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# Effect of Spent Lubricating Oil on Total Antioxidant Power, Total Flavonoid Content and Total Phenolic Content of *Sorghum bicolor (L.) Mooch* and Soyabean *Glycine Max (L.) Merr* Seedlings after 14 Days of Germination

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**ABSTRACT**: Environmental pollution is a growing concern, with its detrimental effects on ecosystems, biodiversity, and human health. Spent lubricating oil (SLO) represents a pervasive environmental pollutant, often finding its way into soil and posing a significant threat to plant life. This study was to investigate effect of the fractions of SLO (water-soluble, WSF; water-insoluble, WISF; and crude whole SLO, cWSLO) and distilled water (DW) respectively. The WSF showed significant ($P<0.05$) decrease in both species of seedlings (Sorghum 15.34 mg GAE/g ± 2.01 and Soya-bean 16.87 mg GAE/g ± 0.86) from results of TAP when compared to that of DW while the cWSLO was most significantly ($P<0.05$) decreased. The TPC also revealed significant ($P<0.05$) decrease from the different fractions when compared to the control (DW) as well as that of the TFC values from the results. The disparities in TPC emphasize the selective influence of different SLO components on the production of phenolic compounds, which could be crucial for plant defense mechanisms against oxidative stress. Thus, the results of the study showed that although the different parameters indicated that SLO can affect the oxidative state of brown sorghum seeds but it can be seen that it may be possible for the plant to utilize some of the water soluble contents of the SLO to aid germination.

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Various studies have reported the adverse effect of petroleum products on plants ranging from reduced germination of seeds, reduced survival of plants to reduced yield of plants (Akinola *et al*., 2004; Andrade *et al*., 2004). Most of the reports on the effects of petroleum products on plants have focused on crude oil, diesel and gasoline (Siddiqui and Adams, 2002; Inoni *et al*., 2006) which get to the nvironment through accidental spillage. However, through the activities of automobile, generator, other machines, and servicing engineers (mechanics) spent oil is discharged to the environment indiscriminately. Spent lubricating oil (SLO) sometimes referred to as waste engine oil is produced by vacuum distillation of petroleum and usually contains chemical additives including amines, phenols, benzenes, calcium, zinc, barium, phosphorus, and sulphur (Lale *et al.*, 2014). It includes mono and multi-grade lubricating oils from petrol and diesel engines, together with gear oils and transmission fluids. Spent auto engine oil is obtained during routine

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maintenance of automobile and power generating engines; and often indiscriminately disposed into gutters, municipal drainage systems, open vacant plots and farms in Nigeria by auto technicians and allied artisans with workshops on the road sides and open places and often indiscriminately disposed into gutters, municipal drainage systems, open vacant plots and farms in Nigeria by auto technicians and allied artisans with workshops on the road sides and open places (Anoliefo and Vwioko, 2005). Various researchers have investigated and reported the ecological toxicity effect of petroleum and spent auto-engine oil (Ahamefule *et al.*, 2015). Oil (petroleum) in soil has deleterious effects on biological, chemical and physical properties of the soil depending on the dose, type of the oil and other factors (Shadrin *et al*., 2020). Oil contamination in soil can disrupt essential physiological processes in plants, including nutrient uptake, water absorption, and photosynthesis. Consequently, this can lead to stunted growth and decreased crop yields.

Among the key biochemical parameters in plants that are sensitive to environmental stressors are Total ntioxidant Capacity (TAC), Total Flavonoid Content (TFC), and Total Phenolic Content (TPC). These parameters are vital for the defense mechanisms of plants against oxidative stress and pathogenic attacks (Schlosberg and Carruthers, 2010). Cereals and legumes have been known to be part of nature's gift that are precious to humanity and mostly referred to as the 'poor man's meat' since they are high in protein (16–50%), essential elements, dietary fibre (10–23%) and vitamins (Maphosa and Jideani, 2017). Hence, the objective of This study was to evaluate the effect of spent lubricating oil (SLO) on total antioxidant power (TAP), total flavonoid content (TFC) and total phenolic content (TPC) of *Sorghum bicolor (L.) Mooch* and Soyabean *Glycine Max (L.) Merr* seedlings after 14 days of germination

## MATERIALS AND METHODS

*Sorghum bicolor* (*S. bicolor*) and *Glycine max* (*G.* max) - (soyabean) seeds were obtained from a local market (Uselu market) in Benin metropolis, Edo state, Nigeria and identified by a botanist in the Department of Plant Biology and Biotechnology, University of Benin and voucher numbers for Sorghum (UBH-S468) and Soyabean (UBH-G628) were generated respectively.

They were cleaned separately to remove any possible contamination and seed viability test was conducted by placing the seeds in containers of water, after 15 minutes, the seeds that sank were taken as viable seeds while those that remained floating were considered as nonviable. The groupings for planting were as thus: Distilled water (DW) - control group 1, water-soluble fraction (WSF) - group 2, water-insoluble fraction (WISF) - group 3 and crude whole spent lubricating oil (cWSLO) - group 4 respectively.

*Total Antioxidant Power (TAP) Estimation*: This assay is also called phosphomolybdate method as described by Sasikumar and Kalaisezhiyen, (2014). The antioxidants present in the extracts reduce the phosphomolybdate (VI) ion to a green phosphomolybdate (V) ion) and in this case, the reactions of sodium phosphate and ammonium molybdate in the presence of $H_2SO_4$ produce the free radical (phosphomolybdate ion). Equal amounts of 4 mM $((NH_4)_2MoO_4)$, 28 mM $Na_3PO_4$, and 0.6 M $H_2SO_4$ were added to produce the standard phosphomolybdate reagent solution. The standard used for this assay was (gallic acid) and was prepared in concentrations of 10, 25, 50, 100, 200, 300, 400, and 500µg/mL while 500µg/mL of plant samples extracts were utilized. To 1mL of extract or gallic acid (as reference) was added to 2 mL of the standard phosphomolybdate solution. It was then incubated in a water bath at 95°C for 90 min. A subtle green colour developed which was then allowed to cool to ambient temperature. After determining the absorbance at 695 nm, the overall antioxidant strength was shown as the corresponding amount of gallic acid (mgGAE/g) in one gram of the extracts.Gallic acid (mgGAE/g) in one gram of the extracts.

*Total Phenolic Content (TFC) estimation*: The total phenolic content of the extract was determined by the Folin–Ciocalteu method (Kaur and Kapoor, 2002). Briefly, 200 µL of crude extract (1 mg/mL) were made up to 3 mL with distilled water, mixed thoroughly with 0.5 mL of Folin–Ciocalteu reagent for 3 min, followed by the addition of 2 mL of 20% (w/v) sodium carbonate. The mixture was allowed to stand for a further 60 min in the dark, and absorbance was measured at 650 nm. The total phenolic content was calculated from the calibration curve, and the results were expressed as mg of garlic acid equivalent per g dry weight.

*Total flavonoid content*: This was determined by the aluminum chloride colorimetric method (Chang *et al*., 2002). In brief, 50 µL of crude extract (1 mg/mL ethanol) were made up to 1 mL with methanol, mixed with 4 mL of distilled water and then 0.3 mL of 5% NaNO2 solution; 0.3 mL of 10% AlCl3 solution was added after 5 min of incubation, and the mixture was allowed to stand for 6 min. Then, 2 mL of 1 mol/L NaOH solution were added, and the final volume of the mixture was brought to 10 mL with double-distilled water. The mixture was allowed to stand for

15 min, and absorbance was measured at 510 nm. The total flavonoid content was calculated from a calibration curve, and the result was expressed as mg quercetin equivalent per g dry weight.

## RESULTS AND DISCUSSION

The ability of plants to thrive and endure in a hostile environment hinge upon their antioxidative potential. Measuring Total Antioxidant Property (TAP) is pivotal, as it reveals the plant's aptitude to counteract the menace of free radicals and protect its delicate cellular structures. Stressors, especially those of an environmental nature, disrupt this delicate balance. Here, TAP serves as an invaluable metric to gauge the influence of SLO on soybean seedlings' antioxidative capacity (Bertrand *et al*., 2019). In this study,as reflected in the tables 1 and 2. The concentration of the total antioxidant power (TAP) and total phenolic content (TPC) of *S. bicolor (L.) Mooch* seeds revealed that, the TAP and the TPC of the seeds were higher in unpolluted soil samples than in polluted soil samples and it decreased with the increase in the concentration of used engine oil on the soil base.This results is supported with the observations made by previous researches (Sharma *et al*. 1980; Aguebor-Ogie *et al*. 2019). This is could be due to the contaminants present in the oil that interfered with the antioxidant activities of the sorghum (Okigbo, 2014).Comparing the total phenolic concentration of the water insoluble, water soluble and whole spent lubricating oil extracts, the concentration of water soluble fraction was found to be higher. This may have been as a result of the low concentration of possible contaminants in the water soluble fraction. The findings of Ekpo *et al.* (2012) and Kayode *et al.* (2009), who noted comparable effects, were supported by this result. The results for TFC demonstrated that the cWSLO competes favorably with the reference standard (quercetin), and the results of WSLO showed a lower concentration when compared to that of the water-insoluble fraction and water soluble fraction. Thus, a decrease in the total flavonoid content of the seeds of *S. bicolor* and *G. max* grown in the cWSLO as compared to the WISF after the 14th days of germination which correlates with report by Adenipeku *et al.* (2008). This surge in flavonoid production, as seen in the WSF when compared to the cWSLO groups, might be perceived as a defense strategy employed by both sorghum and soybean seedlings to ward off the oxidative stress inflicted by cWSLO. However, the identity of these flavonoids and their potential applications in enhancing stress tolerance in crop plants remain shrouded in mystery. This is a realm ripe for dedicated exploration, as these compounds could hold the key to the development of strategies for sustainable agriculture and stress-resilient crops (Castano *et al*., 2017)

**Table 1.** Effect of Spent Lubricating Oil (SLO) and its Fractions on TAP, TPC and TFC of *S. bicolor* (L)

| | Groups<br>Values obtained (concentrations) | | | |
|---|---|---|---|---|
| **Measured Parameters** | **Group 1 (DW)** | **Group 2 (WSF)** | **Group 3(WISF)** | **Group 4 (cWSLO)** |
| Total Antioxidant Property (TAP) (mgGAE/g) | $32.21 \pm 5.65^{a}$ | $15.34 \pm 2.01^{b}$ | $8.01 \pm 0.32^{c}$ | $7.23 \pm 0.16^{d}$ |
| Total Phenolic Content (TPC) (mgGAE/g) | $20.65 \pm 1.92^{a}$ | $16 \pm 2.06^{b}$ | $12.10 \pm 1.10^{c}$ | $9.87 \pm 1.35^{d}$ |
| Total Flavonoid Content(TFC) (mgQE/g) | $7.32 \pm 0.86^{a}$ | $4.98 \pm 0.15^{b}$ | $2.09 \pm 0.11^{c}$ | $1.21 \pm 0.04^{d}$ |

* Different alphabets represent statistical (P<0.05) difference while similar alphabets represent non-significant (P>0.05) difference

**Table 2.** Effect of Spent Lubricating Oil (SLO) and its Fractions on TAP, TPC and TFC of *G. max* (soyabean)

| | Groups<br>Values obtained (concentrations) | | | |
|---|---|---|---|---|
| **Measured Parameters** | **Group 1 (DW)** | **Group 2 (WSF)** | **Group 3 (WISF)** | **Group 4 (cWSLO)** |
| Total Antioxidant Property (TAP) (mgGAE/g) | $24.56 \pm 4.49^{a}$ | $16.87 \pm 0.86^{b}$ | $11.23 \pm 2.39^{c}$ | $3.02 \pm 0.23^{d}$ |
| Total Phenolic Content (TPC) (mgGAE/g) | $32.12 \pm 3.02^{a}$ | $19.16 \pm 3.11^{b}$ | $18.23 \pm 1.76^{c}$ | $8.56 \pm 1.10^{d}$ |
| Total Flavonoid Content(TFC) (mgQE/g) | $14.67 \pm 1.87^{a}$ | $12.64 \pm 1.91^{b}$ | $8.46 \pm 1.11^{c}$ | $6.11 \pm 0.43^{d}$ |

* Different alphabets represent statistical (P<0.05) difference while similar alphabets represent non-significant (P>0.05) difference

*Conclusion*: This study thus threw more light on the fact that spent engine oil, when disposed indiscriminately, becomes unsuitable for germination, hence there is need to enlighten the public on the hazards of indiscriminate disposal of this pollutant into environmental media which will go a long way in ensuring human and environmental health, improved crop propagation and safety, and food security.

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