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Effect of hydrocarbons (Gasoil and spent motor oil) on biochemical parameters of barley (*Hordeum vulgare*)

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Abstract

Petroleum compounds are one of the most commonly encountered pollutants in soils, presenting a stressful environmental factor for plants. The purpose of this work is to study the effect of hydrocarbons (gasoil and spent motor oil) on some biochemical parameters of barley *Hordeum vulgare*. Plantlets were sown in five different concentrations of gasoil (0%, 0.125%, 0.25%, 0.375% and 0.5%) and spent motor oil (0%, 1.25%, 2.5%, 3.75% and 5%) for 75 days in a greenhouse. The results show a significant reduction in the levels of chlorophyll with the gradual increase of gasoil and spent motor oil, noting that the lowest values were obtained at 0.5% gasoil (with a reduction of 59%) and 5% spent motor oil (with 73% of decrease compared to control). The same decrease was observed for proteins and soluble sugars recording that the content of leaves was higher than those of roots. The lowest contents (10.04 $mg.g^{-1}$ of proteins and 14.53 $mg.g^{-1}$ of soluble sugars) were registered at 0.5% and 0,375% gasoil respectively in roots. In addition, the same doses led to a significant increase in proline contents in comparison with the control, recording the highest levels (4.94 and 6.29 $mg.g^{-1}$ dry matter in roots, and 11.97 and 16.78 $mg.g^{-1}$ dry matter in leaves) for the highest doses of gasoil and spent motor oil respectively. What does it mean that gasoil and spent motor oil have an influence on stability of biochemical parameters of barley.

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Introduction

Contamination of the environment as a result of the use of various pollutants by humans has become a global problem affecting both developed and developing countries (Khan, 2005).

The constant increase in the use of hydrocarbons has damaged soil which is subjected to continuous exhaustion in several forms such as soil erosion and depletion of nutriments (Odat and Alshammari, 2011), and thus presents a risk potential for the agricultural circuit (Cunningham and Berti, 1993). Hydrocarbons are widespread in the environment, their main source is oil, but they are also formed by biological or synthetic processes (Weisman, 1998). Gasoil, or diesel, is one of the main products of hydrocarbons (Nwagou *et al.,* 2008), it is phytotoxic at relatively low concentrations and even reduces organic content of soils (Wyszkowski and Ziolkowska, 2008). Engine oils are mainly mixed with hydrocarbons-based oil; their movement contributes to the degradation of environment (Edema *et al.,* 2009). Oil and its different derivatives were found to cause a delay in germination, an induction of early chlorous, and a reduction in shoot and root length and biomass in plants (Minai-Tehrani *et al.,* 2008 and 2012).

The perception of environmental problems has led to many efforts to clean the environment (Leahy and Colwell, 1990). One of these efforts is phytoremediation which is a new, environmentally friendly and promising technique to eliminate contaminants such as hydrocarbons from soil (Luepromchai *et al.,* 2007). The action of plants on the degradation of hydrocarbons can be done in a direct or indirect way. In fact, roots produce peroxidase-type enzymes capable of oxidizing hydrocarbons (Jones *et al.,* 2004). The transfer of organic pollutants to plant aerial parts is an important issue for the safety of human and animal food (Gao and Collins, 2009). In fact, grasses are classified among plants with a good accumulation of trace elements (Kuboi *et al.,* 1986).

Diab (2008) reported an oil degradation rate of 19.9% and 17.6% using *Zea mays* and *Triticum aestivum*, respectively.In this context, our work aims to study hydrocarbons effect (gasoil and spent motor oil) on the content of some biochemical parameters (chlorophyll, proteins, soluble sugars and proline) of Barley *Hordeum vulgare*.

Materials and methods

Application of stress

Soil samples were mixed with hydrocarbons (gasoil and spent motor oil) at different to doses (Table 1).

Polluted soil samples were placed in pots (20 cm high and 15 cm in diameter; 2.5 kg/pot) with a gravel layer at the bottom to ensure good draining.

Plant material

The plant material concerns seeds of barley *Hordeum vulgare* brought from Saïda region (West of Algeria). These seeds were washed and disinfected with 5% sodium hypochlorite for fifteen minutes, then rinsed with distilled water.

Culture methods

Seeds were put to germinate directly in pots to ensure stress since the first day. Watering was done 3 times a week with tap water and a fourth one by nutrient solution of Hoagland, (1938).

After 75 days of growth, plants were removed; roots were rinsed and separated from stems and leaves. All parts are dried for 48 hours at 80°C, and dry samples were crushed to powder for biochemical analyses.

Analyzed parameters

Chlorophyll content is determined by Porra's method, (2002).

Proteins content is determined by Bradford's method, (1976).

Soluble sugars content is determined by the use of Dubois *et al* technique, (1956). The technique of Monneveux and Nemmar (1986) was used to determine levels of proline.

Statistical analysis

Statistical analyses based on the average comparison tests, using 3 repetitions for each dose, were applied with STAT-BOX 6.4 software using ANOVA variance analysis calling for the Newman Keuls test with a Threshold P = 5%.

Results

Effect of gasoil on chlorophyll contents

The results shown in Fig. 1 elucidate a decrease in chlorophyll concentrations with the increase in gasoil doses. Chlorophyll A levels decreased inversely with the concentration of gasoil until it reached its lower level of 498±33µg.g^{-1} at 0.5% compared with the control having a value of 1239±84 µg.g^{-1}.

Table 1. Gasoil and spent motor oil doses in soil.

Hydrocarbons	Concentrations				
Gasoil (G)	0%	0,125%	0,25%	0,375%	0,5%
Spent motor oil (MO)	0%	1,25%	2,5%	3,75%	5%

The same is noted for chlorophyll B, total chlorophyll A+B, and carotenoids.

The statistical analysis shows a highly significant effect of gasoil on chlorophyll content (A, B, A+B and carotenoids) of barley.

Effect of spent motor oil on chlorophyll levels

As shown in Fig. 2, there were a highly significant decrease in chlorophyll A, B, A+B, and carotenoids-xanthophylls in plants grown in soil contaminated with increasing concentration of spent motor oil.

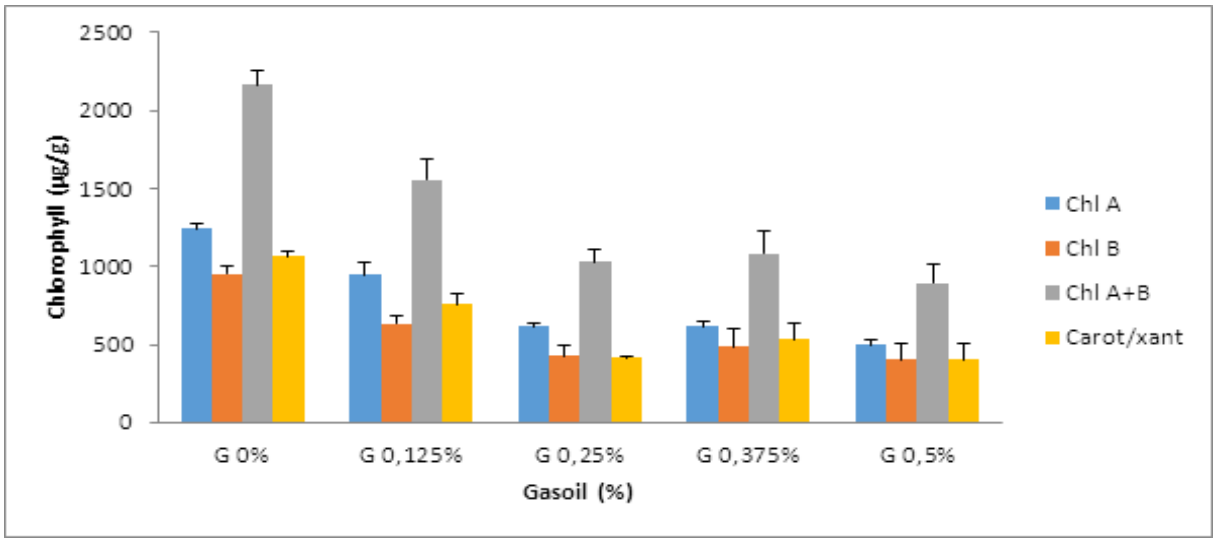

Fig. 1. Chlorophyll contents (µg.g^{-1} fresh matter) of barley *H. vulgare* grown in gasoil-contaminated soil for 75 days.

The levels of chlorophyll A, total chlorophyll, and carotenoids were decreased inversely by the increase in dose concentration, with a reduction of73%, 68%, and 69% respectively in the 5% dose, compared with the control. For chlorophyll B, there was a decrease compared to the control, but the 5% dose there were a slight increase 346±8, compared to 2.5% and 3.75% doses in which chlorophyll B contents were 303±41 and 251±27 µg.g^{-1} respectively.

Proteins content

Effect of gasoil on proteins contents of leaves and roots

Results analysis of the effect of gasoil on proteins content in leaves and roots (Fig. 3) shows the existence of a highly significant effect. Values decrease with the increase of gasoil concentrations, and the contents of leaves are far superior than in roots.

The proteins concentrations in roots decreased in the 0.5% dose by 70% compared to the control, the same was observed in leaves where there is a remarkable fall especially in plants grown in 0.5% gasoil.

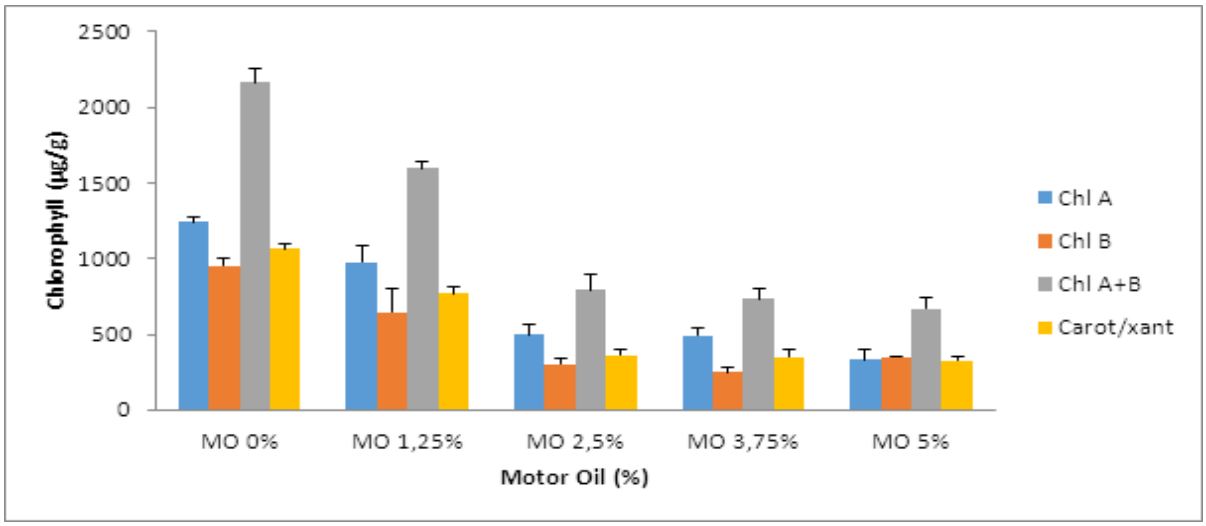

Fig. 2. Chlorophyll contents (µg.g^{-1} fresh matter) of barley *H. vulgare* grown in motor-oil-contaminated soil for 75 days.

The comparison of averages in roots allowed distinguishing 3 homogeneous groups; 0% treatment is the first group, 0.125% dose forms the second group, and the third group is for the doses 0.25%, 0.375% and 0.5%. For leaves, we also have 3 groups, the first is for the control, the second is for treatments 0.125%, 0.25% and 0.375%; In addition, 0.5% dose is the third group.

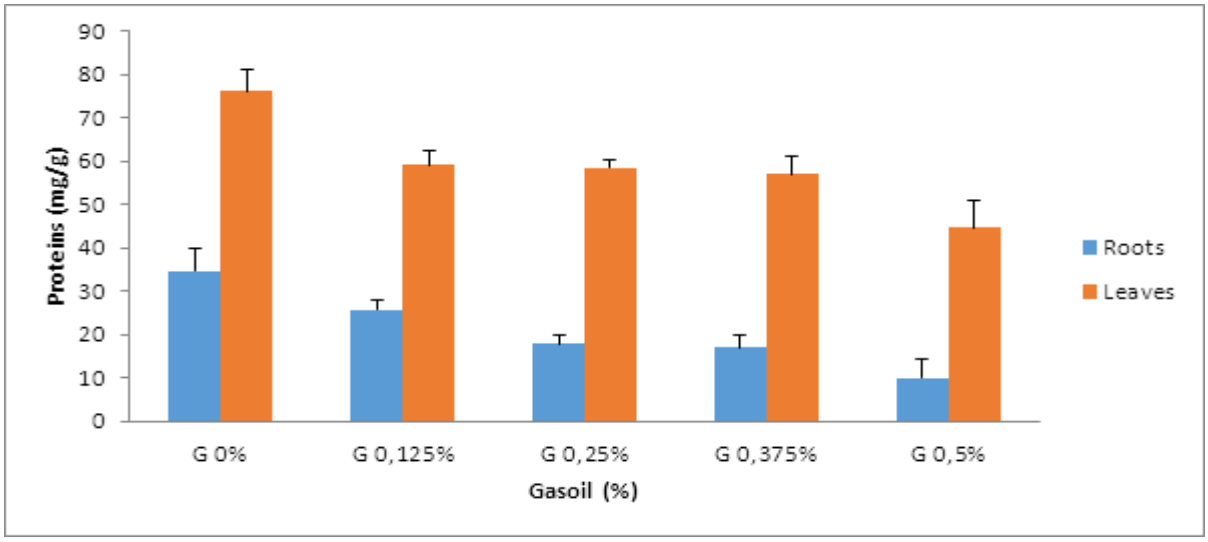

Fig. 3. Proteins content (mg.g^{-1} fresh matter) of barley *H. vulgare* grown in different concentrations of gasoil.

Effect of spent motor oil on proteins contents of leaves and roots

As shown in Fig. 4, the increase in concentration of spent motor oil exerts a negative influence on proteins contents in leaves and roots, with higher values for the leaves than for roots, and a relationship can reach 41% between the two.

The statistical analysis shows a high significant effect with 3 homogeneous root groups, the first is observed for the control, second is for doses 1.25% and 2.5%, and the third is for 3.75% and 5% doses. The same is noted for leaves which are also recorded 3 groups, the dose 0% is the first, 1.25% dose is the second, and the third consists of 2.5%, 3.75% and 5% doses.

Levels of soluble sugars

Effect of gasoil on soluble sugars content of leaves and roots

Results of the effect of gasoil different treatments on soluble sugars content (Fig. 5)

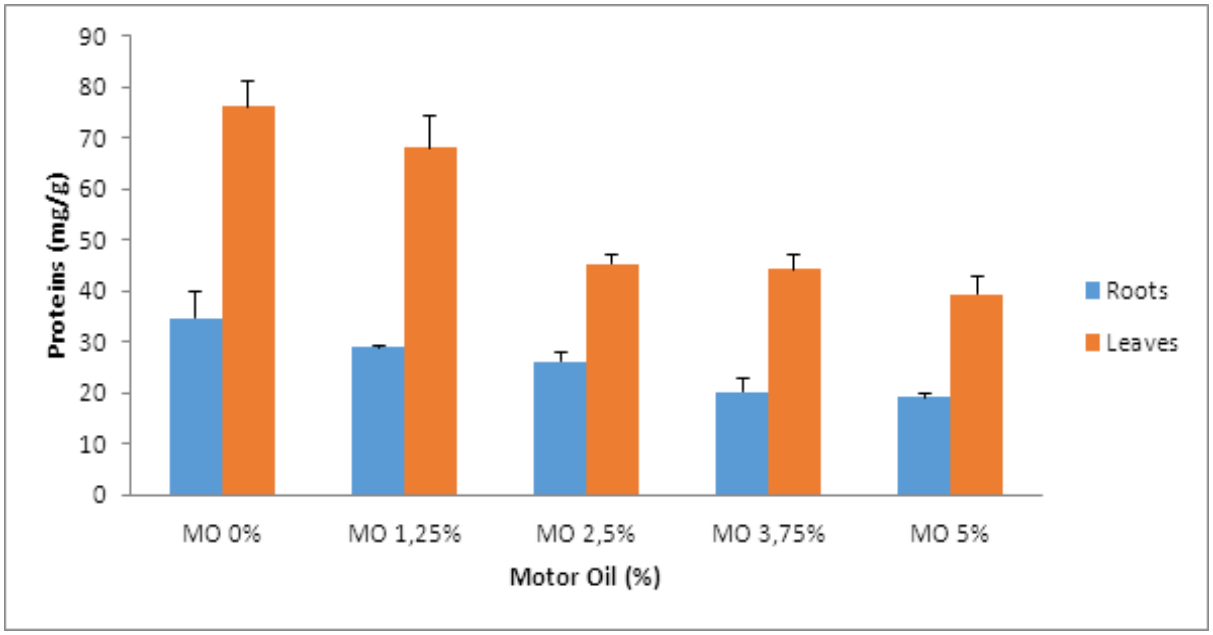

Fig. 4. Proteins content (mg.g^{-1} fresh matter) of barley *H. vulgare* stressed by spent motor oil.

The statistical analysis reveals a highly significant effect for roots, with 5 homogeneous groups. Group (A) is observed for the control, group (AB) for 0.5% dose, group (BC) represents 0.125% dose, and the groups (C) and (D) for 0.25% and 0.375% doses respectively. For leaves, there is a significant decrease with existence of 2 homogeneous groups. The dose 0.125% is in the same group (A) with the 0% dose, in addition, group (B) groups 0.25%, 0.375% and 0.5% doses respectively.

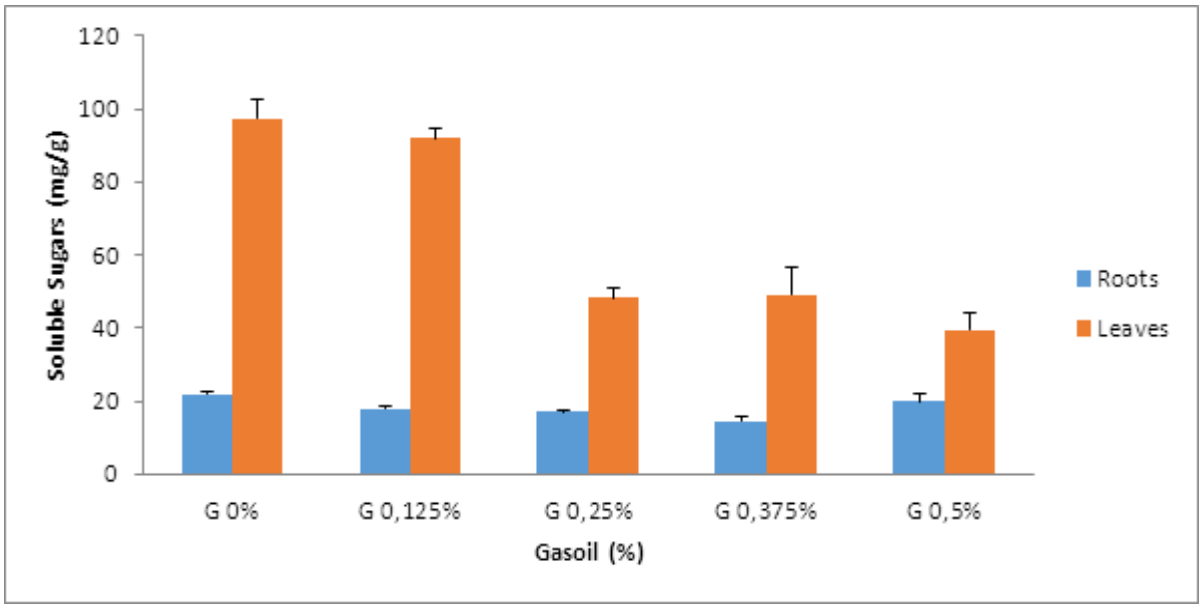

Fig. 5. Soluble sugars content (mg.g^{-1} dry matter) of barley *H. vulgare* grown in soil polluted with gasoil.

Effect of spent motor oil on soluble sugars content of leaves and roots

In Fig. 6, soluble sugars levels in roots increase with the increase in spent motor oil concentrations, except for 1.25% dose where there is a decrease compared to the control. On the other hand, and in the leaves, the same doses exert a reduction in soluble sugars content.

Analysis of variance shows that the presence of spent motor oil induces a highly significant effect on leaves by forming 4 homogeneous groups, (A), (B), (C), (C) and (D) for doses 0%, 1.25%, 2.5%, 3.75% and 5%, respectively. In roots, there is also a significant effect with 4 homogeneous groups, the group (A) represents 5% dose having a value equal to 28.52±2.7 mg.g^{-1}, with an increase of 25% compared to the witness in group (BC).

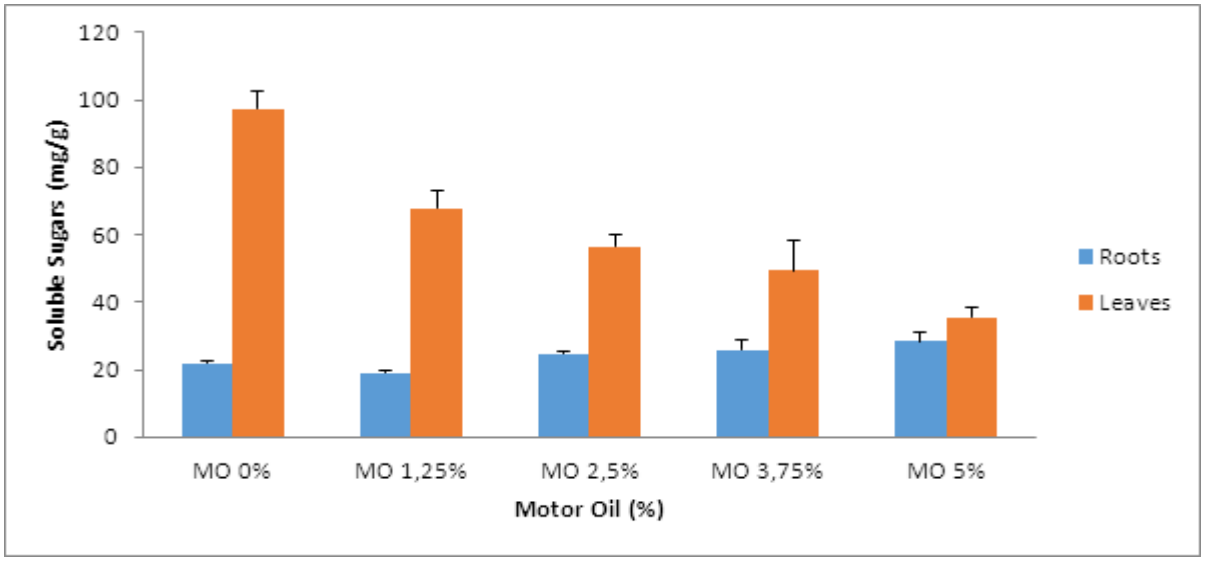

Fig. 6. Soluble sugars content (mg.g^{-1} dry matter) of barley *H. vulgare* grown on soil polluted with spent motor oil.

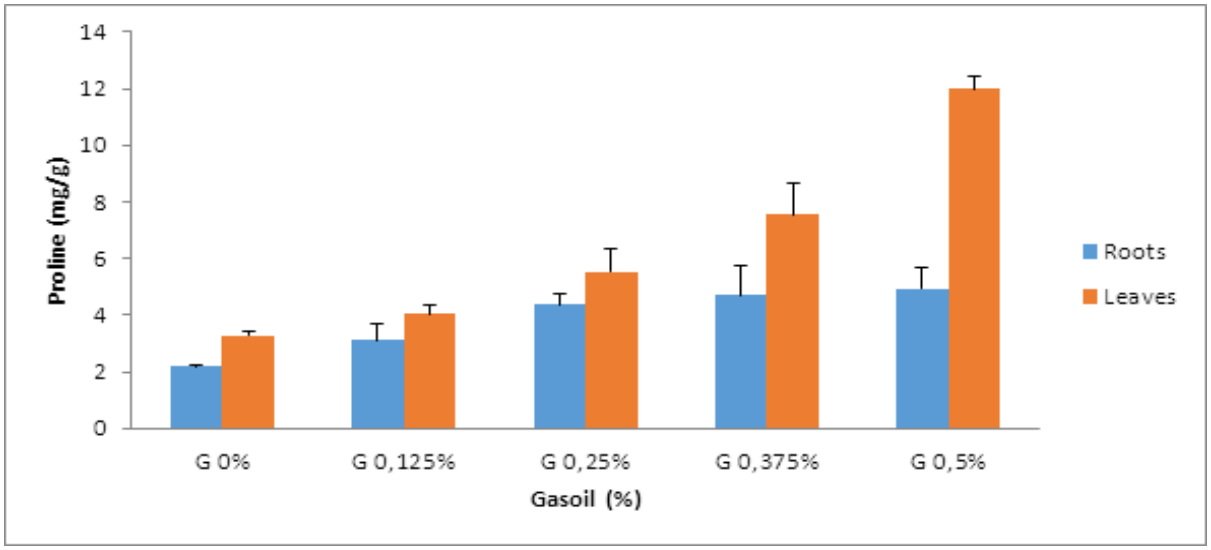

Fig. 7. Proline content (mg.g^{-1} dry matter) of barley *H. vulgare* grown in soil polluted with gasoil.

Proline levels

Effect of gasoil on proline content of leaves and roots

Fig. 7 shows a remarkable and gradual increase in proline levels with the increase in soil-concentrations of gasoil in roots and leaves, with a large proline rate in leaves than in roots and a ratio of up to 137% between the two.

Statistical analysis of the effect of different gasoil-concentrations on proline contents showed a significant effect in roots with observation of 2 homogeneous groups, the first group of plants stressed by 0.5%, 0.375% and 0.25% doses, and the second group includes 0.125% dose and the control. The same significant effect was noted for leaves by forming 4 homogeneous groups and values ranged from 3.27±0.04 mg.g^{-1}for control and 11.97±0.43 mg.g^{-1}for 0.5% dose.

Effect of spent motor oil on proline content of leaves and roots

The results noted in Fig. 8 show that increasing concentrations of spent motor oil in soil exert a highly significant positive influence on proline levels in barley leaves and roots with observation that the ratio between leaves and roots increase with the increase in concentration.

Analysis of variance records 3 homogeneous root groups and a ratio of 144% between 5% dose levels and control. For leaves, there are 4 homogeneous groups, group (A) represents the 5% dose, group (B) is for 3.75% dose, and group (C) groups plants dosed by 2.5%, 1.25% and 0%.

Discussion

Generally, oil-hydrocarbons exert a depressive, or even inhibitory, effect on plant development.

The results of this study showed that gasoil and spent motor oil reduce the contents of biochemical parameters: chlorophyll, proteins and soluble sugars of barley *H. vulgare*. These reductions are inversely proportional to the increase in doses. Studies on the sensitivity of barley *H. vulgare* to hydrocarbons and oil derivatives are rare, especially on biochemical parameters, making interpretation of these results a complex task. In conditions of our experimentation, the reaction of barley *H. vulgare* to gasoil and spent motor oil was made by adaptive modifications from which we retain the following essential points:

Effect of gasoil and spent motor oil on chlorophyll content

A significant decrease in chlorophyll A, B, total, and carotenoids was observed. These results are similar to those obtained by Alkio *et al.,* (2005), who have observed several effects of PAH in *Arabidopsis*, including deformed trichomes and chlorophyll degradation. The most frequent and important symptoms observed in oil-contaminated plants and its by-products include chlorophyll degradation (Baker, 1970; Malallah *et al.,* 1996 and 1998), and a reduction in photosynthesis (Baker, 1970). Salinity stress has a similar effect (decreases chlorophyll levels) on barley (Cheikh *et al.,* 2008), this decrease is partly the cause of the decrease in synthesis of carbohydrates (Levingneron *et al.,* 1995).

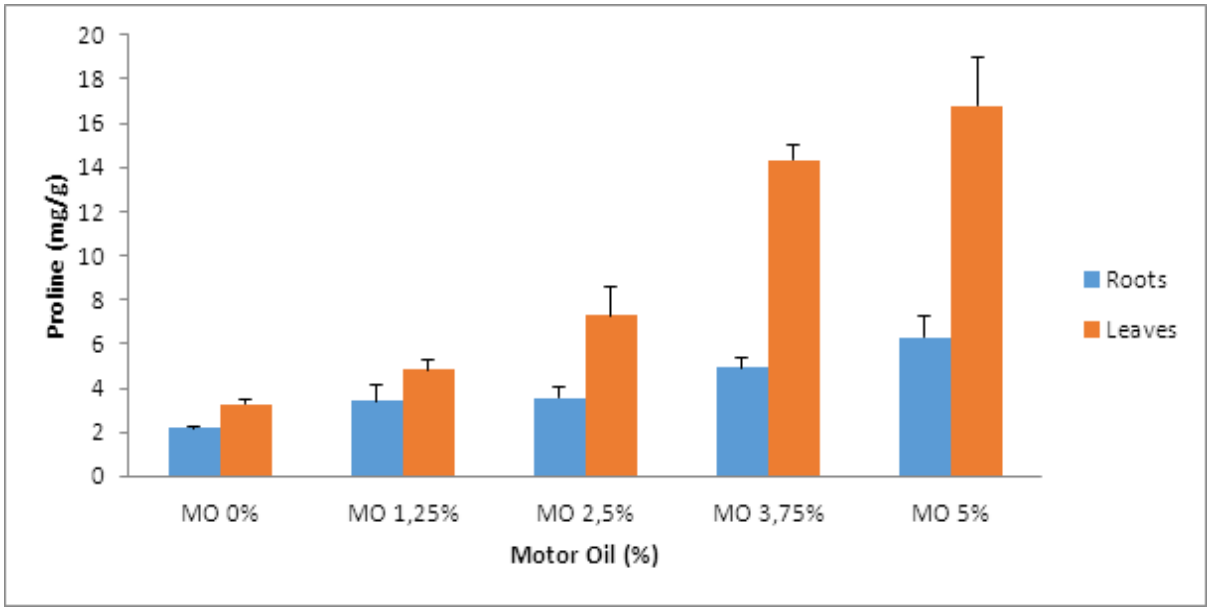

Fig. 8. Proline content (mg.g^{-1} dry matter) of barley *H. vulgare* grown in soil polluted with spent motor oil.

Effect of gasoil and spent motor oil on proteins and soluble sugars content

Our results show a remarkable fall in proteins and soluble sugars content with the increase in gasoil and spent motor oil concentrations, with exception of increase in roots sugars content under effect of spent motor oil, which confirms the explanation of Achuba, (2006) who indicated that molecules of toxic hydrocarbons could inhibit the activities of amylase and starch phosphorylation and thus affect the assimilation of starch.

Effect of gasoil and spent motor oil on proline content

In addition, our study shows that the proline content increases with the increase in concentrations of gasoil and spent motor oil, this finding joins the results of Hassani *et al.,* (2008) on barley due to salinity stress and (Janmohammadi *et al.,* 2013) on wheat leaves as a result of lead effect.

The accumulation of proline, stress-induced, can be the result of three complementary processes: stimulation of its synthesis (Morris *et al.,* 1969; Boggess *et al.,* 1976), Inhibition of oxidation (Stewart *et al.,* 1977; Rayapati and Stewart, 1991) and/or alteration of proteins biosynthesis (Stewart *et al.,* 1977). This accumulation is related to the level of tolerance (Hassani *et al.,* 2008) and allows the protection of cell membrane.

Conclusion

The increased doses of gasoil and spent motor oil induced a decrease in chlorophyll (A, B, A+B, and carotenoids), proteins and soluble sugars with higher values in leaves relative to roots. In addition, there is a proportional accumulation of proline following the increase in hydrocarbons. Finally, our results show a significant effect of gasoil and spent motor oil on the biochemical parameters of barley *H. vulgare*.

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