A Study on The Effect of Different Light Treatment on Some Morphological, Physiological Parameters and Menthol Content of *Mentha Piperita*.

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Abstract: The present study was conducted to assess the effects of Ultraviolet-A (UV-A) 367nm, and White light (W) and ultraviolet+white light (UV-A+W) in addition to control plants in presence of sun light (untreated) on menthol content, some morphological and physiological parameters of *Mentha piperita*. Growth parameters, photosynthetic pigments, total phenol, total indoles, total amino acids, menthol content and DNA were measured. The results showed that a significant increase in height of plants in presence of UV-A irradiation group, as well as increase in total indoles, total amino acids, Whereas UV- A group and UV-A +W group showed a significant decrease in total phenol, leaf area, chlorophyll content, carotenoids concentration and also decreased in menthol content compared to W group and control group respectively.
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Keywords: *Mentha piperita*, Light treatment, Ultraviolet, chlorophyll content, menthol content.

1. Introduction

Peppermint, natural hybrid between Mentha aquatica and Mentha spicata, is one of the most species producing terpenoids. The active constituents of peppermint leaves are about 50% essential oil (meanly menthol), 19% total poly- phenolic compounds, and 7% total hydroxycinnamic **(Duband et al., 1992; Maffei et al., 1999; El-Nagar et al., 2012).** "Peppermint oil contains more than 200 compounds **(Lawrence., 1988)**, most which are monoterpenes and eight step biosynthesis (Menthol), includes a wide spectrum of enzymatic reactions" **(Estepa et al., 2008)**. "The distilled oil is used by the flavoring and pharmaceutical industries for several application" (**Furia et al., 1975; Maffei et al., 1999; Najafi et al., 2012).** "Ultraviolet radiation (UV) is a part of none ionizing region of electromagnetic spectrum which comprises approximately 8-9% of total solar radiation" **(Coohill, 1989; Fredrick, 1993; Hollosy, 2002)**. "The spectrum of UV reaching to the earth surface has been divided into three lower energy; UV-A (320-400nm), which represents about 6.3% of incoming solar radiation and less hazardous part of UV radiation. UV-B (280-320) is particular interest because this wave length represents approximately 1.5% of total spectrum, but can induce a variety damaging effects in plants. UV- C (200-280) nm is extremely harmful to organisms, but not relevant under natural condition of solar irradiation" **(Hollosy, 2002).** UV radiation considered as an important stress factors (especially UV-B) for plants. It damages DNA and it is toxic and mutagenic cell. **(WMO., 1995; Landary., 1997)**. Beside DNA a number of plant molecules such as proteins and lipids strongly absorb UV-B and can induce a specific change in the tissue and whole function **(Caldwell et al., 1989).** Although UV-A has less hazardous part of UV radiation, it has been reported that, UV-A radiation showed some adverse effect on plants **(Krizek., 2004).** "On the other hand, few studies on the effect UV-A radiation on the medicinal plants have been reported. Studies of UV-A responses have been focused mainly on phenyl propanoid synthesis and gene expression" **(Batschauer et al., 1996, Jenkins et al., 1995 ; Christie and Jenkins, 1996).** But little is known about terpene production response to UV-A. Therefore any change in oil chemical composition lead to change in commercial value of essential oil" **(Maffei et al., 1999)**.

The present study aims to investigate the effect of UV-A, W and UV-A+W on the growth parameters, physiological parameters and the active constituent of *Mentha piperita.*

2. Material and Methods

2.1. Plant material and irradiation treatment

In a typical procedure, Rhizomes of *Mentha piperita L.* were transplanted in 16cm plastic pots in control chamber **(Maffei et al., 1999),** Mean temperatures during experimental period were maximum at 39C^{o} and minimum at 23C^{o} and relative humidity was approximately at 51%. Plants were watered every two days regularly to maintain a water regime. The plants were divided into four categories, The first one UV-A group, the second W light group and the third UV-A+W group and fourth one control group. Plants were irradiated for 15 hr/9hr light /dark with an average 15 days **(Parisa et al., 2011)**. Radiation was supplied by two lamps for each group (Luz negra F20/T12 20 watt) for UV-A (PHILIPS 20 watt) for W except plants irradiated with UV-A+W which contain four lamps. TM- 208 solar UV- meters ten mars controlled by quantum version 2 computer in order to evaluate UV-A and W.

2.2. Morphometric measurements

Plant height and total leaf area were record at end of experiment from irradiated plants.

2.3. Photosynthetic pigments content and total indoles

Pigments i.e., chlorophyll a (chla), chlorophyll b (chlb) and caroteinods content were determined according to (Wettstein, 1957). The samples were taken from fresh leaf samples using80% acetone, Total indoles were determined according to (Larsen et al., 1962) using p- dimethyle amino benzaldehyde (PDAB) reagent solution.

2.4. Total phenols content and total amino acids content

Total phenols were calorimetrically determined using Folin-Denis reagent method which described by (Snell and Snell 1953). Total amino acids were determined according to (Moor and Stein, 1954) with reference to argentine stander curve.

2.5. Essential-oil extraction and analysis

After the irradiation, oil was extracted from the plant herb by steam distillation. The essential oils were analyzed Agilent 7000 triple Quad GC Ms.

2.6. Statistical analysis

Data were expressed as mean ± SD. Differences among means were tested for statistical differences by one way analysis of variance (ANOVA). When differences were significant, Data were statistically analyzed using statistical analysis system (SAS, 2006) version 9.0.

2.7. Molecular studies

Inter simple sequence repeats (ISSRs) procedure

PCR reaction was conducted using five primers according to **Williams et al. 1990**.
Their names and sequences are shown in table (1).

Table 1. List of the primer names and their nucleotide sequences used in the study for ISSR procedure.

No.	Primer	Sequence	No.	Primer	Sequence
1	HB10	5` GTGTGTGTGTGTGG 3`	4	HB13	5`CACCACCACGC 3`
2	HB11	5` GAGAGAGAGAGACC 3`	5	HB14	5`GAGGAGGAGGC 3`
3	HB12	5` GTGTGTGTGTGTCC 3`			

3. Results

3.1. Photomorphogenic responses

The UV-A irradiated group showed slight increase in height with respect to UV-A+W group, W light group and control group respectively as shown in figure 1. Plants irradiated with UV-A irradiated group showed a significant decrease in total leaf area with respect to UV-A+W group, W light group and control group respectively as shown in figure 2.

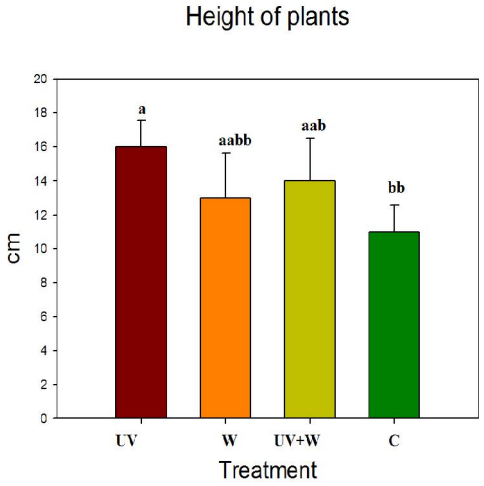

Figure 1. Mean of height for Mentha piperita groups. Data are means of three replications, at 0.05 levels according to t-test. (Means ± SD).

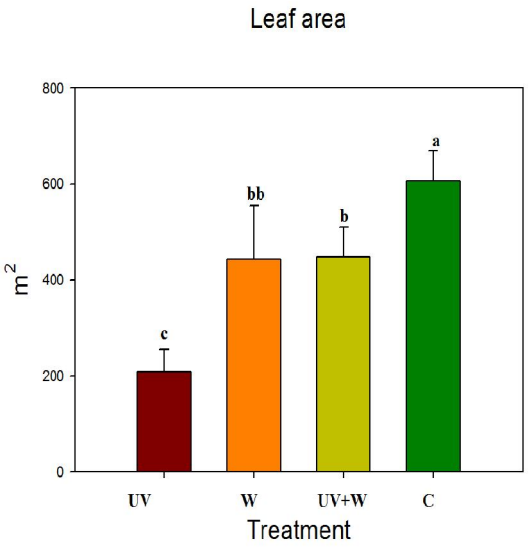

Figure 2. Mean leaf area for Mentha piperita groups. Data are means of three replications, at 0.05 levels according to t-test. (Mean ±SD).

3.2. Pigment content

The content of chlorophyll-a, chlorophyll-b and chlorophyll-T (a+b) showed a significant decrease in presence of UV-A group and UV-A+W group, whereas a significant increase were observed in presence of W light group compared to control group as shown in figure 3. Carotenoids content showed a significant decrease in presence of UV-A group and UV-A+W group, whereas a significant increase were observed in presence of W light group compared to control group as shown in figure 4.

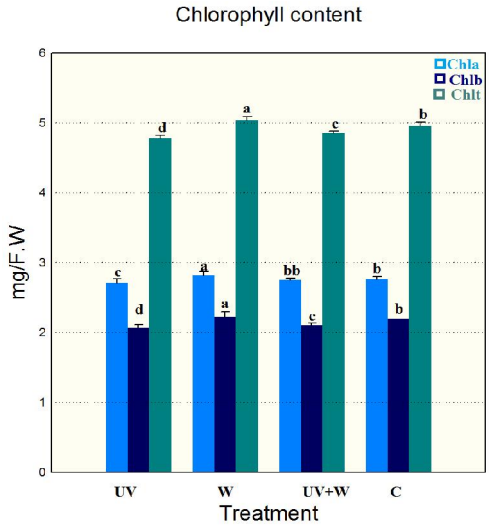

Figure 3. Mean concentration of chlorophyll content for Mentha piperita treated groups. Data are means of three replications, at 0.05 levels according to t-test. (Mean±SD).

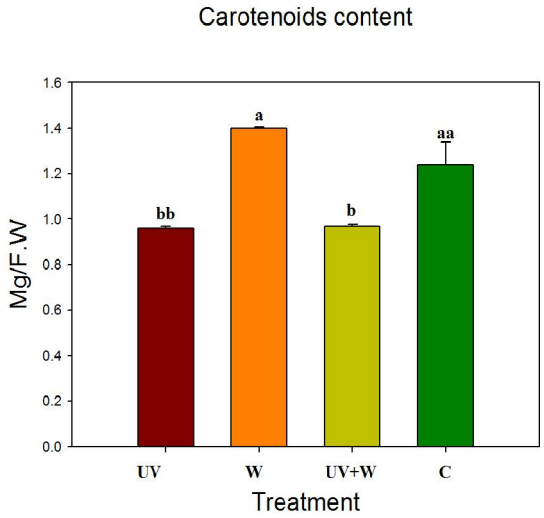

Figure 4. Mean concentration of carotenoids content for Mentha piperita groups. Data are means of three replications, at 0.05 levels according to t-test. (Mean±SD).

3.3. Total amino acids and total indoles content

UV-A irradiated group showed significant increase in total amino acids compared to W group, UV-A+W group and control group respectively (Figure 5). UV-A irradiated group showed significant increase in total indoles, compared to W group, UV-A+W group and control group respectively as shown in figure 6.

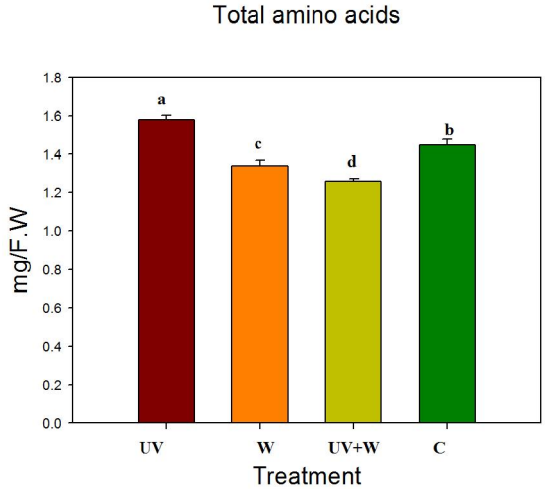

Figure 5. Mean concentration of total amino acids content for Mentha piperita groups. Data are means of three replications, at 0.05 levels according to t-test. (Mean±SD).

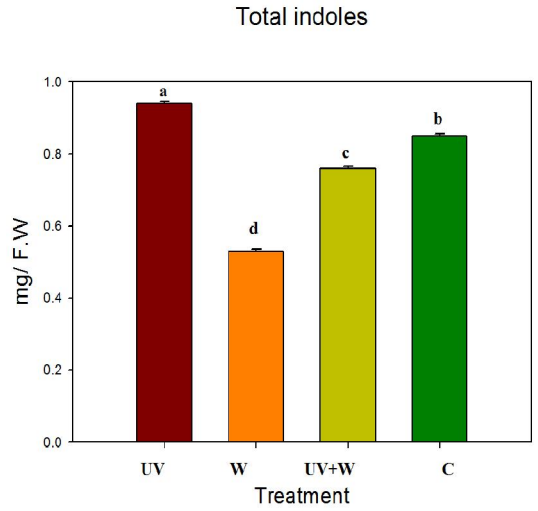

Figure 6. Mean concentration of total indoles content for Mentha piperita treated groups. Data are means of three replications, at 0.05 levels according to t-test. (Mean±SD).

3.4. Total phenolic compounds

Total phenolic compounds showed a significant decrease in presence of UV-A group and UV-A+W group, whereas a significant increase were observed in presence of W light group compared to control group respectively as shown in figure 7.

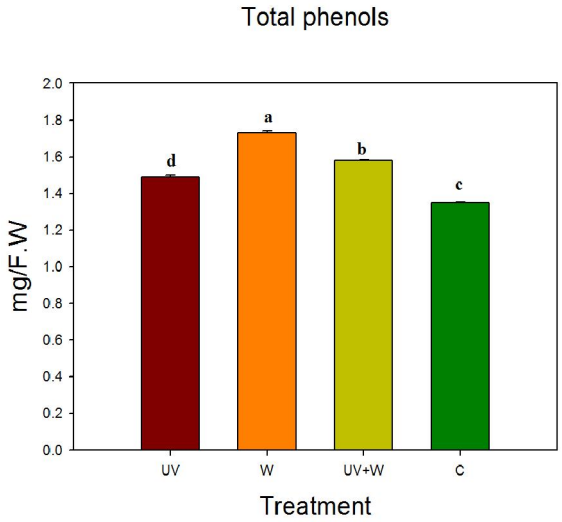

Figure 7. Mean concentration of total phenolic compounds treatment for Mentha piperita groups. Data are means of three replications, at 0.05 levels according to t-test. (Mean±SD).

3.5. Essential oil qualitative variations

UV-A groups showed a significant decrease in menthol content compared to W group, UV-A+W group and control group respectively as shown in figure 8.

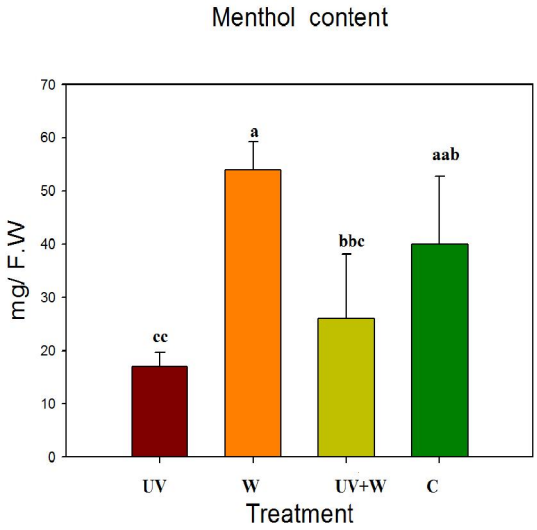

Figure 8. Mean concentration of menthol content for Mentha piperita groups.Data are means of three replications, at 0.05 levels according to t-test. (Mean ± SD).

3.6. Molecular analysis

Identification based on ISSRs

The results are shown in fig (9-13) Primer HB10 resulted in 12 bands with molecular weight from 200 to 115bp. ten bands were monomorphic at 1150, 1000, 985,900, 800, 725, 700, 600, 520 and 370bp. While remaining 2 bands were polymorphic, in which one of them consider as unique band at 300bp. Primer HB11 resulted in 10 bands with molecular weight from 200 to 1050bp. Six bands were monomorphic at 1050, 1020,670,400,300,200 bp, while other four bands were unique bands at 870,770 and 590 bp. Primer HB12 exhibited 13 bands ranging in molecular weight from 280 to 1020bp. nine bands were monomorphic at 1000,830,720,575,560, 495,430, 395 and 370bp. While other four bands were polymorphic which three of them considered as unique bands at 1020,645, 280bp.Primer HB14 resulted in ten bands ranging in molecular weight from 370 to 1200bp.Six bands were monomorphic at 950, 920, 780, 58, 480bp respectively. While other four bands considered as unique bands at 1200, 1150, 700 and 650bp.

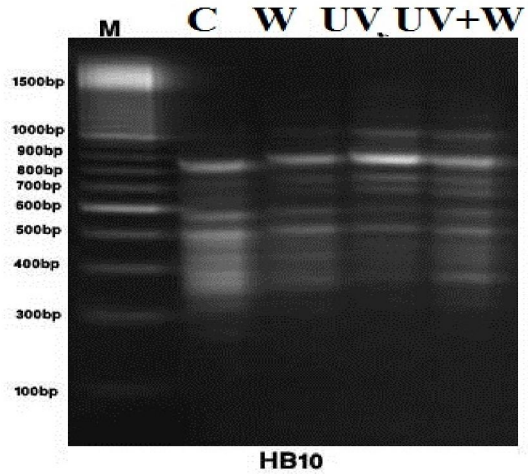

Figure 9. DNA using ISSRs for treatment with primerHB10

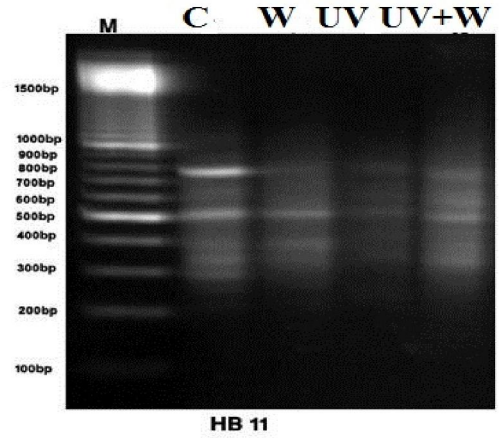

Figure 10. DNA using ISSRs for treatment with primerHB11

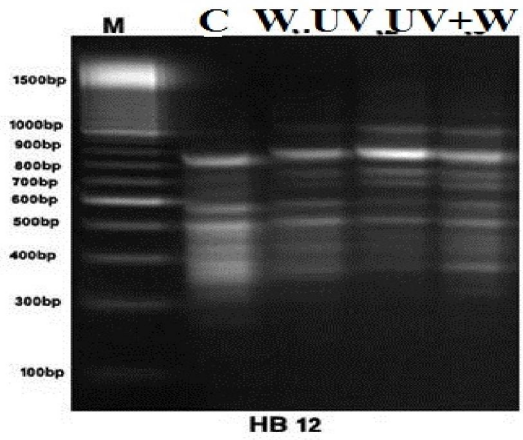

Figure 11. DNA using ISSRs for treatment with primer HB12

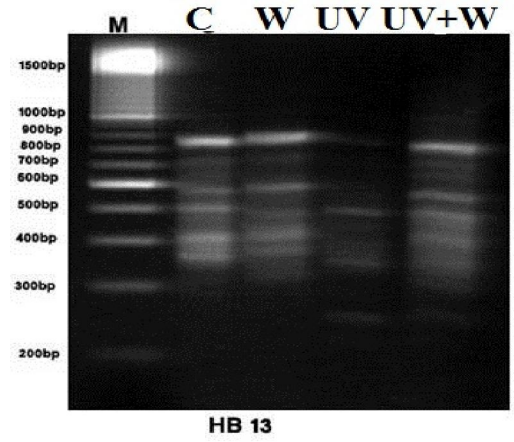

Figure 12. DNA using ISSRs for treatment with primerHB13

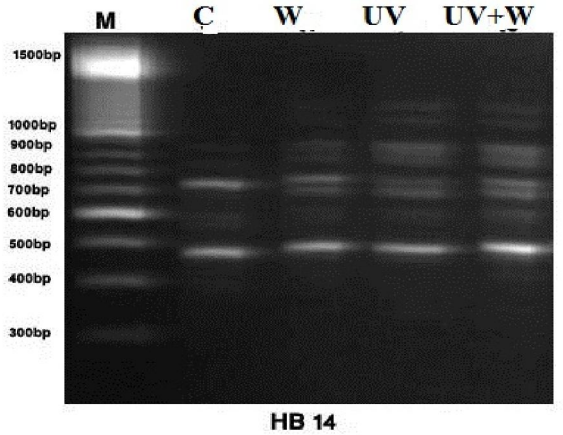

Figure 13. DNA using ISSRs for treatment with primerHB14

4. Discussion

In present investigation, UV-A showed an increase in plant height and reduction in leaf area of peppermint plants compared to control and other treatment. **(Tevini, 1994)** reported that plant growth development are closely related to concentration of some endogenous of plant growth regulators such as IAA. Therefore it is possible that increase or decrease in plant growth is consequence of IAA increase or decrease. Similar changes were found in peppermint plants irradiated with UV-A during night period **(Maffei et al., 1999).** Contrastingly, Blue light irradiation of peppermint caused plant height reduction and no significant difference in total leaf area **(Maffei and Scannerini, 2000).** Contrastingly, **(Parisa et al., 2011)** reported that no significant difference occurred in savory plants irradiated with UV- A. Leaf area showed a significant decrease in plants irradiated with UV-A plants compared to control and other treatment. Reductions in leaf area were found in plants irradiated with UV-B such as

soya bean **(Teramura and Murali, 1986)**, sun flower and cucumber **(Tevini and Teramura, 1989).** The experimental results showed that UV-A caused reduction in content of chla, chlb, chla+b and carotenoids content of peppermint leaves. Similar changes has been reported with previous study which showed that UV-A reduced chla, chlb and chla+b when UV- A was given during night period of peppermint plants. **(Maffei et al., 1999). (Strid et al., 1994)** reported that the lower rates of chlorophyll synthesis resulting from reducing expression gene encoding chlorophyll binding proteins or break down of structural integrity of chloroplasts**.** Carotenoids concentration showed a significant decrease in plants irradiated with UV-A irradiation compared to control plants. It has been reported that, "carotenoids serve a protective function against UV-B radiation **(Rau et al.,1991).** The efficiency in protecting photo system is very important due to their function of efficient quenchers of high energy short wave length radiation. The mechanism by which this accomplished was first proposed to involve a photochemical state change of singlet oxygen to triple form by interaction with carotenoids, removing the potentially dangerous oxygen radical produced in photoxidative process" **(Krinsky, 1979; Mahdavian, 2008).** The result also showed that total phenol content was reduced in plants irradiated with UV-A These results was agreed with previous studies which reported that total phenols was reduced when plants irradiated with UV-A when was given during night. "It is worth mentioning that UV-B can induce in shikmic acid path ways which lead to synthesis of phenolic compounds. The protective function of these compounds against harmful UV-B radiation has been shown in many species, including mutants' deficient either in general pathways of phenyl proponand or flavonids" **(Reuber et al., 1996; Maffei et al., 2000).** The menthol content decreased in presence of UV-A groups compared to other groups. In previous studies, (**Maffei et al., 1999)** reported that "shade-avoidance syndrome caused by night UV-A radiation induced changes in qualitative and quantitative oil composition of peppermint. However, phytochrome is involved in biosynthesis of essential oil component remains to be demonstrated". It has been reported that, UV-B affects terpenoid metabolism of peppermint by inducing changes that leaf senescence. The decrease in menthol depends on menthofuran and methyl acetate increase, even though a significant contribution could be depends up on the accumulation of menthone, the direct precursor of menthol (**Maffei et al., 1999; Croteau, 1987)**.

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