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The Effect of Toxic Substances of Cigarette Filter on the Growth and Some Physiological Characteristics of Tall fescue (*Festuca arundinacea*) in Soil and Hydroponic Media

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Abstract:
A greenhouse experiment was conducted to assess the impacts of discarded filters, either from smoked and non-smoked cigarette filters (shredded and unshredded filter) on fescue plant from poaceae family. This experiment was carried out on the seed germination in the petri dishes and cultivation in the pot and in the hydroponic media. The experiment consisted of four replications and five treatments. Laboratory tests including measuring the chlorophyll a, b and carotenoid, measuring the amount of proline, antioxidant properties were carried out. In the seed germination test, fresh weight of the shoot had significant decrease compared to the control. The amount of chlorophyll a, b and the carotenoid of the hydroponic culture was better influenced than pot culture for the better diffusion of toxic substances. All the cigarette filter treatments caused a significant increase in the antioxidant content in the pot and hydroponic culture, compared to the control. The treatments of shredded and unshredded smoked cigarette filter in pots and hydroponics caused a significant increase in the amount of proline compared to the control conditions. Proline is an osmotic regulator that shows the positive response of the plant under the stress conditions of toxic substances from these filters.

Keywords: cellulose acetate, cigarette filter, tall fescue, toxic substance, environmental impact

ENVIRONMENTAL IMPLICATION

Cigarette filters have a significant environmental impact, and not in a good way. 95% of cigarette filters are made of plastic material (cellulose acetate) that may appear like cotton. It is non-biodegradable and may remain toxic for up to ten years. Cigarette butts scattered along green spaces, sidewalks, roadsides and beaches. About 4.5 trillion cigarettes are littered each year. Littered cigarette butts leach toxic chemicals such as arsenic and lead, to name a few into the environment and can contaminate water.

INTRODUCTION

Tall fescue is known also as Festuca arundinacea from poaceae family. This is a perennial plant with thick and deep main roots. Tall fescue has a short underground stem and is considered a tall plant. The leaves of this plant are dark green or shiny. Its panicle is 10 to 30 cm long and there are 5 to 7 seeds in each panicle (Hannaway et al., 1999). Tall fescue is a perennial plant, which can tolerate a wide range of environmental stressors, including strongly acid to alkaline conditions, and its deep root system allows it to grow in wetlands and other low-lying areas. This plant is an introduced grass found in the Pacific Ocean, northwest wetlands, reclaimed tidal lands, and the mid-west coast of South Korea (Lee et al., 2022). This plant has positive characteristics such as high fodder yield, long grazing season, relatively good seed production, adaptability to a wide range of soil conditions, suitable cold resistance and soil protection. Due to its thick, strong, deep and wide fibrous filaments, tall fescue reduces soil erosion (Hannaway et al., 1999).

Cellulose acetate filters were used in cigarettes in the 1950s following the increasing number of smokers. There was convincing scientific evidence that smoking results into lung cancer and other serious diseases (Novotny et al., 2009). Cigarette contains higher levels of a cancercausing substance. The heavy metals and trace elements in cigarette butt leachate are the cause of toxicity in the organisms. Although the presence of various compounds in cigarettes and their filters has been extensively studied, few reports have been presented on the amount leached into aquatic medium. The occurrence of heavy metals in cigarettes is largely due to the soil conditions in which tobacco is being cultivated. Apart from that, using pesticides, insecticides and herbicides, the addition of casing materials to tobacco leaves and dried leaves, and the use of brightening agents on wrapping paper produce most of the new chemicals (Michael et al., 2022). Cigarette filter contains dangerous substances including chromium, nickel, cadmium, arsenic and lead, which are carcinogenic and dangerous materials for humans and animals (Hazbehiean et al., 2022). Cigarette filters cause pollution in different areas, swallowing by birds, animals and sea creatures can result into death or some problems. The washing of cigarette filters by rainfall and passing through waterways causes the release of toxic chemicals such as nickel, lead acetone, cadmium and arsenic in the water and soil affects organisms. (Hazbehiean et al., 2022).

MATERIALS AND METHODS

Plant Growth and Treatments

For this research, pots and hydroponic medium were treated in the research greenhouse of the biology department of Shiraz University, in the winter 2021 and the spring 2022, and the related experiments were also conducted in the laboratory. Based on the performed studies, the preparation of samples and their treatment was as follows.

To test seed germination, 50 seeds of tall fescue in petri dish with paperfilter under and above the seeds were used in the laboratory. There were 5 treatments including control, unsmoked unshredded cigarette filter, unsmoked shredded, smoked unshredded, and shredded smoked cigarette filter each with 4 replicates. Each petri dish had five cigarette filters. The seeds were wet with distilled water and number of seed germinated were calculated on the 10th day.

In pot cultivation, 20 pots (each 2 kg) with the suitable soil (sand, garden soil and leaf fertilizer with the same amount) were used and the same size of 10 seedlings were planted in each pot. Totally 30 cigarette filters were added to each pot in three times 10th, 20th and 40th days after seedlings planting and on the 54th day of planting, the seedlings were collected.

In hydroponic culture the germinated seeds were used. The containers of the same size and shape with the capacity of four liters of water were choose. The eight holes on the top of the containers were formed and the surroundings of the containers painted with black color so that the roots of the plant were placed in the darkness. Twenty containers were filled each with four litters of water, then added one milliliter of micronutrients solution, and ten milliliters of macronutrients solution for every liter of water. The seedlings were placed in the holes at the head of the containers so that the root was at the bottom and the stem was at the top. The glass wool was used to fix the plant. The eight plants were put in each hole so 3 that each container had 64 plants. Like the previous experiment, five treatments and four repetitions were used. The treatments were added the in two stages, 20 cigarette filters on the 2nd day of hydroponic culture, and 30 cigarette filters on the 6th day of hydroponic culture. The containers were refilled with Hoagland solution whenever they were empty. The plants were collected on the 28th day of experiment.

Measurement of Shoot and Root Fresh Weight

the shoot of the plant was cut from pot and hydroponic cultures and also the root from hydroponic culture. Then measured their weight before placing in liquid nitrogen.

Measurement of Chlorophyll and Carotenoid Concentration

Leaf tissue (0.01 g) was taken and ground with 80% acetone, then poured into the falcon and reached to a volume of 10 ml. The solution was centrifuged for 10 minutes at 6000 rpm. The upper layer was used to measure chlorophyll and carotenoid.

To measure the absorbance of the solutions, the spectrophotometer (SHIMADZU 160A, Japan) was used with the 663 nm for chlorophyll a, 645 nm for chlorophyll b, and 470 nm for carotenoid (Arnon, 1967).

The following formulas were applied to calculate chlorophyll a, b and carotenoids.
Chlorophyll a = 12.7 × A663 - 2.69 × A645 (mgg-1)
Chlorophyll b = 22.9 × A645 - 4.68 × A663 (mgg-1)
Carotenoid = [(1000 × A470 - 1.82 × Chl a - 85.02 × Chl b) /198] (mgg-1)

Measurement of Proline in the Shoot

Proline content in the shoot was measured using ninhydrin acid in accordance with Bates method (Bates et al., 1973). Increased absorption is directly associated with the amount of proline. The shoot (0.05 g) was weighed and placed in a mortar, 10 ml of sulfosalicylic acid 3% was gradually added to it and ground well to create a uniform solution. The solution was poured into the falcon and placed in the centrifuge at 1500 rpm for 10 minutes. The upper layer was separated and it was considered as the base extract for proline measurement. Two ml of base extract, plus two ml of pure glacial acetic acid, with two ml of ninhydrin reagent were poured into the test tube and shaken to mix well.

The test tube was placed in a BainMarie with aluminum cover at a temperature of 95°C. After one hour, the samples were removed from the water bath and transferred directly to the ice bath to be cool, and the reaction was terminated. After cooling, it was transferred to room temperature, then four ml of toluene were added to the solution and mixed by vortex for 30 seconds, this caused the two phasing of the contents of the tube. After 20 minutes, the optical absorbance of the upper layer was read at of 520 nm using a spectrophotometer (SHIMADZU 160A, Japan). The amount of proline was calculated by the standard curve, as milligrams per liter, but the final data was presented by changing the unit using the following calculation in μmoles proline per gram.

[(μg proline/ml × ml toluene) / 115.5 μg/μmole] / [(g sample) / 5 = μmoles proline/g of fresh weight

Antioxidant Measurement

Antioxidant potential was determined using the stable DPPH radical in accordance to the method of Shimada et al. (1992). First, 150 μL of the plant base extract was added to each foil wrapped falcon containing 2850 μL of a 0.004% DPPH solution, and there was a control solution that contained no extract and only contained 150 μL of 100% methanol and 285 microliter solution of

0.004% DPPH. The control solution and the sample solution were placed in the dark for one hour and then its absorbance was read at 517 nm by a spectrophotometer (SHIMADZU 160A, Japan).

RESULTS AND DISCUSSION

The Impacts of Toxic Substances of Cigarette Filter on the Seed Germination

The percentage of seed germination under the treatments of shredded unsmoked cigarette filter, shredded smoked cigarette filter and shredded smoked cigarette filter has decreased significantly compared to the control. The reasons of this culture are growth inhibitory stressed of heavy metals and other toxic components of tobacco. Cigarette tobacco proved that it is toxic at the seed germination stage, and could be toxic at both vegetative and reproductive stages of plant growth (Haq et al., 2018). Cigarette filters are made of cellulose acetate, which is a plastic product (Novotny et al., 2009). Cellulose acetate present in cigarette filter mitigates the seed germination (Fig. 1).

The Impacts of Toxic Substances of Cigarette Filter on the Fresh Weight

In the pot, the fresh weight of tall fescue in cigarette filter treatments had decreased compared to control conditions. This reduction was observed from unshredded unsmoked cigarette filter, unshredded smoked cigarette filter, shredded unsmoked cigarette filter and shredded smoked cigarette filter, respectively. In the hydroponic medium, all the treatments had a significant reduction compared to the control conditions. The fresh weight of the unshredded unsmoked cigarette filter, the shredded unsmoked cigarette filter, the unshredded smoked cigarette filter and the shredded smoked cigarette filter showed significant decrease compared to the control conditions, respectively. The stress of toxic substances and heavy metals leads to plant cell damage and the accumulation of metal ions in the plant, which disrupts the ionic cell homeostasis (Yadav, 2010). Heavy metal toxicity reduces the movement of sucrose and water and increases oxidative damage (Haider et al., 2021). Also, cellulose acetate in cigarette filter causes negative effects on plant growth (Fig. 2).

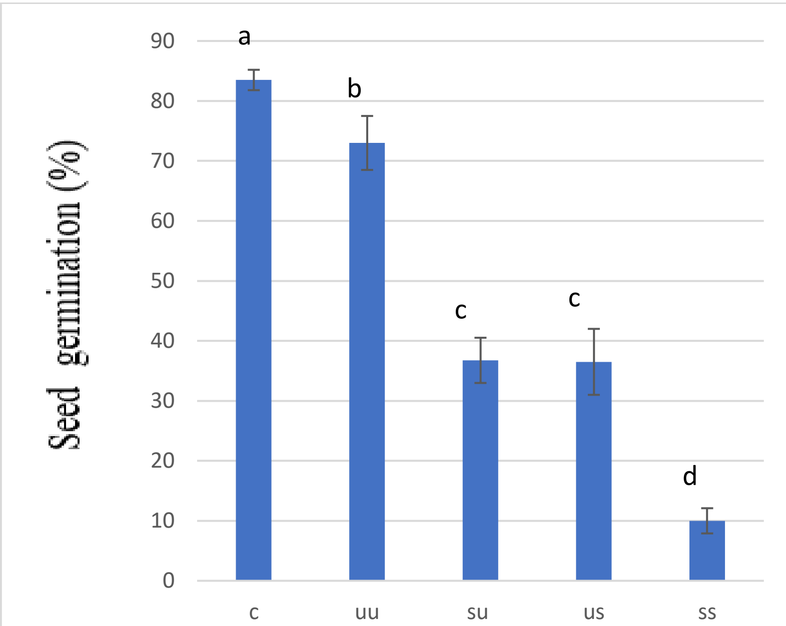

Figure 1- The impacts of toxic substances of cigarette filter on the germination of tall fescue seeds. Each number is the average of four repetitions of ±SE. Different letters indicate a significant difference at level p

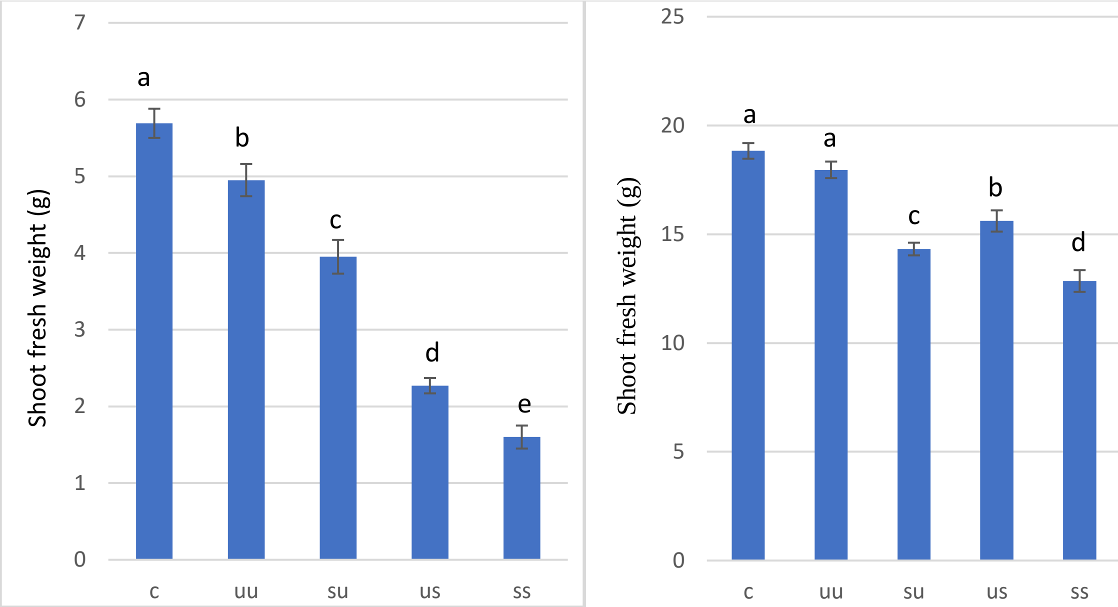

Figure 2- The impacts of toxic substances of cigarette filter on the fresh weight of the shoot of tall fescue in hydroponics (left) and pots (right) media. Each number is the average of four repetitions of ±SE. Different letters indicate a significant difference at level p

Effects of Toxic Substances of Cigarette Filter on Photosynthetic Pigments

In pots, cigarette filter treatments did not have a significant impact on the content of chlorophyll a and b, but the amount of carotenoids decreased significantly under the conditions of shredded and unshredded smoked cigarette filter treatments compared to the control (Fig. 3). It shows the effect of toxic substances of smoked cigarette filter. In hydroponic medium, the treatments of shredded unsmoked cigarette filter, unshredded smoked cigarette filter and shredded smoked cigarette filter significantly decreased their chlorophyll a content compared to the control. The content of chlorophyll b in the treatments of unshredded smoked cigarette filter and shredded smoked cigarette filter compared to the control conditions was significantly decreased. The amount of carotenoids in the treatment of shredded smoked cigarette filter and unshredded smoked cigarette filter has been significantly reduced compared to the control (Fig. 4). The difference between the content of chlorophyll a and b in pot and hydroponic media can be related to better diffusion of toxic substance in hydroponic medium from cigarette filter and absorption to the plant. The toxicity of toxic substances and heavy metals in photosynthesis can prevent the activity of different photosynthetic enzymes and chlorophyll biosynthesis, damage the chloroplast membrane system, and interfere with the process of photosynthetic electron transport (Aggarwal et al., 2012).

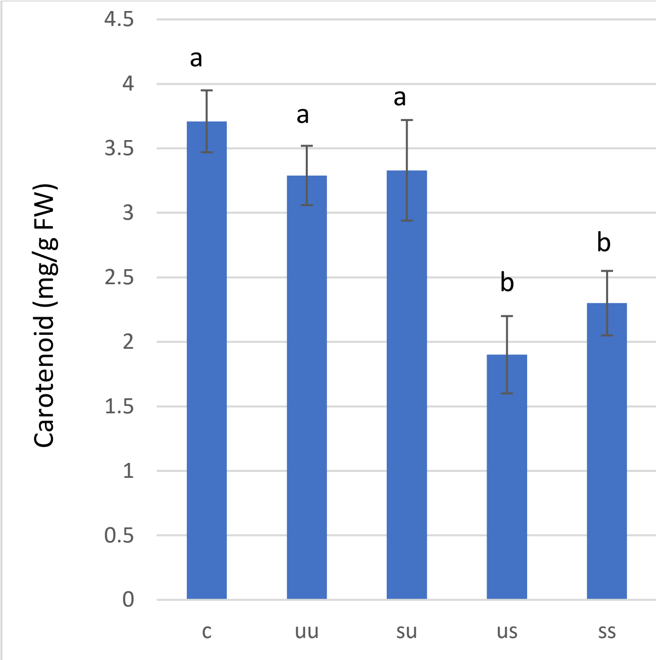

Figure 3- Effects of toxic substances of cigarette filter on carotenoids of tall fescue in pots. Each number is the average of four repetitions of ±SE. Different letters indicate a significant difference at level p

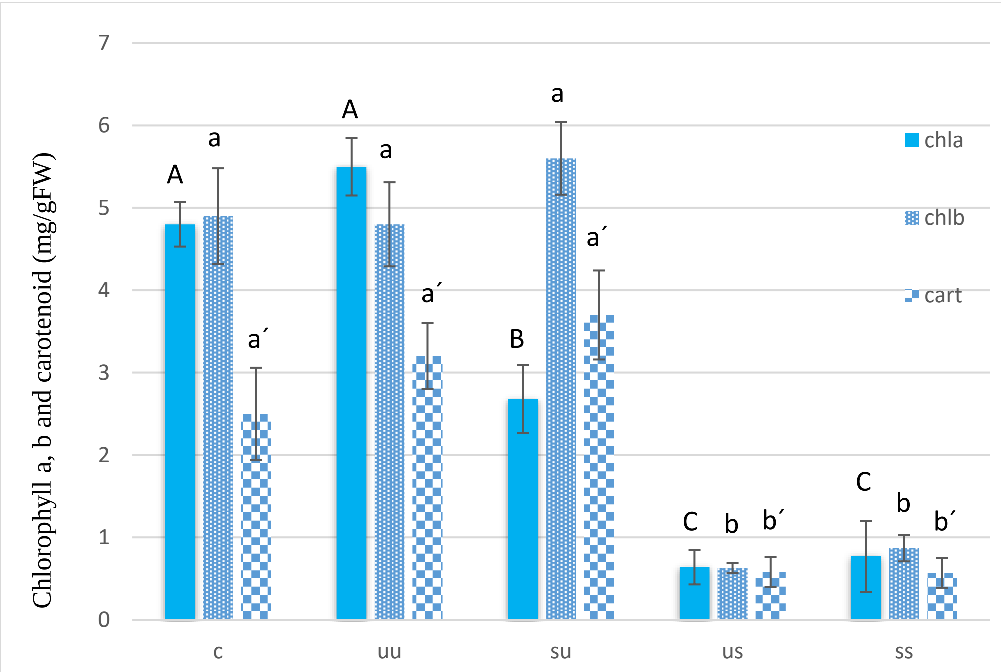

Figure 4- Effects of toxic substances of cigarette filter on chlorophyll a, chlorophyll b and carotenoids of tall fescue in hydroponics. Each number is the average of four repetitions of ±SE. Different letters indicate a significant difference at level p

Effects of Toxic Substances of Cigarette Filter on the Proline Content

Both in pots and in hydroponics, the treatments of shredded smoked cigarette filter and unshredded smoked cigarette filter have significantly increased compared to the control of the proline content (Fig. 5). It shows the effect of toxic substances in smoked filter. Plants accumulate large amounts of different types of compatible solutes in response to various plant stresses.

Compatible solutes are low molecular weight, highly soluble organic compounds, which are usually non-toxic at high cellular concentrations. These solutes protect plants against stress and adjust cellular osmosis, detoxify ROS, protect membrane integrity, stabilize enzymes and proteins. These include proline, sucrose, polyols, and ammonium compounds such as glycine (Hayat et al., 2012).

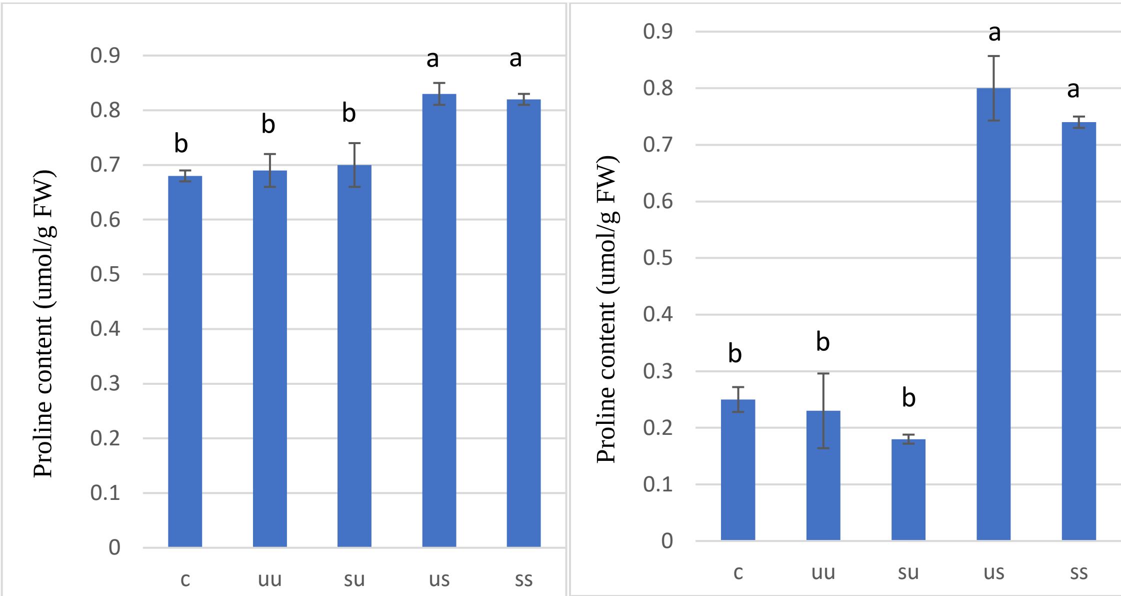

Figure 5- Effects of cigarette filter toxic substances on the proline content of tall fescue in hydroponics (left) and pots (right) media. Each number is the average of four repetitions of ±SE. Different letters indicate a significant difference at level p

The Impact of Toxic Substances of Cigarette Filter on the Antioxidant Content

In the pots, the amount of antioxidants has increased significantly in all cigarette filter treatments, compared to the control. In the treatment of unshredded unsmoked cigarette filter, shredded unsmoked cigarette filter, and smoked unshredded and shredded smoked cigarette filter, the amount of antioxidants increased significantly compared to the control. In the hydroponics, all the treatments from unshredded unsmoked cigarette filter, shredded unsmoked cigarette filter, unshredded smoked cigarette filter, and shredded smoked cigarette filter had a significant increase in their antioxidant content compared to the control (Fig. 6). Stress produces reactive oxygen species (ROS) that the plant must deal with. Thus, the plant uses enzymatic and non-enzymatic antioxidants, and antioxidant defense is of great importance to protect the plant from oxidative stress. Under stress conditions, antioxidant defense is increased and accumulation of secondary metabolites is occurring as a defense mechanism (Hasanuzzaman and Fujita, 2012).

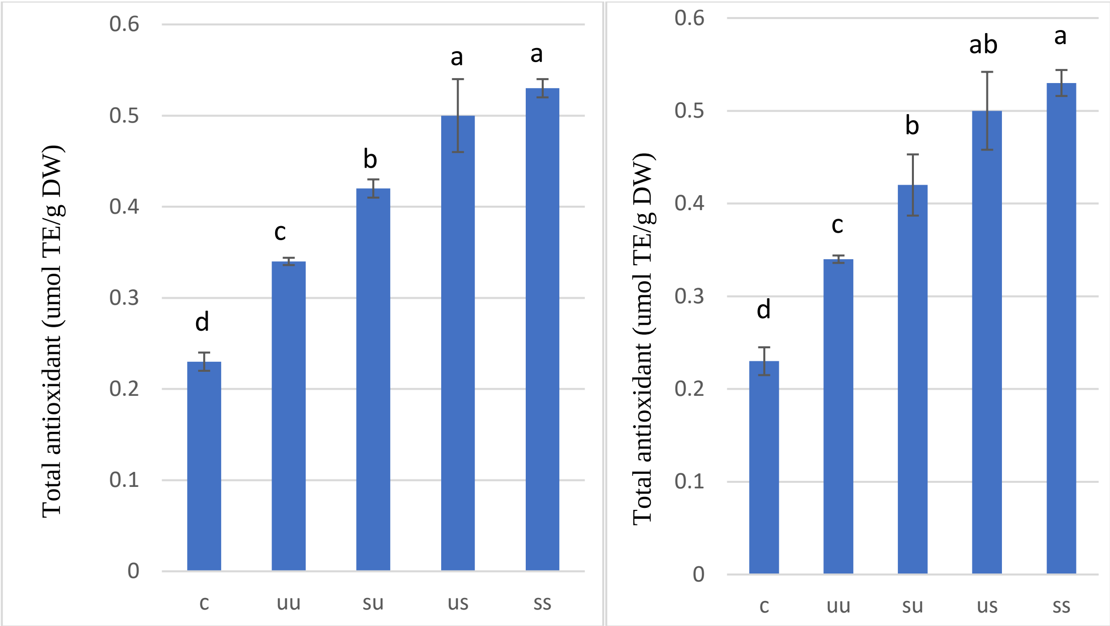

Figure 6- Effects of cigarette filter toxic substances on the antioxidant content of tall fescue in pots (left) and hydroponics (right). Each number is the average of four repetitions of ±SE. Different letters indicate a significant difference at level p

CONCLUSION

In the seed germination test, fresh weight of the shoot of the plant in the hydroponic culture and the fresh weight of the shoot of the tall fescue in the pot the treatments had significant decrease compared to the control. The amount of chlorophyll a, b and the carotenoid of the hydroponic culture was better influenced than pot culture for the better diffusion of toxic substances. All the cigarette filter treatments caused a significant increase in the antioxidant content in the pot and hydroponic culture of the tall fescue, compared to the control, which indicates that the plant has shown a positive response under stress conditions to cope with the stress. The treatments of shredded and unshredded smoked cigarette filter in pots and hydroponics caused a significant increase in the amount of proline compared to the control conditions. Proline is an osmotic regulator that shows the positive response of the plant under the stress conditions of toxic substances from these filters.

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