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Investigation the effects of UV radiation on physiological characteristics of *Moringa oleifera in vitro and in vivo*.

Abstract

Moringa Oliefera seeds were treated with UV light type A, B and C for 30min. Seedlings length, Number of plants, number of axillary buds, number of adventitious buds and number of apical buds were recorded 34 days after sowing the treated seeds in addition to the control *in vivo*. Sterilized seeds were cultured *in vitro* on MS agar medium containing 4.0 mg/l BA for seed germination, then shoot explants were cut into small pieces and placed on the MS agar medium supplemented with 0.5 mg/l BA and 2.5 mg/l 2, 4-D. The percentage of callus induction, callus fresh weight were determined about six weeks after inoculating. Also proline and carbohydrate concentrations were determined for intact plant and callus cultures. Results showed that the physiological parameters which studied *in vivo* reduced significantly at UV-B recording 1.5, 1.8, 3.4, 2.8 and 19.3cm No. germinated plants, No. Adventitious buds, No. axillary buds, No. apical buds and seedlings height respectively. While UV-B recording the highest mean values in relation to percentage of callus induction, callus fresh weight and proline concentration (100%, 112mg and 9.7µM/g respectively) compared to the control (72.3%, 93.3mg and 7.3µM/g respectively). A significant reduction in the mean carbohydrate concentrations observed in all UV treatments in both intact plants and callus cultures compared with control.

Introduction

Moringa oleifera is the most widely cultivated species of the genus *Moringa*, which is the only genus in the family Moringaceae (Azra, 2011). It is a fast-growing and it can reach a height of 10–12 m (32-40 ft). They grow on slender, hairy stalks in spreading or drooping later flower clusters which have a length of 10–25 cm. Flowering begins within the first six months after planting. In seasonally cool regions, flowering only occurs once a year between April and June. In more constant seasonal temperatures and with constant rainfall, flowering can happen twice or even all year-round. The fruit is a hanging, three-sided brown capsule of 20–45cm size which holds dark brown, globular seeds with a diameter around 1cm. The seeds have three whitish papery wings and are dispersed by wind and water (Iqbal and Bhanger, 2006). Many parts of the moringa are edible. Regional uses of the moringa as food vary widely that include immature seed pods, leaves, oil pressed from the mature seeds and roots (Olson and Carlquist, 2001). Seed pods/fruits; even when cooked by boiling; remain particularly high in vitamin C (which may be degraded variably by cooking) and are also a good source of dietary fiber, potassium, magnesium, and manganese (Olson and Carlquist, 2001). Moringa seed oil also has potential for use as a biofuel (Rashid *et al*., 2008). The roots are shredded and used as a condiment with sharp flavor qualities deriving from significant content of polyphenols (Atawodi *et al*., 2010). Ultraviolet (UV) light is electromagnetic radiation with a wavelength from 400nm to 10nm, UV is traditionally divided into three wavelengths. UV-C (200-280 nm) is extremely harmful to living organisms, but not relevant under natural conditions of

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solar irradiation. UV-B (280-320 nm) is of particular interest because although this wavelength represents only approximately 1.5 % of the total spectrum, it can have a variety of damaging effects in plants. UV-A (320-400 nm) represents approximately 63% of the incoming solar radiation and is the least hazardous part of UV radiation (Hollosy, 2002). UV spectrum has effects both beneficial and harmful to human health (Haigh, 2007). Strong absorption of UV-B photons by biologically important macromolecules i.e. proteins and nucleic acids has a large effect on plant and animals metabolisms (Hediat *et al*., 2011). The effects of UV light on plants include inhibited growth, morphological changes and increase in the level of phenolic pigments (Brzezinska *et al*., 2006). Inhibition of photosynthesis belongs to the key factors responsible for physiological disorders and a decrease in the biomass of crop plants. The deleterious effect of UV-B on the efficiency of this process can be attributed to specific reductions in expression of important photosynthetic genes, a reduction in Rubisco activity, changes in ion permeability of thylakoid membranes, and in the level of chlorophyll and carotenoids (Ines *et al*., 2007). The effect of UV-B radiation have been well-documented on barley, wheat, oats, maize, soybean and cotton (Gao *et al*., 2003). Saradhi *et al*. (1995) concluded that the level of proline in the seedling increased significantly with increase in UV-B exposure time. In addition, it has been suggested that exposure to ultraviolet radiation reduces plant growth vigor, chlorophyll contents, carotenoids, amino acids, proteins, total sugars and starch, UV radiation induced the accumulation of flavonoids, proline, copherol and ascorbate contents (Carlettia *et al*., 2003). *Moringa oleifera* callus induction was a greatly influenced by temperature, nutrients, pH and addition of ascorbic acid in the growing medium, Furthermore, the seeds contain an essential oil (Bennett *et al*., 2003). Stephenson and Fahey (2004) reported that 20% success rate of germination of immature seeds with subsequent shoot development from the epicotyl meristematic tissues of *M. oleifera* Lam. cultured on Murashige and Skoog (MS) semi-solid medium (Murashige and Skoog 1962) amended with 1 mg/L benzylaminopurine (BAP) and 1 mg/L gibberellic acid (GA3). Islam *et al*. (2005) initiated shoot proliferation from juvenile shoots cut into nodal sections of *M. oleifera* Lam. on MS solid basal medium supplemented with either 1 mg/L or 1.5 mg/L benzylaminopurine (BAP). Initially, callus developed on the cut ends of some node sections 1 wk after inoculation, which differentiated into small shoots. Maximum number of shoots was observed in 1 mg/L BAP amended culture medium, which further increased when repeatedly subcultured on the same media formulation.

Materials and methods

Seeds treatments with UV radiation.

Seeds were treated with UV light in a different wave length including A, B and C for 30min (Abd El-Kadder *et al*., 2014).

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Germination of seeds *in vivo*.

Seeds of *M. oleifera* were treated with UV radiation type A, B and C. The seedlings were raised inside a greenhouse in Biotechnology Dept., Al- Nahrain University. Seedlings length, Number of plants, number of axillary buds, number of adventitious buds and number of apical buds were recorded 34 days after sowing.

Medium preparation

Murashige and Skoog (MS) medium were prepared and supplemented with sucrose, myo-inositol and growth regulators. The pH of the medium was adjusted to 5.8 using 0.1N NaOH or 0.1N HCl, then 8g/l agar was added to the medium. The medium was dispensed into 15x2.5cm tubes (10 ml/tube).

Medium sterilization

The medium was sterilized by autoclaving.

Seed sterilization, germination and callus induction *in vitro*

Seeds of *M. oleifera* were washed with running water for 10 minutes; surface sterilized with 30% Clorox solution for 30 minutes, and then rinsed four times with sterile distilled water. The seeds after dipping in 95% ethanol, and removing the seed coats were cultured on MS agar medium containing 4.0mg/l BA for seed germination, then shoot explants were cut into the small pieces and placed on the MS agar medium supplemented with 0.5 mg/l BA and 2.5 mg/l 2, 4-D for six weeks. All cultures were incubated at 25 ± 2ºC. (Oraibi, 2016). callus induction frequency (%) was calculated using the following formula (Yousif, 2002).

Callus induction frequency (%) = No. seeds produced callus/total seeds cultured x100.

Measurement of callus fresh

Callus fresh weight was measured after six weeks of subculturing into a callus growth medium (Ahmad, 2008).

Determination of proline concentration in *M. oleifera* cultures

Proline concentrations were determined according to Bates *et al.* 1973. A quantity of 10mg dry weight of plant tissues was homogenized with 3% sulfosalicylic acid, The filtrate was mixed with 2ml of glacial acetic acid and ninhydrin reagent and incubated at 100°C for 30min. The samples were rigorously mixed with 4ml toluene, light absorption of toluene phase was estimated at 520nm using spectrophotometer. Proline concentration was determined and expressed as µM/g dry weight

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Preparation of proline standard curve

Proline standard curve was plotted by using different concentrations of proline 1, 2, 4, 6, 8 and 10µg/ml. Then 2ml of glacial acetic acid and ninhydrin reagent were added to each proline concentration, and incubated at 100°C for 30min. The samples were rigorously mixed with 4ml toluene, light absorption of toluene phase was estimated at 520nm using spectrophotometer (fig. 1).

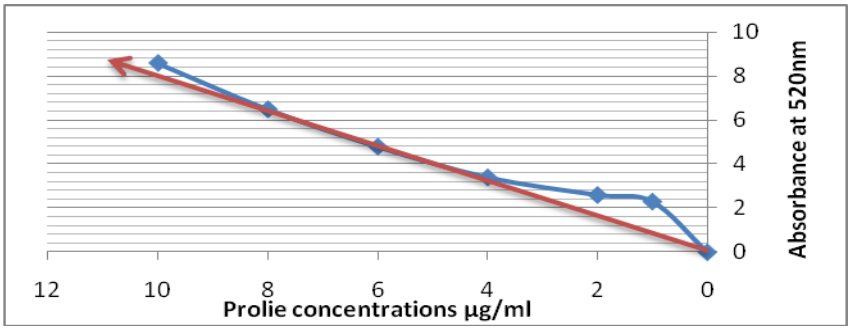

Figure 1: Standard curve of proline.

Determination of carbohydrate concentrations *M. oleifera* cultures

Total sugar content (carbohydrate concentrations) was determined without the identification of specific sugar components based on the method of phenol sulfuric acid (Herbert *et al*., 1971). A quantity of 10mg dried plant tissues was homogenized with deionized water, the extract was filtered, and then treated with 1ml of 5% phenol and 1ml of 98% sulfuric acid, the mixture was incubated at 30°C for 20min then absorbance at 485nm was determined by spectrophotometer. Concentrations of soluble sugar were expressed as µg/g dry weight.

Preparation of glucose standard curve

Glucose standard curve was drawn by preparing the following glucose concentrations 10, 20, 40, 60, 80 and 100mg/ml, then 2ml was taken from each concentration and treated with 1ml of 5% phenol and 1ml of 98% sulfuric acid. The mixture was incubated at 30°C for 20 min then absorbance at 485nm was estimated by spectrophotometer (fig. 2)**.**

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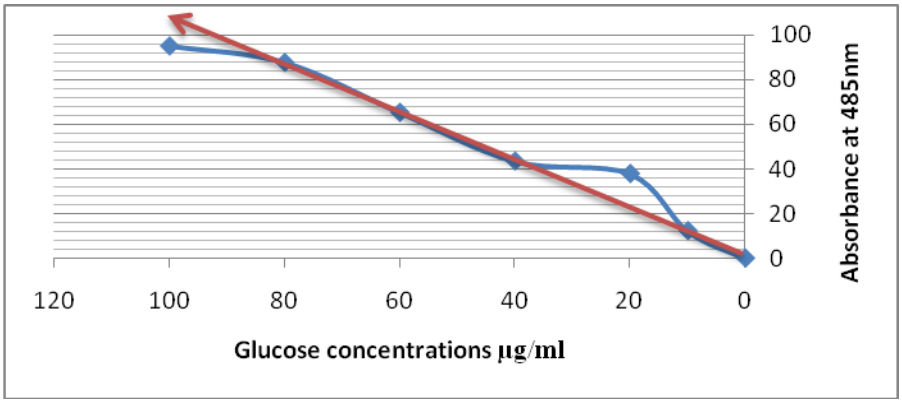

Figure 2: Standard curve of glucose for determination of reducing sugars according to Herbert *et al*. (1971).

Results and discussion

Effect of UV radiation types A, B and C on some physiological characteristics of *M. oliefera in vivo*.

Results displayed in table 1 shown that the studied parameters significantly reduced in UV-B treatment recording 1.5, 1.8, 3.4, 2.8 and 19.3cm No. germinated plants, No. Adventitious buds, No. axillary buds, No. apical buds and seedlings height respectively compared with control (0.0) which recording 4.6, 4.8, 7.8, 6.1 and 24.7cm for the same parameters respectively. This results was agreement with those obtained by K. Zuk-Golaszewska1 *et al*. (2003) who reported that treatment of some plant species with UV-B lead to reduction in plant growth, biomass and UV-B radiation caused chlorophyll degradation but has no impact on quality of the plants. Hediat *et al*. (2011) reported that reduction in plant growth and biomass accumulation due to UV-B exposure was found in several trees and crop species. Negative impact of enhanced UV-B radiation on cotton growth included reduction in height, leaf area, total biomass and fiber quality and growth reduction is mediated through leaf expansion, which is a consequence of the UV-B radiation effects on the rate and duration of both cell division and elongation (Gao *et al*., 2003). UV-A and UV-C show no significant differences compared with control. Figure 3 describe the effect of UV radiation type A, B and C on different physiological characteristics of *Moringa oleifera in vivo* after 34 days of sowing seeds under greenhouse conditions.

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Table 1. Effect of UV radiation type A, B and C on the mean of different physiological characteristics *in vivo* after 34 days of sowing seeds under greenhouse conditions.

UV (nm)	No. germinated plants	No. adventitious buds	No. Auxiliary buds	No. apical buds	Seedlings height (cm)
0.0	4.6	4.8	7.8	6.1	24.7
A	2.8	4.4	4.7	4.1	26.1
B	1.5	1.8	3.4	2.8	19.3
C	2.0	3.2	5.2	3.7	21.8
LSD 0.05	3.1				

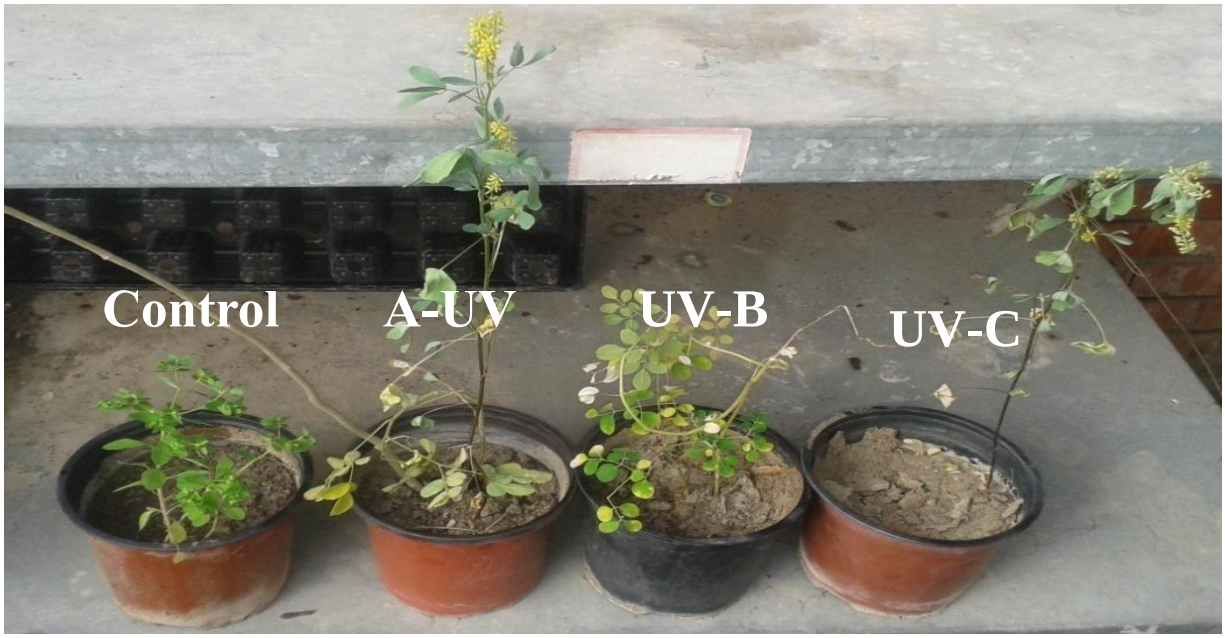

Figure 3. Effect of UV radiation type A, B and C on different physiological characteristics of *Moringa oleifera in vivo* after 34 days of sowing seeds under greenhouse conditions.

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Effect of UV radiation types A, B and C on mean % callus induction and callus fresh weight.

Results shown in table 2 reveal that a significant increase resulted in the % callus induction in UV-B treatment with mean value 100% compared with control (72.3%). While there was no significant differences recorded between treatments in the mean callus fresh weight compared with control, but the highest value obtained in UV-A and UV-B recording 102.7mg and 112.0mg respectively as shown in table 3. These results was agreement with those obtained by Abd El-Kadder *et al*. (2014) who reported that ultraviolet radiation significantly affected callus growth in term of fresh weight, they also investigated that irradiation of UV for 30min lead to increasing the callus growth up to 17.3 %. Figure 4 Describe the differences of callus cultures which originated from cotyledons germinated from seeds treated UV radiation type A, B, C in additional to the control and showing the changes in the callus mass of *Moringa oleifera* which grown on MS medium for four weeks.

Table 2. Effect of UV radiation type A, B and C on the mean % callus induction, after inoculating explants onto solid MS medium for four weeks, n=10.

UV (nm)	% Callus induction
0.0	72.3
A	48.0
B	100.0
C	42.2
LSD 0.05	25.75

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Table 3. Effect of UV radiation type A, B and C on the mean callus fresh weight (mg), after inoculating explants onto solid MS medium for four weeks, n=10.

UV (nm)	Callus fresh weight (mg)
0.0	93.3
A	102.7
B	112.0
C	82.7
LSD 0.05	37.83

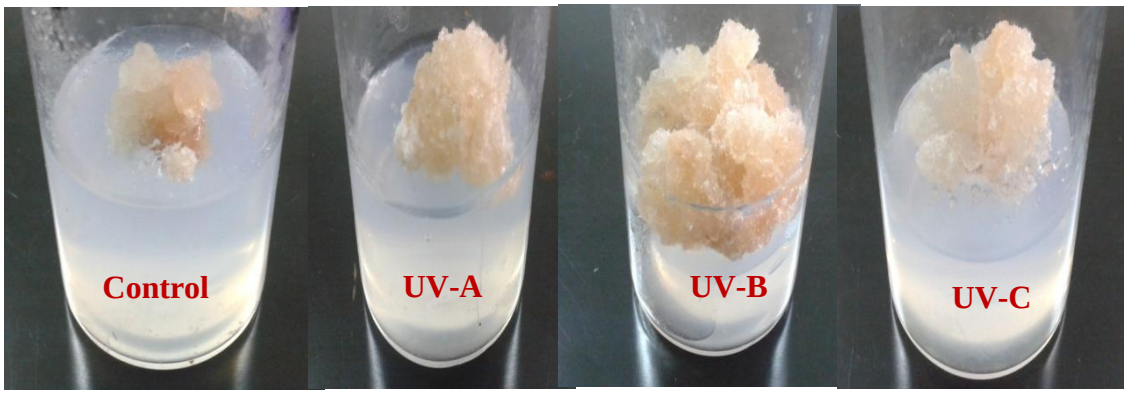

Figure 4. *Moringa oleifera* callus cultures originated from cotyledons germinated from seeds treated UV radiation type A, B, C in additional to the control, showing the changes in the callus mass grown on MS medium for six weeks.

Effect of UV radiation types A, B and C on mean proline and carbohydrates concentration.

Figure 5 exhibits that a significant increase in the mean proline concentration extracted from callus tissues reached 9.2, 7.4, 9.7 and 8.3µM/g for 0.0, UV-A, UV-B and UV-C respectively compared with those obtained from intact plant parts (7.3, 6.1, 9.5 and 6.4µM/g respectively) and the highest mean proline concentration was recorded in UV-B treatment for both intact plant and callus cultures, these results are in accordance with those of Riksa and Rizkita (2014) who reported that UV radiation is a useful technique to enhance secondary metabolites production. Also Hediat *et al*. (2011) reported that decreasing ultraviolet wave length induced a highly significant increase in the level of proline in both root and shoot of all tested plants and from the

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results obtained, it is suggested that proline can protect cells against damage induced by ultraviolet radiation.

Ritarani *et al*. (2010) reported that Living organisms are exposed to diverse forms of environmental stress including changes in temperature, water content, osmolarity , pH , oxidation , nutritional starvation and chemical compounds. Under severe stress conditions, cellular macromolecules such as proteins, nucleic acid and membranes are seriously damaged and lead to growth inhibition or cell death etc. Proline is an important amino acid which is also known as a stress substrate. It is believed to have multiple functions as it stabilizes proteins and membranes and scavenge reactive oxygen species.

Figure 6 shows that a significant reduction in the mean carbohydrate concentrations occurred in all UV treatments in both intact plants and callus cultures compared with control, as well as mean carbohydrate concentrations extracted from callus cultures was higher than those extracted from intact plant. These results is in agreement with those of Yousif (2002) who reported that the reduction in carbohydrate concentrations may because that plant cells tolerate stress by using carbohydrates as an energy source to combat stress.

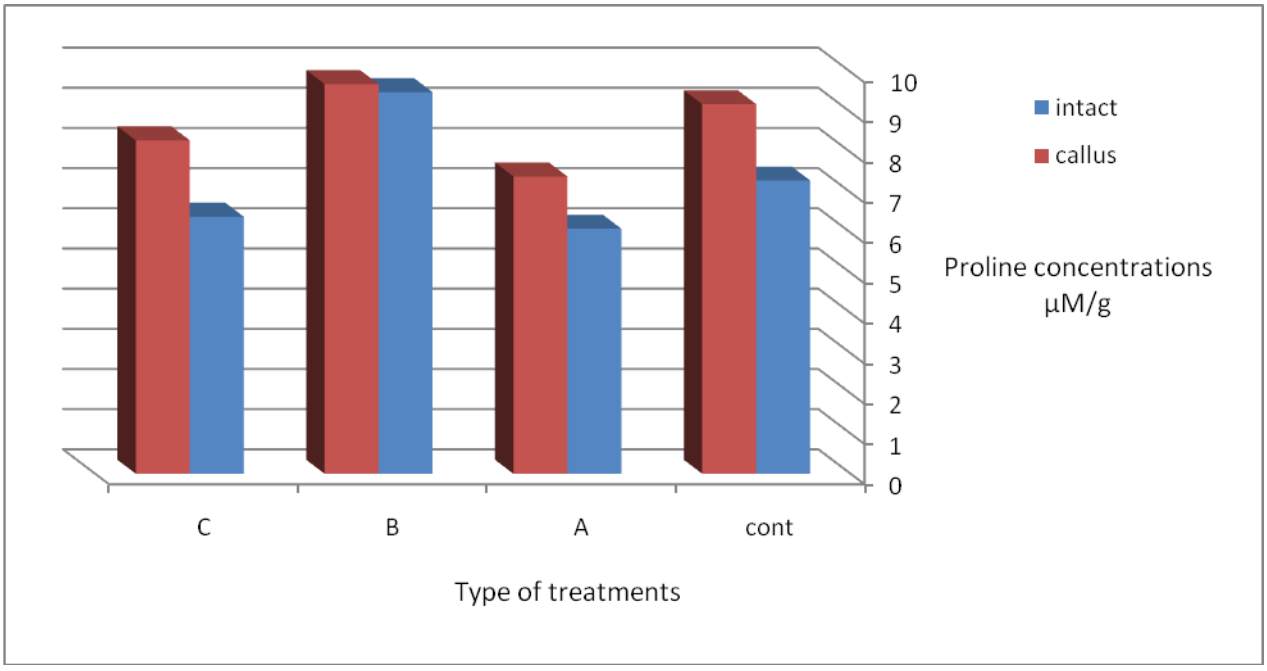

Figure 5. Effect of UV radiation on proline concentration in *Moringa oleifera* tissues *in vitro* and *in vivo*.

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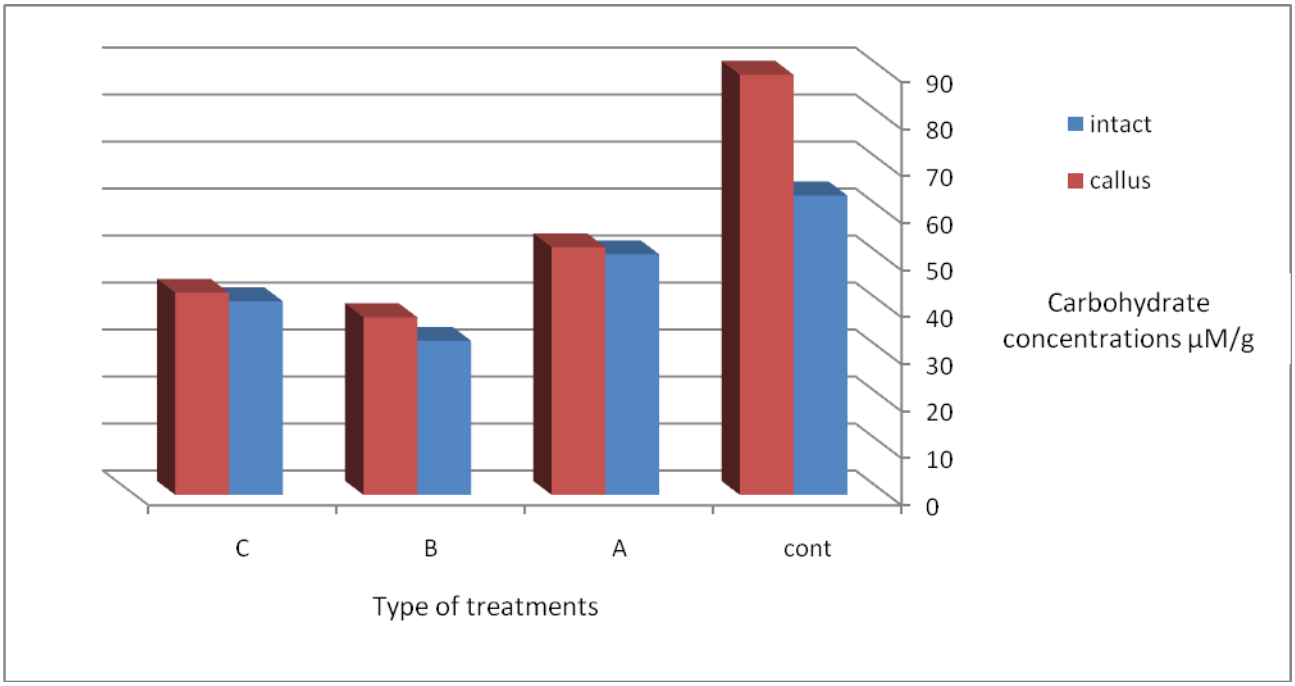

Figure 6. Effect of UV radiation on carbohydrate concentrations in *Moringa oleifera* tissues *in vitro* and *in vivo*.

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