each treatment and labeled properly. Plant adaptation potential was monitored through plant height, number of leaves and leaf area. Plant height, Plant height was determined by measuring the height of the plants with a measuring tape. Data was analyzed using Analysis of Variance (ANOVA) with the aid of SPSS software version 25.

Table 1: Treatment Concentrations

Treatment	Contamination (Spent oil)	Amendment (Mycorrhizal Fungi)
Control	No contamination	No amendment
Treatment 1	100ml	15g of mycorrhizal fungi
Treatment 2	200ml	15g of mycorrhizal fungi
Treatment 3	100ml	No amendment
Treatment 4	200ml	No amendment
Treatment 5	No contamination	15g of mycorrhizal fungi

3. RESULT

3.1 Plant Height

Result for plant height of okra seedlings exposed to spent oil contaminated soil amended with mycorrhizal fungi is shown in table 4.1. The result reveals that there were variations on plant height across the different treatments. Plants raised in uncontaminated soil + mycorrhizal fungi were tallest in height (18cm by 22 DAP). It also revealed that treatments 3 and 4 (100ml and 200ml spent oil contamination without amendment) suffered stunted growth rate compared to the control and the amended treatments.

3.2 Number of Leaves

All the treatments maintained a significant leaf number except treatments 3 and 4 which were 100ml and 200ml spent oil contaminated soils without amendment. Some leaves in these treatments dropped prematurely at 17 DAP, hence caused a reduction in the number. The number of leaves for the control experiment increased as the day progressed. Treatments 1, 2 and 5 showed similar increase to the control.

3.3 Leaf Area

Leaf area for all treatments showed an increase as the day progressed. Plants in treatment 5 (Uncontaminated soil + mycorrhizal fungi) showed the largest leaf surface area. It reached 51.25cm² at 22 DAP compared to the control which was 49.17cm² at 22 DAP. This was followed by treatment 3 and 1 which were 42.16cm² and 40.53cm² 22 DAP respectively. Treatment 2 (200ml of spent oil contamination + Amendment) exhibited the smallest leaf area.

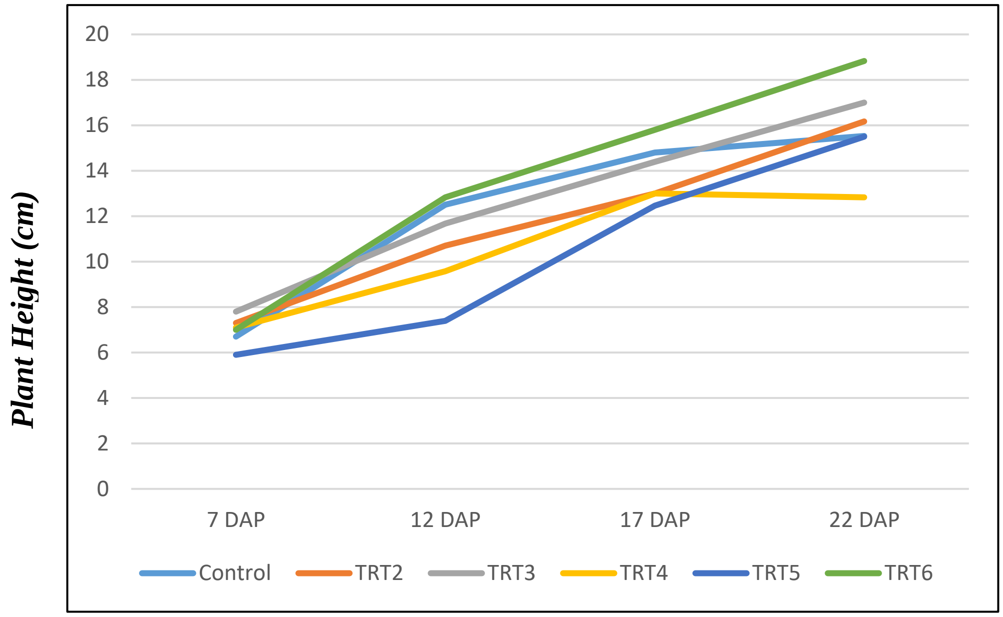

Fig. 1: Plant Height for Abelmoschus esculentus

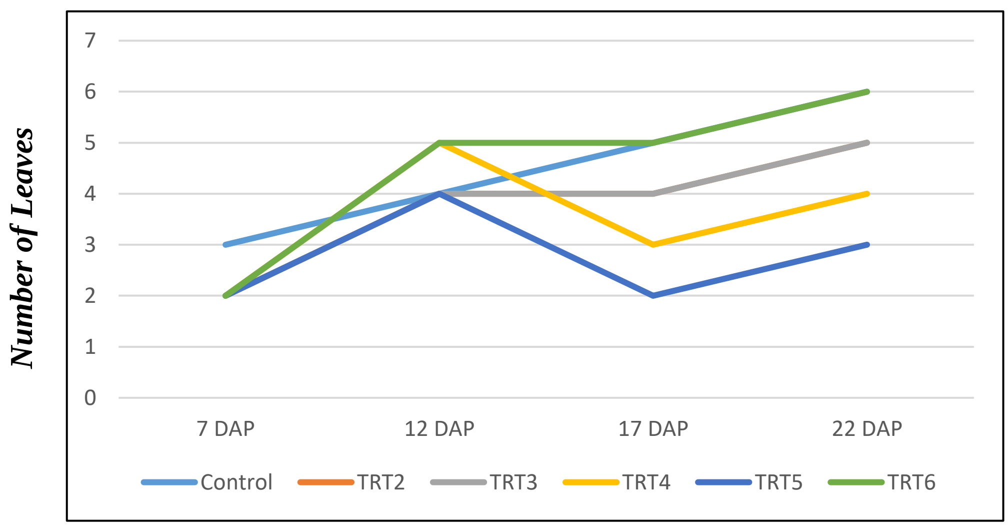

Fig. 2: Number of Leaves for Abelmoschus esculentus

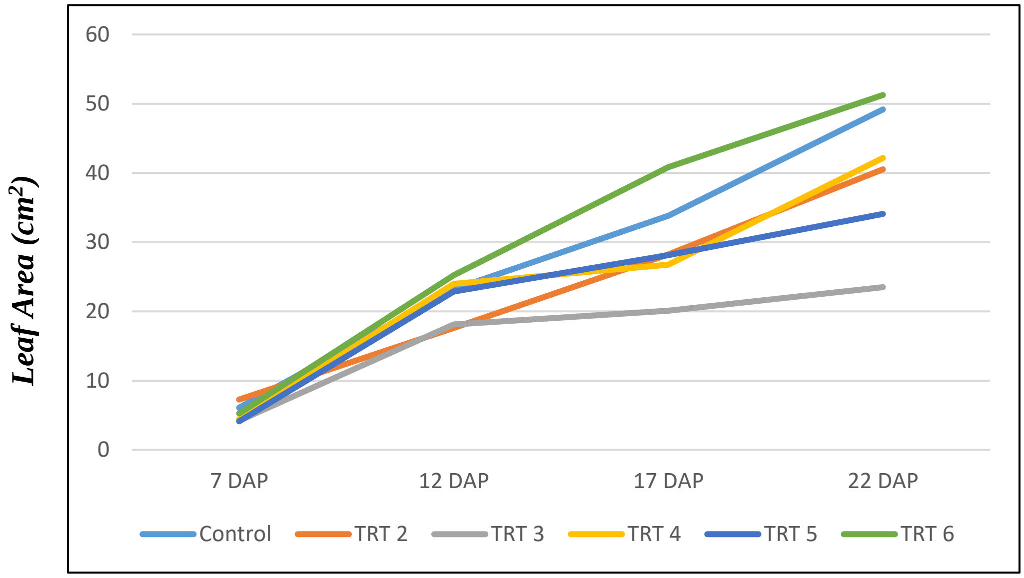

Fig. 3: Leaf Area for Abelmoschus esculentus

Plate 1: Contaminated and uncontaminated Abelmoschus esculentus plants

4. DISCUSION

Observations on the plant height of *Abelmoschus esculentus* under different treatments reveals that plant height varied significantly across different treatments. As depicted in Figure 4.1, plants in the uncontaminated soil amended with mycorrhizal fungi exhibited the greatest height, indicating that the presence of mycorrhizal fungi substantially enhances plant growth in optimal soil conditions. Consistent with Laranjeira et al (2021), who found that mycorrhizal associations improve nutrient uptake and plant vigor. This suggests that the symbiotic relationship between the fungi and the plant roots plays a crucial role in nutrient uptake and overall growth performance, particularly in unpolluted environments. Conversely, the treatments involving spent oil contamination without any amendment demonstrated the slowest growth rates, emphasizing the detrimental effects of oil pollution on plant development. This aligns with findings of Sagaya *et al*. (2023) who highlighted the inhibitory effects of soil contaminants on plant development. The gradual improvement of plant height in contaminated soils amended with mycorrhizal fungi supports findings by Kuyper et al (2021), who documented that mycorrhizal fungi can help plants cope with environmental stressors by enhancing water and nutrient acquisition. However, the slower growth compared to the uncontaminated amended treatment suggests that while mycorrhizal fungi can mitigate some of the adverse effects of contamination, they may not fully overcome the toxic impacts of heavy pollutants, as also noted by Gómez-Sagasti *et al*. (2021) in their study on mycorrhiza-assisted phytoremediation.

The result on the number of leaves indicates that while most treatments maintained a consistent number of leaves, treatments with 100ml and 200ml spent oil contamination without amendment, experienced reduction in leaf number by 17 days after planting (DAP). This was in contrast to the control and amended treatments, which showed a steady increase in leaf count. These findings are consistent with previous research by Sagaya et al (2023), who found that oil contaminants induce plant stress, leading to leaf loss, and with Ibrahim et al (2023), who reported that amendments, such as mycorrhizal fungi, enhance plant growth and resilience under adverse conditions.

Result on leaf area (Fig. 4.3) shows that leaf area increased across all treatments as the days progressed. Treatment with uncontaminated soil + mycorrhizal fungi exhibited the largest leaf surface area, reaching 51.25 cm^2 at 22 DAP, compared to the control, which had 49.17 cm^2 at 22 DAP. This result aligns with previous research indicating that mycorrhizal fungi significantly enhance leaf surface area by improving nutrient uptake and overall plant vigor (Machineski *et al*., 2018). Treatment 3 and 1 followed, with leaf surface areas of 42.16 cm^2 and 40.53 cm^2 at 22 DAP, respectively. This is consistent with studies suggesting that while contaminated soil impacts plant growth, amendments can mitigate some negative effects, leading to improved leaf surface areas compared to untreated contaminated soils (Chen *et al*., 2022). Smallest leaf area exhibited by Treatment 2 supports previous research by Ogbo et al (2009) that highlights the severe negative impact of oil contamination on plant leaf development, even when amendments are applied, as the pollutants still inhibit optimal growth.

6.1. Conclusion

Based on these findings, the potential of mycorrhizal fungi to enhance plant resilience in spent oil polluted soil has been demonstrated. Mycorrhizal fungi significantly improved stress resistance and overall plant growth, enabling okra to thrive in adverse conditions where contamination would typically hinder its growth. This highlights the importance of bioremediation techniques in agriculture, offering a sustainable approach to cultivating crops in polluted soils, thereby contributing to food security and environmental restoration.

It is recommended that mycorrhizal fungi should be widely adopted as a bioremediation strategy in soils contaminated with spent oil and future research should explore the synergistic effects of multiple remediation strategies on soil health and crop performance.

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