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

Abstract

Moringa Oliefera seeds were treated with UV light type A, B and C for 30 minutes. 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~~percentage of callus induction and callus fresh weight ~~were determined~~were determined about six weeks after inoculating. Proline and carbohydrate concentrations were also determined for intact plant and callus cultures. Results showed that the physiological parameters 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. UV-B recorded 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 concentration was 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 fast-growing and it can reach a height of 10–12 m (32-40 ft). They grow on slender, hairy stalks in spreading 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 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 (280-200 nm) is extremely harmful to living organisms, but not relevant under natural conditions of solar irradiation. UV-B (320-280 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 (400-320 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) reported significant increases in the level of proline in the seedling 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 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 a 20% success rate of germination of immature seeds with subsequent shoot development from the epicotyl meristematic tissues of *M. oleifera* 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* 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 week after inoculation, which later 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. Therefore, the aim of these experiments was to study the effect of different UV radiation wavelengths (UV-A, UV-B and UV-C) on some physiological parameters of an important medicinal plant as *Moringa oleifera*.

Materials and methods

Seeds treatments with UV radiation.

Three groups of *M. oleifera* seeds were treated with UV radiation for 30 min, each group consist of 50 seeds. The first group was treated with UV-C radiation (254 nm~~) which~~) ~~-~~produced by a germicidal lamp (G30T8 30 W, Philips, Holland). The ~~S~~second group was treated with UV-B radiation using a UV-B lamp, F875-UV-B 8 W, Bio-Rad, Hercules, CA, USA with a cellulose acetate filter to remove all wavelengths < 280 nm for ~~-~~UV-B treatment and a polyester filter was used to remove all wavelengths < 320 nm for UV-A treatment. The fourth group was the control (without UV treatment). Seeds were sown in ~~a distled~~distilled water for 15 minutes, then washed three times and spread in sterile petri dishes under the UV Lamps.

Germination of seeds *in vivo*.

Each seeds group whether from irradiated or non-irradiated was divided into 25 pots, ~~as~~ and 2 seeds were cultured in each pot. Seedlings were raised inside a greenhouse in Biotechnology Departmentt~~.~~, 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~~, 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 (Viseclave-MACS-1100, Korea) at 15 Ibs pressure and 121 °C for 15 min.

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. After dipping the seeds in 95% ethanol, and removing the seed coats, they were cultured on MS agar medium containing 4.0mg/l BA for seed germination. Seedlings were then cut into small pieces (expalnt) and placed on the MS agar medium supplemented with 0.5 mg/l BA (Benzyl adenine) and 2.5 mg/l 2,4-D (2,4-Dichlorophenoxyacetic acid) for six weeks. All cultures were incubated at 25 ± 2º~~C (~~C (Oraibi, 2016). Callus induction frequency (%) was calculated using the following formula:

Callus induction frequency (%) = No. explants produced callus/total explants cultured x100 (Yousif, 2002).

Measurement of callus fresh weight

Callus fresh weight was measured after six weeks of subculturing into a callus growth medium using sensetive balance.

Determination of proline concentration in *M. oleifera* cultures

Proline concentrations were determined according to Bates *et al.* (1973). 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 and light absorption of toluene phase was estimated at 520nm using spectrophotometer (Analog- 305634, Japan). Proline concentration was determined and expressed as µM/g dry weight.

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).

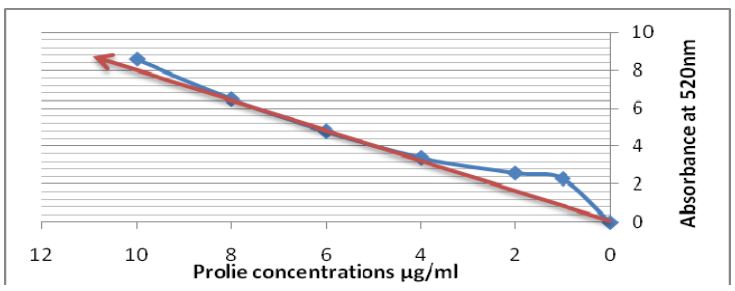

Figure 1: Standard curve of proline.

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). 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 plotted 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~~% sulfuric acid. The mixture was incubated at 30°C for 20 min then absorbance at 485nm was estimated by spectrophotometer (fig. 2)**.**

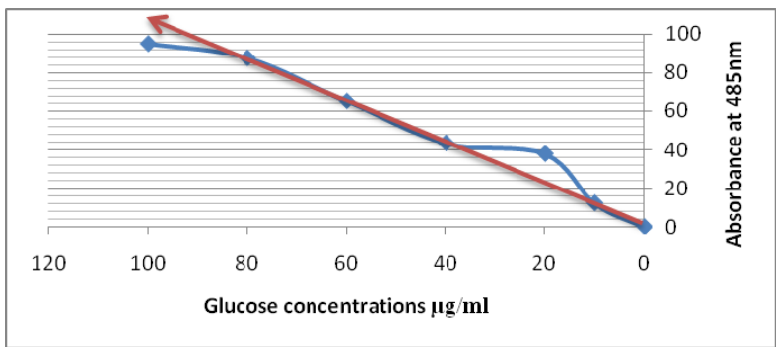

Figure 2: Standard curve of glucose for determination of reducing sugars according to Herbert *et al*. (1971).

Experimental design and statistical analysis

The experiments were designed as factorial experiments with a completely randomized design**.** Analyses were done using the SPSS var. 12 software. Differences between means were determined and least significant differences were compared at $P \leq 0.05$ (Steel and Torrie, 1982).

Results and discussion

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

Results displayed in table 1 show 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 and Upadhaya (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~~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 shows the effect of UV radiation type A, B and C on different physiological parameters of *Moringa oleifera in vivo* after 34 days of sowing seeds under greenhouse conditions.

Table 1. Mean ~~E~~effect of UV (nm) type A (400-320 nm), B (320-280 nm) and C (280-200 nm) on ~~the mean of~~ different physiological characteristics *in vivo* after 34 days of sowing seeds under greenhouse conditions. n=30.

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				

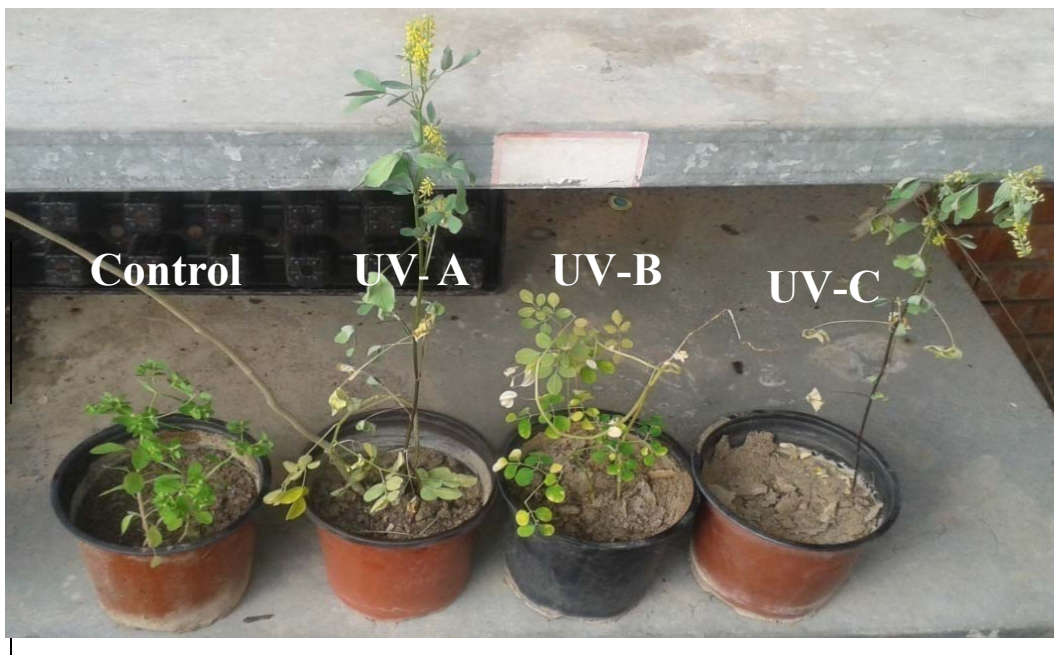

Figure 3. Effect of UV radiation type A, B and C on the ~~morphological characteristics~~morphological characteristics (vegetative changes) of *Moringa oleifera in vivo* after 34 days of sowing seeds under greenhouse conditions.

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

Results shown in table 2 reveal that there was a significant increase in the mean % callus ~~induction recorded~~induction recorded ~~at~~ for the UV-B ~~treatment with~~treatment with a 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 was 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. Mean ~~E~~effect of UV (nm) type A (400-320 nm), B (320-280 nm) and C (280-200 nm) on ~~the mean~~ % callus induction, after inoculating explants onto solid MS medium for six 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

Table 3. Mean ~~E~~effect of UV radiation type A, B and C on ~~the mean~~ callus fresh weight (mg), after inoculating explants onto solid MS medium for another six 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

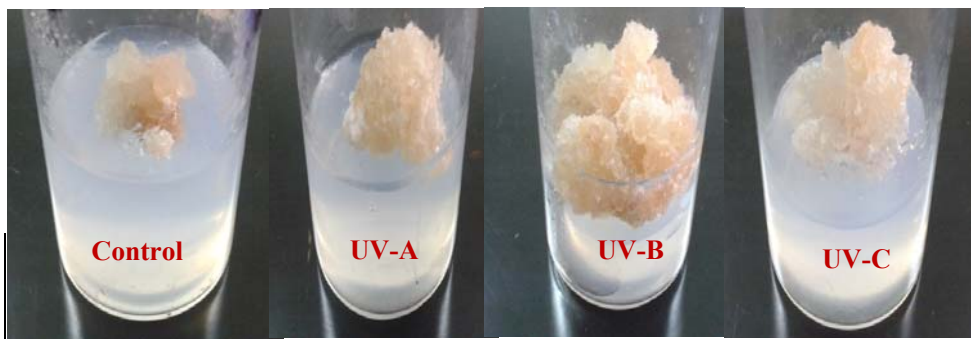

Figure 4. *Moringa oleifera* callus cultures originated from cotyledons germinated from seeds treated UV radiation type A, B, C in additional to the control, showing the changes in the callus mass grown on MS medium for six weeks.

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

Figure 5 exhibits that there was a significant increase in the mean proline concentration extracted from callus tissues which reached 9.2, 7.4 and 8.3µM/g for 0.0, UV-A and UV-C treatment, respectively, compared with those obtained from intact plant parts (7.3, 6.1 and 6.4µM/g, respectively). UV-B treatment recording highest mean proline concentration either in callus tissues or in intact plant parts (9.7 and 9.5 µM/g respectively). Although there were no significant differences obtained in the mean proline ~~concentration in~~concentration in UV-B treatment between callus tisses and intact plant, these results are in accordance with those of Riksa and Rizkita (2014) who reported ~~that UV~~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 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 scavenges 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, Mean carbohydrate concentrations extracted from callus cultures were 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 be due to the fact that plant cells tolerate stress by using carbohydrates as an energy source to combat stress, therefore resulting in lower carbohydrate levels.

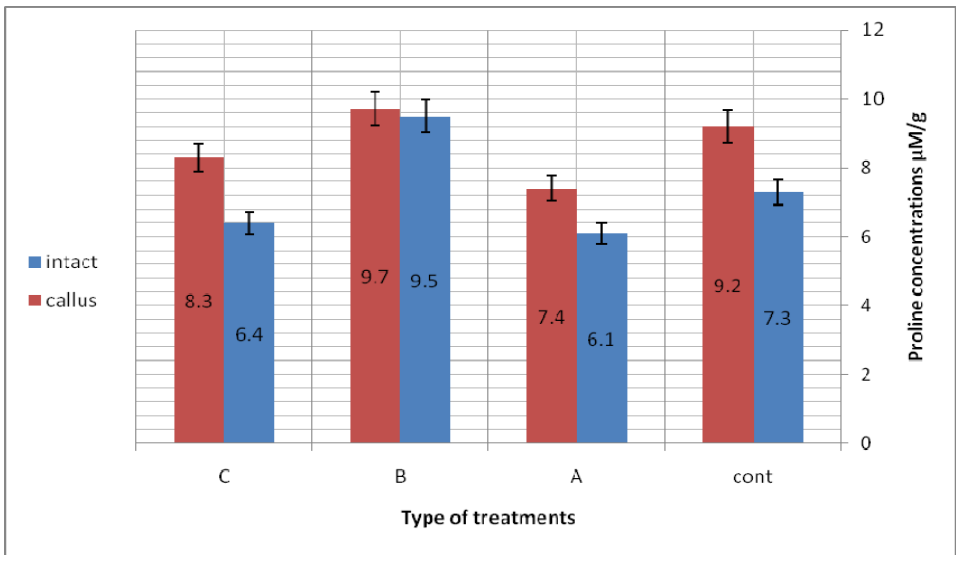

Figure 5. Effect of UV radiation on proline concentration in *Moringa oleifera* tissues *in vitro* and *in vivo*.

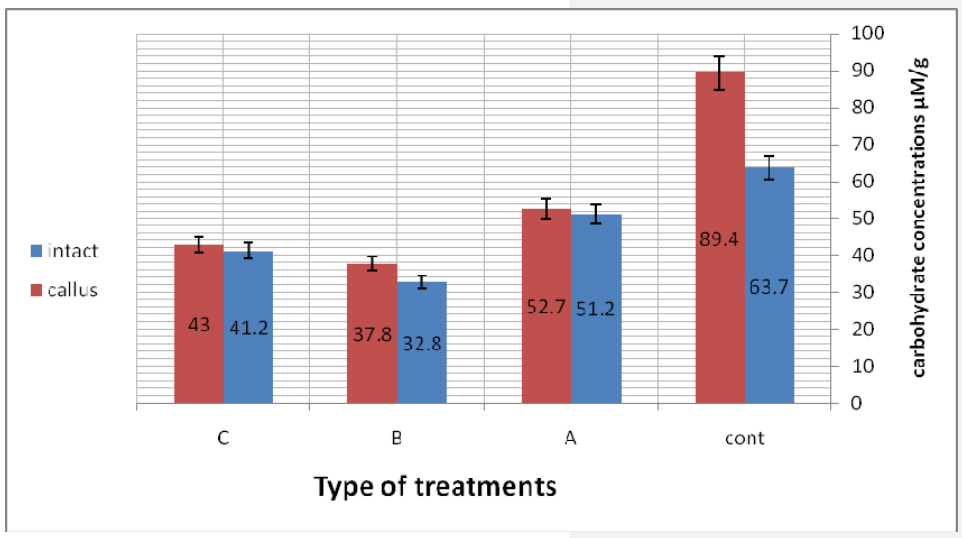

Figure 6. Effect of UV radiation on carbohydrate concentrations in *Moringa oleifera* tissues *in vitro* and *in vivo*.

Conclusions

An explosion of interest in the numerous therapeutic properties of *Moringa oleifera* over the last decade has led to numerous *in vitro* and *in vivo* trials. Moringa is used for treatment anemia, rheumatism, asthma, cancer, constipation, diabetes, diarrhea, epilepsy, stomach pain, stomachand intestinal ulcers, intestinal spasms, headache, heart problems, high blood pressure, kidney stones, fluid retention and thyroid disorders. Moringa is also used to reduce swelling, increase sex drive (as an aphrodisiac), boost the immune system, and increase breast milk production. Some people use it as a nutritional supplement or tonic. Moringa is sometimes applied directly to the skin as a germ-killer or drying agent. It is also used topically for treating pockets of infection, gum disease, snakebites, warts, and wounds. Oil from moringa seeds is used in foods, perfume, and hair care products. Therefore, because of the great scientific and medical importance of this plant and the limited research that involves the beneficial aspects of ultraviolet radiation. The results exhibited the ability of UV- B radiation to cause a significant increase in such physiological parameters as percentage of callus induction, callus fresh weight and proline concentration, where specific wavelengths can be used to increase the production and improve the quality of important medicinal plants.

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