Jewel Journal of Scientific Research (JJSR) 6(1&2): 184-193, 2021
©Federal University of Kashere-Nigeria (Print ISSN: 2384 – 6267, Online ISSN 2756-651X)
jjsr.fukashere.edu.ng

Physiological responses of photosynthetic and respiratory rates of some leafy vegetables to Spent engine oil contamination

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Abstract
Indiscriminate disposal of Spent engine oil (SEO) is a threat to food security in Nigeria. Therefore, this study evaluated effects of SEO on photosynthetic and respiratory rates of *Telfairia occidentalis, Corchorus olitorius* and *Amaranthus hybridus* leaves. The experiment was laid using a complete randomized design with five replicates. The vegetables were sprayed with 20, 40, 60, 80 and 100 mgL^{-1} SEO. Water served as control. Respiratory and photosynthetic rates of leaves of the vegetables were determined 2, 3, 4, 5 and 6 weeks after treatments. Data were analyzed using Statistical Analysis System. Means were separated using Duncan's Multiple Range Test at 5%. Respiratory rates (6.3 x10^{-5}) were significantly higher ($p<0.05$) in *T. occidentalis* treated with 100 mg L^{-1} compared with lower concentrations of the SEO. Also, significant reduction (6.9 x10^{-5}) was recorded in the photosynthetic rates of *T. occidentalis* treated with 100 mg L^{-1} while photosynthetic rate (11.4 x10^{-5}) of *T. occidentalis* showed significant increase. Furthermore, 100 mg L^{-1} produced significant increase (5.6 x10^{-5}) in the respiratory rates of *C. olitorius* while photosynthetic rate (5.7 x10^{-5}) showed significant decrease in *C. olitorius* treated with lower concentrations of the oil. Respiratory rate (7.7 x10^{-5}) showed significant increase in *A. hybridus* treated with 100 mg L^{-1} while photosynthetic rate of the vegetables treated with 100 mg L^{-1} SEO revealed significant reduction (5.1 x10^{-5}). In conclusion 100 mg L^{-1} SEO had negative effects on respiration and photosynthetic rates of leaves of vegetables therefore the vegetables should not be cultivated in oil contaminated land area.

Keywords: Photosynthetic rate: Respiratory rate: physiological responses, Spent engine oil; Vegetables; Contamination

Received: 16th Aug, 2021 *Accepted:* 11th Oct., 2021 *Published Online:* 25th Dec., 2021

Introduction

Improper disposal of spent engine oil (SEO) into vacant lands and water bodies by motor mechanics operators, individuals as wells as industries have been identified as a potential environmental threat to fauna and flora, soil quality, food security, climate change, agricultural practices and human health in Nigeria. Many of the oils are generated after servicing and subsequent draining of oil from automobiles and generator engines used domestically or industrially. These resulted in environmental contamination and disruption of natural habitats of organisms found in the environment (Sharifi *et al.*, 2007; Adu *et al.*, 2015). Also, additives are formed in the contaminated soil due to production processes, these additives are eventually incorporated into the oil to boost the viscosity, thermal and oxidation stability of oil (Agbogidi *et al.*, 2005) which when exposed to the atmosphere or absorbed by the vegetables makes them toxic to herbivores and other consumers of the vegetables (Adu *et al.*, 2015).

In addition, contamination of the natural environment by petroleum-derived substances contributes to the degradation of agricultural land and changes the physiological processes of plants cultivated on the affected lands (Olayinka and Arinde, 2012). According to Odjegba and Idowu (2002), germination of *Amaranthus hybridus* seeds was significantly affected by spent engine oil-polluted soil and eventually resulted in low sustainable production of vegetables for nutritional requirements of many households in developing nations such as Nigeria.

Studies by Wenying *et al.* (2017) also revealed that degradation of chlorophyll, alterations in the stomata mechanisms and reduction in photosynthesis and respiration rates (Wang *et al.*, 2017), accumulation of toxic substances or their by-products in vegetal tissue, decrease in size as well as less production of biomass (Daniel-Kalio and Pepple, 2006; Adenipekun *et al*, 2008) were the major common symptoms of plants contaminated with oil. Also, growth retardation and chlorosis of leaves coupled with dehydration of plants and water deficiency were other physiological processes associated with responses of plants to oil contamination Adu *et al.* (2015). This contamination is a critical threat to many agricultural and horticultural practices and has consequentially made many arable lands not unfit for sustainable cultivation of vegetables for human consumption. Based on the harmful effects of SEO on the physiology of plants, this study was conducted to evaluate the contamination effect of the used engine on photosynthetic and respiratory rates of *A. hybridus*, *T. occidentalis* and *C.olitorius*

Materials and Methods

Sources of experimental materials

Seeds of *A. hybridus*, *T. occidentalis* and *C. olitorius* used for this study were collected from nearby farms in Abeokuta. Visual identification and authentication of the vegetables were carefully done by a plant taxonomist, Professor Aworinde D. O of Ondo State University of Science and Technology, Okiti Pupa, Ondo State Nigeria.

SEO was collected from auto-mechanic villages at Mile 6 road, Idi Aba and Camp, Osiele, Abeokuta, Ogun State. SEO from the two locations were thoroughly mixed and allowed to stay for five days and thereafter used to constitute five different concentrations of SEO contaminant levels i.e. 20; 40; 60; 80; 100 mgL^{-1} using water as diluent. This was achieved using syringes which ensured accurate quantity of the SEO concentration. 20 ml of SEO was added to 980 ml of water and made up of 20 mgL^{-1} SEO while 40 mgl^{-1} was achieved by adding 40 ml of SEO to 960 ml of water diluent. Similar procedures were repeated for 60, 80 and 100 mgL^{-1} SEO concentrations. Water served as control.

The experiment was carried out at the teaching and research botanical garden of the Federal University of Agriculture, Abeokuta.

Collection of Soil Sample

Topsoil was randomly collected from the *Tectona grandis* plot, Federal University of Agriculture, Abeokuta and mixed thoroughly to ensure uniform distribution of nutrients. Also, pebbles and stones were removed from the soil and thereafter the soil was put into 90 perforated black polythene bags and watered for five days after which the polythene bags filled with soil were divided into three per vegetable.

Experimental design of the study

Each of the divisions was arranged into 6 groups of five replicates using a randomized complete block design. Seeds of *A. hybridus*, *T. occidentalis* and *C. olitorius* were separately sowed in each polythene bag containing the soil in situ. Each treatment was separated from the next by 0.5 m row spacing. The seedlings were thinned to five per polythene bag after emergence. SEO application commenced three weeks after planting when the vegetables have been established.

Each vegetable was treated daily with approximately 4litres of concentrations 20, 40, 60, 80, 100 mgL^{-1} v/v oil/water at the same time once daily from the second week to the sixth week after planting. Control vegetables were watered daily with equal amount of water only (4litre) for the same period. The experiment was monitored for respiratory and photosynthetic rates of the vegetables

Effect of SEO Contamination on Respiratory and Photosynthetic Rates of Leaves of *T. occidentalis, C. olitorius* and *A. hybridus*

Respiratory rate:

Sach's half leaf method described by Kadiri (1999) was employed. Five leaf discs (area $1.4cm^2$) were carefully bored out at dawn (5:00 am) with a cork borer from half part of a leaf still intact on treated and control vegetables. The treated and control vegetables were transferred immediately into a dark room. The leaf discs bored out were collected in envelopes and dried in the oven at 80^0C to constant weight; their dry weights were recorded and designated as W_1. Five hours later, still in the darkroom, the treated and the control vegetables were harvested with another set of 5 leaf discs (area $1.4cm^2$) bored out of the other half part of the leaf that was earlier bored for both treated and control vegetables. The bored-out leaf discs were dried in the oven at 80^0C to constant weight. Their dry weights were recorded and designated W_2.the entire procedure was replicated five times for each sample and the respiratory rate of the vegetables were calculated using the formula

$$\text{Respiratory rate} = \frac{(W1 - W2)}{1.4\text{cm2} \quad 5\text{hr}}\ x\ 1$$

Photosynthetic rate

Photosynthetic rate was determined in a manner similar to that of respiratory rate by modifying methods of Thoday (1909) and Neales and Incoll (1968). Five leaf discs (area $1.4cm^2$) were bored out from half part of a leaf on a treated and control vegetables that were used in determining respiratory rate. This was done immediately after determining the respiratory rate and with treated and control plant still in the dark. The leaf discs were also kept in envelopes and dried in the oven at 80^0C to constant weight, their weight was recorded and designated as W3. The treated and control plants were immediately transferred outdoor in the sun. Five hours later in the sun, another set of 5 leaf discs were bored out from the half parts of the leaf that was earlier bored on both the treated and control plants. The bored-out leaf discs were dried in the oven at 80^0C to constant weight; their dry weights recorded and designated W4. The entire procedure was repeated five times and photosynthetic rate was calculated using the mathematical relationship below;

$$\text{Photosynthetic rate} = \frac{(W1-W2)g + (W4-W3)g\ x\ 1}{1.4\text{cm2} \quad 5\text{hr}}$$

Unit of Photosynthetic rate is $gcm^{-2}h^{-1}$

W1 and W2 were Inputs from respiration.

Data Analysis:

Data were analysed using Statistical Analysis System (SAS, 2013, Version 9.3.SAS Institute Inc., Cary, NC., USA). Means were separated using Duncan's Multiple Range Test at 5% level of significance ($p < 0.05$)

Results

Effects of spent engine oil on respiratory and photosynthetic rates of *A. hybridus, T. occidentalis* and *C. olitorius* are presented in table 1. Results revealed that various concentrations of spent engine oil studied produced significant effects on respiratory and photosynthetic rates of *T. occidentalis* leaves. It was also noticed that with inclusion of concentrations of SEO, both respiratory and photosynthetic rates of *T. occidentalis* leaves showed significant difference ($p < 0.05$). Concentration of SEO produced significant increase ($p < 0.05$) on the respiratory rate of the vegetable while significant reduction ($p < 0.05$) was observed in the photosynthetic rate of the vegetable with inclusions of concentrations of engine oil over the period of investigation. Further investigation revealed that respiratory rates (6.3 $x10^{-5}$) were significantly higher ($p < 0.05$) in *T. occidentalis* treated with 100 mg L^{-1} compared with values of the parameter in the vegetable treated with lower concentrations of the SEO. Significant reduction (6.9 $x10^{-5}$) were also recorded in the photosynthetic rates of *T. occidentalis* treated with 100 mgL^{-1} while photosynthetic rates (11.4 $x10^{-5}$) of the vegetable showed significant increase ($p<0.05$). Also, respiratory rates recorded in the leaves of *C. olitorus* showed significant increase ($P < 0.05$) while photosynthetic rates showed significant decrease ($p<0.05$) with inclusion in concentrations of the treatments.

Furthermore Also, 100 mg L^{-1} produced significant increase ($P<0.05$) (5.6 $x10^{-5}$) in the respiratory rates of *C. olitorius* while photosynthetic rate (5.7 $x10^{-5}$) showed significant decrease in *C. olitorius* treated with other treatments (Table 2). Similar observations were noticed on the effects of the concentrations of SEO on respiratory and photosynthetic rates of *A. hybridus.* Furthermore, respiratory rate (7.7 $x10^{-5}$) showed significant increase in *A. hybridus* treated with 100 mgL^{-1} while photosynthetic rate of the vegetable revealed significant reduction ($p<0.05$) (5.1 $x10^{-5}$) in the vegetable treated with 100 mg L^{-1} (Table 3).

Discussion

Spent engine oil produced several effects on leaves of *T. occidentalis*, *C. olitorius* and *A. hybrids* investigated. The consistent increase recorded in respiration and decrease in photosynthetic rates of the vegetables as a result of contamination of used oil may be attributed to a number of factors such as hydrophobic nature of oil which might have prevented intake of water from air and soil by surface area of root hairs due to thin coat that surrounds it.

Contact between leaves of the vegetables and spent engine oil film might have prevented chlorophyll and other photosynthetic pigments present in the leaves from absorbing light energy and becomes activated for photosynthetic activities. Also, the contamination might have caused the oil to interfere with oil semi-permeable membrane of roots of the vegetables thereby preventing absorption some mineral nutrients from the soil. This observation is consistent with findings of Onwusiri *et al.* (2017) and Thanh *et al.* (2018) who reported reductions in the chlorophylls, mineral and protein contents in *Telfairia occidentalis* and *Acacia raddiana* seedlings grown in contaminated soils. Also, Adenipekun and Kassim (2006) and Ekpo *et al.* (2014) reported that engine oil affected products of photosynthetic products such as protein, ash and fat contents in *Celosia argentea* leaves. Bioaccumulation of the oil on the root membrane of root of the vegetables may inhibit absorption of nutrients needed for formation of pigments that can efficiently trap energy for effective photosynthetic activities. Oil film may also impair absorption of water from the soil or atmosphere as one of the factors required for photosynthesis to occur in the leaves of plants.

Reduction in the photosynthetic rates reported in the present study may be attributed to ability of hydrophobic layer of oil to cover meristematic regions of roots of the vegetables where exudation, osmosis, mycorrhizal association with roots with microbes and other physiological processes of plants are taken place. However, hydrophobic layer over the root membrane as a result of spent engine oil may have limiting effect on water and nutrients absorption necessary for food synthesis in the leaves of the vegetables. This observation corroborates findings of Omosun *et al.* (2008); Odjegba and Atebe (2007); Cleusa *et al.* (2011 and Onwusiri, *et al.* (2017). Studies of Umebese and Awoniyi (2001) showed that oil contamination is toxic to plants and can create unfavourable conditions for nutrients uptake and make some nutrients such as nitrogen unavailable.

Milivojevic *et al.* (2006); Agbogidi *et al.* (2007); Rahman *et al.*, (2007); Li *et al.* (2008) and Ogbuehi *et al.*, (2010) reported reduction in proximate contents of cassava and maize due to impairment of photosynthetic pigments through cell injury and disruption of metabolism, decreased in enzymatic activities in photosynthetic metabolism and disruption of transportation of photosynthetic rates as well as stomata closure of affected plant grown in soil contaminated with spent engine oil Contact between oil and plant tissues, might be responsible for high respiration or reduction recorded in the photosynthetic rates of the vegetables.

Table 1: Effect of spent engine oil on respiratory and photosynthetic rate ($gcm^{-2}h^{-1}$) in the leaf of *Telfairia occidentalis* weekly from 2 to 6 WAT

Oil concentration. (mgL^{-1})	**Respiratory rate ($gcm^{-2}h^{-1}$)**					**Photosynthetic rate ($gcm^{-2}h^{-1}$)**				
	2WAT	**3WAT**	**4WAT**	**5WAT**	**6WAT**	**2WAT**	**3WAT**	**4WAT**	**5WAT**	**6WAT**
0	2.3×10^{-5}b	2.9×10^{-5}b	3.4×10^{-5}b	4.0×10^{-5}bc	4.3×10^{-5c}	14.6×10^{-5}a	13.4×10^{-5}a	12.6×10^{-5}a	11.4×10^{-5}a	11.4×10^{-5}a
20	3.1×10^{-5}ab	3.1×10^{-5}b	3.7×10^{-5}ab	4.6×10^{-5}b	4.9×10^{-5b}	13.1×10^{-5}ab	12.0×10^{-5}ab	9.4×10^{-5}b	9.7×10^{-5}ab	9.1×10^{-5}ab
40	2.9×10^{-5}ab	3.7×10^{-5}ab	4.0×10^{-5}ab	4.9×10^{-5}b	4.9×10^{-5b}	12.0×10^{-5}ab	11.1×10^{-5}ab	7.1×10^{-5}bc	8.9×10^{-5}ab	9.1×10^{-5}ab
60	3.4×10^{-5}ab	4.0×10^{-5}ab	4.3×10^{-5}ab	4.9×10^{-5b}	5.1×10^{-5ab}	12.3×10^{-5}ab	10.0×10^{-5}ab	6.6×10^{-5}c	8.3×10^{-5}b	8.3×10^{-5}ab
80	4.0×10^{-5}ab	4.6×10^{-5}ab	4.6×10^{-5}ab	5.1×10^{-5ab}	5.7×10^{-5ab}	10.6×10^{-5}ab	9.4×10^{-5}ab	5.7×10^{-5}c	7.1×10^{-5}b	7.4×10^{-5}b
100	4.3×10^{-5}a	4.9×10^{-5}a	5.1×10^{-5}a	5.4×10^{-5a}	6.3×10^{-5a}	9.4×10^{-5}b	8.3×10^{-5}b	5.1×10^{-5}c	6.9×10^{-5}b	6.9×10^{-5}b
S.E.±	1.6×10^{-6}	1.8×10^{-6}	2.6×10^{-6}	1.2×10^{-5}	1.1×10^{-5}	2.1×10^{-5}	2.2×10^{-5}	1.3×10^{-5}	1.5×10^{-5}	1.7×10^{-5}

Means followed by different superscripts on the same columns are significantly different ($p < 0.05$) according to Duncan's Multiple Range Test

Table 2: Effect of spent engine oil on respiratory and photosynthetic rate ($gcm^{-2}h^{-1}$) in the leaf of *Corchorus olitorius*

Oil conc. (mgL^{-1})	**Respiratory rate**					**Photosynthetic rate**				
	2WAT	**3WAT**	**4WAT**	**5WAT**	**6WAT**	**2WAT**	**3WAT**	**4WAT**	**5WAT**	**6WAT**
0	2.9×10^{-5}b[3]	2.9×10^{-5b}	2.9×10^{-5}bc	3.1×10^{-5}b	2.1×10^{-5b}	15.1×10^{-5}a	10.9×10^{-5}a	13.1×10^{-5}a	12.9×10^{-4}a	11.1×10^{-4}a
20	3.4×10^{-5}ab	4.0×10^{-5ab}	3.3×10^{-5}c	3.4×10^{-5}b	3.7×10^{-5b}	13.1×10^{-5}a	9.7×10^{-5}ab	10.0×10^{-5}ab	11.4×10^{-4}ab	9.1×10^{-5}ab
40	3.7×10^{-5}ab	4.3×10^{-5ab}	3.4×10^{-5}bc	4.3×10^{-5}ab	4.0×10^{-5ab}	12.6×10^{-5}ab	8.9×10^{-5}abc	8.9×10^{-5}b	9.7×10^{-5}ab	8.0×10^{-5}ab
60	4.3×10^{-5}ab	4.3×10^{-5ab}	4.0×10^{-5}abc	4.9×10^{-5}ab	5.0×10^{-5ab}	11.1×10^{-5}ab	7.7×10^{-5}bc	8.6×10^{-5}b	8.9×10^{-5}ab	7.1×10^{-5}ab
80	4.4×10^{-5}a	4.6×10^{-5ab}	5.4×10^{-5}ab	5.1×10^{-5}a	5.2×10^{-5ab}	8.6×10^{-5}b	6.6×10^{-5}c	7.4×10^{-5}b	8.0×10^{-5}b	6.6×10^{-5}ab
100	4.4×10^{-5}a	5.0×10^{-5a}	5.6×10^{-5}a	5.1×10^{-5a}	5.6×10^{-5a}	8.3×10^{-5}b	6.3×10^{-5}c	6.9×10^{-5}b	7.7×10^{-5}b	5.7×10^{-5}b
S.E.±	1.2×10^{-5}	1.3×10^{-6}	2.1×10^{-6}	1.2×10^{-5}	1.3×10^{-5}	2.1×10^{-5}	1.5×10^{-5}	1.8×10^{-5}	2.2×10^{-5}	2.2×10^{-5}

Means followed by different superscripts on the same columns are significantly different ($p < 0.05$) according to Duncan's Multiple Range Test

Table 3: Effect of spent engine oil on respiratory and photosynthetic rate ($gcm^{-2}h^{-1}$) in the leaf of *Amaranthus hybridus*

Oil conc.	Respiratory rate					Photosynthetic rate				
(mgL^{-1})	2WAT	3WAT	4WAT	5WAT	6WAT	2WAT	3WAT	4WAT	5WAT	6WAT
0	1.1×10^{-5b}	2.0×10^{-5b}	3.1×10^{-5}b	3.4×10^{-5}b^{3}	4.0×10^{-5}b	16.0×10^{-5}a	16.0×10^{-5}a	13.7×10^{-5}a	10.3×10^{-5}a	10.0×10^{-5}a
20	1.7×10^{-5b}	2.0×10^{-5b}	3.4×10^{-5}ab	4.3×10^{-5}ab	5.4×10^{-5}ab	13.7×10^{-5}ab	13.4×10^{-5}ab	12.3×10^{-5}a	8.3×10^{-5}ab	8.9×10^{-5}ab
40	1.6×10^{-4b}	2.3×10^{-5ab}	4.0×10^{-5}ab	4.6×10^{-5}ab	5.7×10^{-5}ab	12.9×10^{-5}ab	12.6×10^{-5}abc	11.4×10^{-5}ab	8.0×10^{-5}ab	8.3×10^{-5}ab
60	2.6×10^{-5ab}	2.3×10^{-5ab}	4.9×10^{-5}ab	5.7×10^{-5}ab	6.3×10^{-5}ab	12.0×10^{-5}ab	10.6×10^{-5}bc	10.3×10^{-5}ab	6.9×10^{-5}b	6.9×10^{-5}ab
80	2.6×10^{-5ab}	2.6×10^{-5a}	4.9×10^{-5}ab	5.7×10^{-5}ab	7.1×10^{-5}a	10.6×10^{-5}b	9.4×10^{-5}bc	10.0×10^{-5}ab	6.3×10^{-5}b	6.3×10^{-5}ab
100	2.9×10^{-5a}	2.6×10^{-5a}	5.1×10^{-5}a	6.0×10^{-5}a	7.7×10^{-5}a	9.4×10^{-5}b	8.6×10^{-5}c	7.7×10^{-5}b	6.0×10^{-5}b	5.1×10^{-5}b
S.E.±	1.0×10^{-6}	1.2×10^{-6}	1.4×10^{-6}	1.2×10^{-5}	1.4×10^{-5}	2.1×10^{-5}	2.0×10^{-5}	2.0×10^{-5}	1.6×10^{-5}	1.8×10^{-5}

Means followed by different superscripts on the same columns are significantly different ($p < 0.05$) according to Duncan's Multiple Range Test

Also, studies of Agbogidi and Ejemeta (2005), Agbogidi and Eshegbeyi (2006) and Agbogidi and Illondu (2013) revealed that reduction in dry mass accumulation following spent oil application on garden soil showed that hydrocarbons from oil contaminated soils accumulate in the chloroplast of plant leaves. However, reduction observed in photosynthetic rates in the present study may indicate that the oil posed obstruction to nutrient partitioning and their movement via phloem vessels. In addition, significant increase recorded in respiratory rates of leaves of the vegetables may be an indication that the oil has impaired respiratory structures such as lenticels in the roots and stomata in the leaves of the vegetables. This assertion corroborates submissions of Adel *et al* (1980) who postulated that mechanical injury to plant tissues or introduction of toxins at certain concentrations to plants has tendency to increase the rate of respiration in plants. Findings of Hass (1917) on effect of viscous and highly refined oil in the rate of respiration of citrus trees showed that respiration is greatly increased and remains abnormally high in the tree grown in oil contaminated soil. High respiratory rates recorded could also be that the oil has affected the biological oxygen demand level, thereby interfering with normal gaseous exchange in the vegetables

Odejimi and Ogbalu, (2006) and Kayode *et al.,* (2009) reported penetration of crude oil into pore spaces of terrestrial vegetation impeded photosynthesis and that other physiological processes of the vegetation and consequently suffocated the vegetation as a result of exclusion of air by the oil. Similarly, Anoliefo and Edegbai (2000) Osubor *et al.,* (2003) observed that polluted soil with spent engine oil experienced physiological drought in terms of disruption of plant water relation and root respiration which are necessary for growth of plants. Also, high rates of respiration rates recorded in this study may be regarded as one of the indicators of cellular metabolic activity such as oxidation of organic compound especially simple carbohydrate, storage of organic compound and a reduction in its intensity as well as decreases in the overall metabolic rate of the plant (Mello *et al*, 2010) However, results of this study on the effects of spent engine oil surrounding ecosystems as important for decision makers regarding phytoremediation efforts after soil contamination with SEO (Nothers *et al.,* 2017).

Conclusion

This study showed that spent engine oil has harmful effects on respiration and photosynthetic rates of *A. hybridus, T. occidentalis* and *C. olitorius* leaves, therefore, the study recommends that automobile mechanic centres should be sited in government approved areas to avoid contamination of agricultural products being consumed by people. People should desist from indiscriminate disposal of spent engine oil on farmlands to prevent reduction in the physiological processes of plants.

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