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Effect of different fractions of spent lubricating oil on some anti-oxidant properties and anti-oxidant enzymes of radicle and stem of germinating tomato (*Solanum lycopersicum*)

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ABSTRACT: The total flavonoid, ascorbic acid and phenolic contents, ferrous ion chelating ability (FICA), glutathione peroxidase (GP_x) and superoxide dismutase (SOD) activities of the stem and radicle of germinating tomato (*Solanum lycopersicum*) grown in different fractions of spent lubricating oil (SLO) contaminated soil were assayed. The SLO was fractionated into different fractions viz: water soluble fraction (WSF), water insoluble fraction (WISF) and whole spent lubricating oil (WSLO) which were used to contaminate 100g of soil for planting the tomato (*S. lycopersicum*) seeds and samples were collected within a period of seven (7) days interval. Radicle ascorbic acid content was found to be significantly ($P<0.05$) reduced after the germinating period of 21 days when compared to the control while the stem ascorbic acid showed the same significant ($P<0.05$) reduction. The flavonoid content was found to be significantly ($P<0.05$) increased after the 21 days of germination as compared to the control. The stem and radicle phenolic contents showed a significant ($P<0.05$) reduction as compared to the control samples while the FICA was slightly significantly ($P<0.05$) decreased after 21 days of germination. The stem and radicle GP_x activity was significantly ($P<0.05$) increased while the SOD activity was slightly significantly ($P<0.05$) decreased after 21 days of germination as compared to day 7. The results of the study shows that although the different parameters indicated that SLO can affect the oxidative state of the tomato plant 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.

Key words: Glutathione peroxidase, *Solanum lycopersicum*, flavonoid, lubricant

Introduction

Lubricating oil, produced by vacuum distillation of crude oil (Kalichevsky and Peter, 1960), is an essential product of petroleum that aids the reduction of frictional forces between contacting metal surfaces of an engine. The spent lubricating oil (SLO) is usually obtained after servicing and subsequent drainage from engines and generators (Anoliefo and Vwioko, 2001; Ogbo *et al*., 2006).

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Service stations in most parts of Nigeria find it difficult to properly dispose of the SLO hence large volume is indiscriminately disposed on plots, land, sewage, and drainage ditches (Odjegba and Sadiqi, 2002) thus increased pollution incidents in the environment are more wide spread than pollution with crude oil (Atuanya, 1980).
It is widely agreed that toxicity of lubricating oils have greater effects on germination, growth and yield of crops (Esenowo and Umoh, 1996). There is evidence that antioxidant supplements promote disease and increase mortality in humans (Bjalakovic *et al*., 2007; Ristow and Zarse, 2010). It was previously proposed on a hypothetical basis that free radicals may induce an endogenous response culminating in more effective adaptations which protect against exogenous radicals (and possible other toxic compounds) (Tapia, 2006).

Generally, a plant's cell try to keep the concentration of reactive oxygen species (ROS) at the possible low level because they are more reactive than molecular oxygen (O_2) (Diaz *et al*., 2001) and they react with almost every organic constituent of the living cell. However, in plants, algae, and cyanobacteria, ROS are also produced during photosynthesis (Demmig-Adams and Adams, 2002) particularly under conditions of high light intensity (Krieger-Liszkay, 2004). This effect is partly offset by the involvement of carotenoids in photo-inhibition, which involves these antioxidants reacting with over reduced forms of photosynthetic reaction centers to prevent the production of ROS (Kerfeld, 2004).

Tomatoes (*S. lycopersicum*) are eaten all freely throughout the world and their consumption is believed to benefit the heart, among other organs basically they contain the carotene, lycopene, one of the most powerful natural antioxidants (Freedman *et al*., 2008; Zhang *et al*., 2009). However, over the years, mechanical advancement has occurred which has led to an increased environmental pollution and in turn, a negative impact on food and food crops and limited information is available for spent lubricating oil toxicity on tomato seedling (*S. lycopersicum*) germination. Consequently, this work was aimed at studying some oxidant and antioxidant activities in the germination of tomato seedlings in a controlled condition.

Materials and Method

The tomato seedlings (*S. lycopersicum*) were obtained from a local supplier. Upon obtaining, they were cut open and the seeds removed and placed in water and then separated out on a petri dish. The Spent Lubricating Oil (SLO) was gotten from a nearby mechanic workshop and was separated into its fractions as described by Anderson *et al*., (1974). The fractions were labeled as Water Soluble Fraction (WSF), Water Insoluble Fraction (WIF) and Whole Spent Lubricating Oil (WSLO) after separation. Cotton wool in petri dishes was used as soil to mimic the soil. They were treated with either distilled water (Control); WSF; WISF, of the spent lubricating oil; or with the WSLO. This treatment was done to mimic conditions on farmlands at site of pollutions or farmlands close to pollution sites and represent 0.03% contamination. With six (6) seeds planted per petri dish, eighteen (18) viable seeds of the *S. lycopersicum* were planted for each treatment (i.e. each triplicate). The total phenol content was determined by the method of McDonald *et al*. (2001), ferrous ion (Fe^{2+}) chelating ability was determined by the method of Minotti and Aust (1987), total flvanoid content was determined by the method of Meda *et al*. (2005) and that of ascorbic acid was determined by the method of Benderitter *et al*. (1998). The stem and radicle superoxide dismutase (SOD) activity was determined by the method of Misra and Fridovich (1972) while that of glutathione peroxidase (GP_x) activity was determined by the method of Sato *et al*. (1978).

Statistical analysis

All results are reported as means of four replicates (Mean ± SEM). All results were compared with respect to control. Analysis of variance (ANOVA) was carried out to compare mean values. Differences at $P<0.05$ were considered as significant.

Results and Discussion

Disposal of spent lubricating into gutters, water drains, open vacant plot and farmlands is a common practice especially by auto-mechanics and individuals who change oil from their generators and vehicles (Edebiri and Nwaokwale, 1981). Plants are exposed to incomplete reduced oxygen species which are toxic by-products, generated at low levels in non-stressed plant cells in chloroplast and mitochondria and extra amount of reactive oxygen species (ROS) occur under stressed conditions like contamination with heavy metals which are contained in SLO (Diaz *et al*., 2001; Sahw et al., 2004)

Previous study showed that the plant, *Phaseolus vulgaris* leaves exposed to cadmium ion (Cd^{2+}) contains more phenolics than the control plants after being sprayed with copper sulphate (Diaz *et al*., 2001). Thus, an increase in the total phenol content (Table 1 and 2) of the radicle of *S. lycopersicum* grown in the WSF and WISF contamination was seen after the second and third week of germination which correlates with report by Parry *et al*. (1994).

Synthesis of isoflavonoids and their flavonoids are induced when plants are infected or injured or under low temperature and low nutrient conditions (Winkei-Shirley, 2002). This was reflected in the result obtained in this study as presented in Table 1 and 2. Study has also shown that selective chelation of heavy metals is relevant to bioremediation (Rama and Prasad, 1998). This is not surprising since this study revealed an increase in Fe^{2+} chelating ability in the stem and radicle of the tomato plant (Table 1 and 2).

Ascorbic acid production is one of the important mechanisms by which plants scavenge free radical that causes lipid peroxidation and thus protects against oxidative stress (Padayatty *et al*., 2003). Our study did show that there is possible a compromise in the production of ascorbic acid (Table 1 and 2) leading to the decrease in the ascorbic content as the germination progressed.

Glutathione peroxidase (GP_x) is known to occur in a wide variety of plants, animals, and microorganisms (Flohe and Gunzler, 1984). In cells, glutathione is maintained in the reduced form by the enzyme, glutathione reductase and in turn reduces other metabolites and enzyme systems, such as ascorbate in the glutathione-ascorbate cycle, GP_x and glutaredoxins as well as reacting directly with oxidants (Meister, 1994). Thus, it can be seen from the result of this study, the increase in the GP_x activity as the germination progresses (Table 3). The SOD activity was seen to be increased after day 14 and 21 of germination as compared to the control (Table 3) (Ke *et al*., 2011). This could possibly be an alternative means of defense for the stem and radicle against ROS as the ascorbic acid content reduced.

Although, the reason for the beneficial effects ascorbate on symbiotic performance have been unclear, the report of Dalton *et al*. (1986) support a role of ascorbate in a system for the prevention of peroxide damage which was reflected in the decrease in the ascorbic acid content as the germination progressed (Table 2 and 3).

From the results, if, however, the increase in the antioxidant enzymes activities observed in the stem and radicle (Table 3) reflects the overall ability of the tomato to produce ascorbic acid, then the vitamin may be available to offer protection to both the radicle and stem relative to the Fe^{2+} chelating ability (Table 1 and 2).

Therefore, it can be seen that the *S. lycopersicum* plant may be able to adapt and utilize some water soluble contents of SLO but in the process, may bioaccumulate some to a toxic level which may contribute to low yield of food crops and especially root plants.

Table 1: Ascorbic acid content, total phenolic content, total flavonoid content and ferrous ion (Fe^{2+}) chelating ability of the radicle of the tomato (*S. lycopersicum*) after germinating in cotton wool contaminated with spent lubricating oil

Parameters	Groups			
	Control	**WSF**	**WISF**	**WSLO**
7days after germination				
Ascorbic acid (mg/g)	3.93 ± 0.16^{a}	4.29 ± 0.28^{b}	4.91 ± 0.45^{c}	4.98 ± 0.47^{d}
Total phenol (mg/g)	4.95 ± 0.65^{a}	4.75 ± 0.27^{b}	4.29 ± 0.27^{c}	4.08 ± 0.15^{d}
Total flavonoid (mg/g)	0.62 ± 0.02^{a}	0.31 ± 0.01^{b}	0.83 ± 0.02^{c}	0.46 ± 0.01^{d}
Fe^{2+} chelating ability (mg/g)	7.22 ± 0.72^{a}	7.09 ± 0.43^{a}	6.45 ± 0.74^{b}	6.01 ± 0.23^{b}
14 days after germination				
Ascorbic acid (mg/g)	3.82 ± 0.58^{a}	2.50 ± 0.30^{b}	$2.61 \pm 0.45^{b,c}$	1.96 ± 0.57^{d}
Total phenol (mg/g)	1.86 ± 0.16^{a}	3.48 ± 0.18^{b}	2.81 ± 0.48^{c}	1.19 ± 0.29^{d}
Total flavonoid (mg/g)	2.20 ± 0.10^{a}	$2.84 \pm 0.51^{a,b}$	2.77 ± 0.09^{c}	4.30 ± 0.30^{d}
Fe^{2+} chelating ability (mg/g)	8.35 ± 0.27^{a}	9.77 ± 0.01^{b}	7.63 ± 0.25^{c}	6.37 ± 0.34^{d}
21 days after germination				
Ascorbic acid (mg/g)	2.28 ± 0.30^{a}	2.35 ± 0.55^{b}	1.71 ± 0.28^{c}	1.52 ± 0.26^{d}
Total phenol (mg/g)	1.87 ± 0.02^{a}	$1.89 \pm 0.29^{a,b}$	2.91 ± 0.06^{c}	1.25 ± 0.03^{d}
Total flavonoid (mg/g)	1.28 ± 0.01^{a}	2.81 ± 0.15^{b}	2.67 ± 0.31^{c}	1.94 ± 0.01^{d}
Fe^{2+} chelating ability (mg/g)	8.09 ± 0.36^{a}	7.76 ± 0.25^{b}	6.80 ± 0.06^{c}	6.06 ± 0.39^{d}

Values are Mean ± SEM, n = 36. Means of the same row followed by different superscripts differ significantly (P<0.05) while means of the same row followed by same superscript do not differ significantly (P>0.05).

Table 2: Ascorbic acid content, total phenolic content, total flavonoid content and ferrous ion (Fe^{2+}) chelating ability of the stem of the tomato (*S. lycopersicum*) after germinating in cotton wool contaminated with spent lubricating oil

Parameters	Groups			
	Control	**WSF**	**WISF**	**WSLO**
7days after germination				
Ascorbic acid (mg/g)	3.91 ± 0.10^{a}	4.17 ± 0.06^{b}	4.67 ± 0.10^{c}	4.95 ± 0.04^{d}
Total phenol (mg/g)	5.36 ± 0.04^{a}	4.45 ± 0.04^{b}	4.79 ± 0.01^{c}	5.33 ± 0.09^{a}
Total flavonoid (mg/g)	0.077 ± 0.003^{a}	$0.036 \pm 0.007^{a,b}$	$0.129 \pm 0.003^{b,c}$	0.142 ± 0.030^{d}
Fe^{2+} chelating ability (mg/g)	8.97 ± 0.42^{a}	8.99 ± 0.39^{b}	$8.99 \pm 0.39^{b,c}$	7.26 ± 0.56^{d}
14 days after germination				
Ascorbic acid (mg/g)	3.78 ± 0.06^{a}	1.38 ± 0.04^{b}	1.66 ± 0.19^{d}	1.07 ± 0.02^{d}
Total phenol (mg/g)	3.78 ± 0.26^{a}	1.38 ± 0.04^{b}	5.66 ± 0.11^{c}	2.84 ± 0.25^{d}
Total flavonoid (mg/g)	0.553 ± 0.015^{a}	0.298 ± 0.002^{b}	0.639 ± 0.085^{c}	0.652 ± 0.018^{d}
Fe^{2+} chelating ability (mg/g)	8.86 ± 0.22^{a}	7.35 ± 0.08^{b}	9.00 ± 0.04^{c}	6.94 ± 0.39^{d}
21 days after germination				
Ascorbic acid (mg/g)	2.41 ± 0.07^{a}	2.09 ± 0.01^{b}	1.30 ± 0.02^{c}	$1.50 \pm 0.02^{c,d}$
Total phenol (mg/g)	2.96 ± 0.05^{a}	2.57 ± 0.13^{b}	$2.77 \pm 0.13^{b,c}$	2.52 ± 0.08^{d}
Total flavonoid (mg/g)	0.258 ± 0.008^{a}	0.388 ± 0.062^{b}	$0.240 \pm 0.006^{a,c}$	$0.235 \pm 0.001^{a,d}$
Fe^{2+} chelating ability (mg/g)	8.99 ± 0.25^{a}	8.83 ± 0.15^{b}	8.60 ± 0.07^{c}	8.42 ± 0.38^{d}

Values are Mean ± SEM, n = 36. Means of the same row followed by different superscripts differ significantly (P<0.05) while means of the same row followed by same superscript do not differ significantly (P>0.05).

Table 3: Glutathione peroxidase (GP_x) and Supaeroxide dismutase (SOD) activities of the stem and radicle of the tomato (*S. lycopersicum*) after germinating in cotton wool contaminated with spent lubricating oil

Parameters	Groups			
	Control	**WSF**	**WISF**	**WSLO**
7days after germination				
Radicle				
GP_x (Unit/min/mg protein)	0.34 ± 0.017^{a}	$0.24 \pm 0.001^{a,b}$	$0.36 \pm 0.027^{a,c}$	$0.34 \pm 0.027^{a,c,d}$
SOD (Unit/min/mg protein)	0.19 ± 0.005^{a}	0.16 ± 0.006^{a}	0.19 ± 0.005^{a}	0.21 ± 0.011^{b}
Stem				
GP_x (Unit/min/mg protein)	0.72 ± 0.006^{a}	0.25 ± 0.035^{b}	$0.23 \pm 0.015^{b,c}$	0.49 ± 0.013^{d}
SOD (Unit/min/mg protein)	0.15 ± 0.017^{a}	0.18 ± 0.003^{a}	0.17 ± 0.002^{a}	0.15 ± 0.003^{a}
14 days after germination				
Radicle				
GP_x (Unit/min/mg protein)	1.50 ± 0.09^{a}	$1.59 \pm 0.12^{a,b}$	1.43 ± 0.21^{c}	$1.38 \pm 0.25^{c,d}$
SOD (Unit/min/mg protein)	1.06 ± 0.01^{a}	1.03 ± 0.02^{a}	1.52 ± 0.01^{b}	1.52 ± 0.02^{b}
Stem				
GP_x (Unit/min/mg protein)	1.18 ± 0.14^{a}	1.68 ± 0.02^{b}	1.05 ± 0.09^{c}	1.02 ± 0.09^{d}
SOD (Unit/min/mg protein)	1.04 ± 0.03^{a}	1.72 ± 0.08^{b}	1.67 ± 0.24^{c}	$1.76 \pm 0.02^{b,d}$
21 days after germination				
Radicle				
GP_x (Unit/min/mg protein)	3.06 ± 0.03^{a}	3.33 ± 0.05^{b}	2.18 ± 0.01^{c}	1.66 ± 0.04^{d}
SOD (Unit/min/mg protein)	1.53 ± 0.03^{a}	1.45 ± 0.01^{b}	1.45 ± 0.05^{b}	1.48 ± 0.08^{b}
Stem				
GP_x (Unit/min/mg protein)	2.41 ± 0.13^{a}	2.77 ± 0.35^{b}	2.66 ± 0.04^{c}	2.04 ± 0.03^{d}
SOD (Unit/min/mg protein)	1.42 ± 0.01^{a}	1.45 ± 0.02^{a}	1.47 ± 0.03^{a}	1.46 ± 0.01^{a}

Values are Mean ± SEM, n = 36. Means of the same row followed by different superscripts differ significantly ($P<0.05$) while means of the same row followed by same superscript do not differ significantly ($P>0.05$).

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References

Anderson, J.W., Neff, J.M., Cox, B.A., Tatem, H.E. and Hightower, G.M. (1974) Characteristics of dispersions and water soluble extracts of crude oils and their toxicity to estuarine crustaceans and fish. Mar. Biol. 27: 75 – 88.

Anoliefo, G.O. and Vwioko, D.E. (2001) Tolerance of *Chromolena odorata* (L) K & R grown in soil contaminated with spent lubricating oil. Journal of Tropical Bioscience. 1(1): 20 – 24.

Atuanya, A.I. (1980) Effects of waste engine oil pollution on physical and chemical properties of soil. Nigerian Journal of Applied Sciene. 5: 55 – 176.

Benderitter, M., Maupoil, V., Vergely, C., Dalloz, F., Briot, F. and Rochette, L. (1998) Studies by electron paramagnetic resonance of the importance of iron in the hydroxyl scavenging properties of ascorbic acid in plasma. Fundamentals of Chemical Pharmacology. 12: 510 – 516.

Bjelakovic, G., Nikolova, D., Gluud, L., Simonetti, R. and Gluud, C. (2007) Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: Systemic review and meta-analysis. JAMA. 297(8): 842 – 857.

Dalton, D.A., Russell, S.A., Hanus, F.J., Pascoe, G.A.and Evans, H.J. (1986) Enzymatic reactions of ascorbate and glutathione that prevent peroxide damage in soybean root nodules. Proc. Natl. Acad. Sci. 83: 3811 – 3815.

Demmig-Adams, B. and Adams, W. (2002) Antioxidants in photosynthesis and human nutrition. Science. 298(5601): 2149 – 2153.

Diaz, J., Bernal, A., Mar, F. and Merrino, F. (2001) Induction of shikimate dehydrogenase and peroxidase in pepper (*Caspium annum* L) seedlings in response to copper stress and its relation to lignifications. Plant Sci. 161: 179.

Edebiri, R.A.O. and Nwaokwale, E. (1981) Control of pollution from internal combustion engine used lubricant. Pp. 71 – 75.

Esenowo, G.J. and Umoh, N.S. (1996) Effects of used engine oil pollution of soil on germination and yield of *Zea mays* L. Transaction of the Nigerian Society for Biological Conservation. 2: 23 – 38.

Flohe, L. and Gunzler, W.A. (1984) Assays of glutathione peroxidase. *Methods Enzymol*. 105: 114 –121.

Freedman, N.D., Park, Y. and Subar, A.F. (2008) fruit and vegetable intake and head and neck cancer risk in a large United States prospective cohort study. International Journal of Cancer. 122(10): 2330 – 2336.

Kalichevsky, V.A. and Peters, P. (1960) Petroleum, our man of work. The raw material, crude petroleum and natural gas in petroleum products handbook. (Guthrie, V.B. ed). Pp. 1 – 22.

Ke, L., Zhang, C., Guo, C., Lin, G.H. and Tam, N.F. (2011) Effects of environmental stresses on the responses of mangrove plants to spent lubricating oil. Mar. Pollut. Bull. 63(5-12): 385 – 395.

Kerfeld, C. (2004) Water-soluble carotenoid proteins of cyanobacteria. Archives of Biochemistry and Biophysics. 430(1): 2 – 9.

McDonald, S., Prenzler, P.D., Autolovich, M. and Robards, K. (2001) Phenolic content and antioxidant activity of olive extracts. Food Chem. 73:73 – 84.

Meda, A., Lamien, C.E., Romito, M., Milligo, J. and Ncoulma, O.G. (2005) Determination of the total phenolic, flavonoid and proline contents as well as their radical scavenging activity. Food Chem. 91: 571 – 577s.

Meister, A. (1994) Glutathione-asacorbic acid antioxidant system in animals. Journal of Biological Chemistry. 269(13): 9397 – 9400.

Minotti, G. and Aust, S.D. (1987) The requirement for iron (III) in the initiation of lipid peroxidation by iron (II) and hydrogen peroxide. J. Biol. Chem. 262:1098 – 1104.

Misra, H.P. and fridovich, I. (1972) The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem. 247: 3170 – 3175.

Odjegba, V.I. and Sodiqi, A.O. (2002) Effect of spent engine oil on the growth parameters, chlorophyll and protein levels of *Amaranthus hybridus* L. The Environmentalist. 22: 23 – 28.

Ogbe, M.E., Okhuoya, J.A. and Anaziah, O.O. (2006) Effect of different levels of lubricating oil on the growth of *Pleurotus tber-regium*. Fries Singer. Nigerian Journal of Botany. 19(2): 266 – 270.

Padayatty, S.J., Katz, A.W., Yaohui, E.C.K. and Peter, K.L. (2003) Vitamin C as an antioxidant: Evaluation of its role in disease prevention. Journal of American College of Nutrition. 22(1): 18 – 35.

Parry, A.D., Tiller, S.A. and Edwards, R. (1994) The effects of heavy metals and root immersion on isoflavonoids metabolism in Alfalfa (*Medicago sativa* L). Plant Physiol. 106: 195.

Ristow, M. and Zarse, K. (2010) How increase oxidative stress promotes longevity and metabolic health: The concept of mitochondrial hormesis (mitohormesis). Experimental Gerontology. 45(6): 410 – 418.

Sato, M., Ramarathman, N., Suzuki, Y., Ohkubo, T., Takeuchi, M. and Ochi, H. (1978) Variety differences in the phenolic content and superoxide radical scavenging potential of wines from different sources. Journal of Agricultural and Food Chemistry. 44: 37 – 41.

Tapia, P. (2006) Sublethal mitochondrial stress with an attendant stoichiometric augmentation of reactive oxygen species may precipitate many of the beneficial alterations in cellular physiology produced by caloric restriction, intermittent fasting, exercise and dietary phytonutrients. 66(4):832 – 843.

Winkei-Shirley, B. (2002) Biosynthesis of flavonoids and effects of stress. Curr. Opin. Plant Biol. 5: 218.

Zhang, C.X., Ho, S.C., Chen, Y.M., Fu, J.H., Cheng, S.Z. and Lin, F.Y. (2009) Greater vegetable and fruit intake is associated with lower risk of breast cancer among Chinese women. International Journal of Cancer. 125(1): 181 – 188.