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Using U. V. C Ray for Inducing Resistance Against *Tobacco mosaic virus*

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ABSTRACT

The research discusses the use of flashes of ultraviolet-C rays in inducing resistance against viral pathogens and the possibility of its employment in covered houses or garden nurseries. A number of tomato and tobacco plants of type *Nicotiana tabacum*, uder protected cultivation conditions, were exposed to UV-C rays at wavelengths 200-280 nm using an electronic LED lamp, for 5 and 10 minutes, all plants were inoculated 48 hours after the last exposure to UV rays. The number and size of local lesions were calculated on the leaves of inoculated tobacco plants, as well as monitoring the development of symptoms on tomato plants inoculated with the virus for 12 days of inoculation, and the virus concentration was estimated based on the amount of absorbance at 405 nm by ELISA test. The results indicated that the ultraviolet rays used enhanced the plant's resistance to viral infection through a noticeable increase in the enzyme peroxidase, as it reached (59220 nanometers) in the treated plants compared to the untreated plants that were (28,016 nanometers). The irradiation for the first five minutes had a higher effect than the longer irradiation that lasted for ten minutes, in addition to that the irradiation for intermittent periods and for a short period was better than the continuous exposure for one time. It was found that the leaves far from the radiation exposure area acquired an inducible character of resistance against the pathogen.

Keywords: Tobacco mosaic virus; UV-C; plant's resistance; DAS-ELISA; peroxidase enzyme.

INTRODUCTION

Nowadays, it has become necessary to find alternatives of insecticides or decreasing using them to minimize their damaging effect on the environment. These alternatives might include the chemical inducers of the plant defenses which are currently used on a large scale. Nevertheless, several disadvantages accompany their use, especially their instability under the field conditions. Therefore, tendency towards using the more-stable physical inducers with the possibility of blending them easily with the more-stable with other methods of treatment including the chemical or the biological (Urban et al, 2018). It was observed that the increase of the light intensity is a main result due to the climate changes. The light intensity is considered one of the important factors in photosynthesis and also plays a vital role in the reaction of the plant with the pathogens including viruses. Little is known about the effect of the environmental factors on the spread in the plant and through the seeds in particular. Many hypotheses indicated that light intensity enhances the resistance of the plant against the infection (Ballaré, *et al*., 2011). Thus, the environmental conditions that minimize the virus severity without affecting the plant proliferation enhances the resistance processes.

It was observed that the two types of the Ultra violet ray B and C increase the plant resistance against the pathogens. This ray operates through a light path that activates mitogen, which is responsible for activating the Kinase enzyme in the treated plants and this enzyme is considerably similar to the enzyme produced by several pathogens (Urban *et al*., 2018).

MATERIALS AND METHODS

Ultra violet ray source:

The lamps used were bought from the local market and they include (15) diodes (LEDs) that are characterized with producing 20mW electrical pulses. Plants were exposed to UV ray inside a cooled space (the fridge was used for this purpose) to increase the efficiency of exposure. Using the French made lamps could reach 100 megawatt/cm^2, which equals 1 kilowatt/m^2 (2214 µmol photons m^{-2} s^{-1}) from the surface of the lamp with a window of 5 cm in the bottom of the electrical mixer sphere, as shown in Fig. (1).

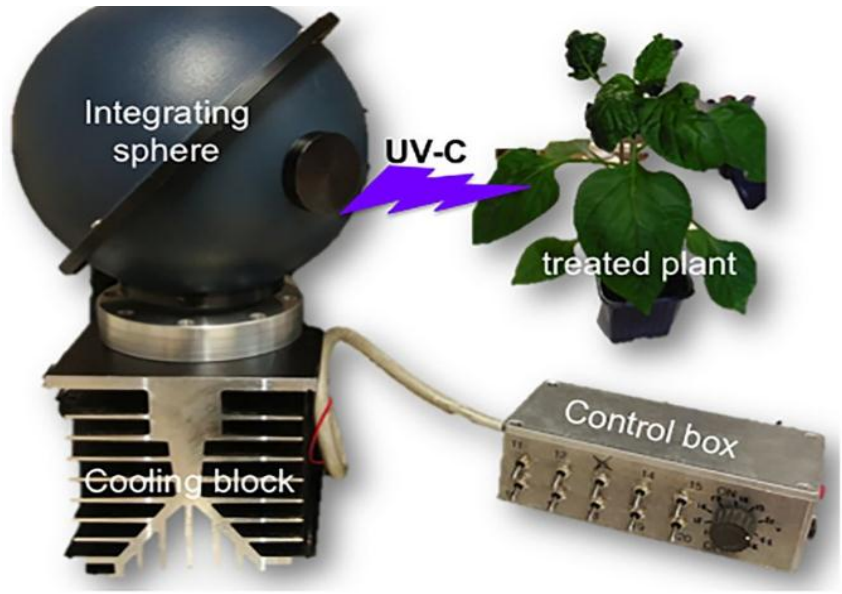

Fig. 1: The device used to provide the plants with the ultra violet ray. The internal part contains 15 UV-C lamps as a circular shape. The 5 cm-radius spherical window is removed and the rays is directed to the leaves straightforward.

Cultivating tobacco and tomato plants:

The test plants were cultivated in plastic house in the Department of Plant Protection, College of Agriculture and Forestry / Mosul University using plastic pots (of 20×20 cm) sterilized by 5% sodium hypochlorite, filled with soil mixed with petmos (a ratio of 2:1) and sterilized by the autoclave. The plants were distributed to each pot (one / two plat per a pot). The plants were sprayed weekly with a fertilizer (N: P: K 20:20:20) 200g/100 liter of water. All the plants were covered with muslin to protect them from insects and sprayed every ten days with the insecticide Confider SL 200 (Imidacloprid) with a ratio of 1.25 ml/liter of water to guarantee the extermination of the insects.

The source of the virus, the preparation, infection and the diagnosis:

Samples of infected leaves of tomato plants showed mosaic symptoms and deformation were collected from tomato fields in AlRasheediya and Wanah districts near Mosul city Fig. (2). The samples were brought to the laboratory and a viral inoculum was prepared from them. Several tobacco plants were inoculated using the mechanical inoculation. The inoculated plants were kept in the greenhouse after taking all the precautions and treatment procedures. Two weeks later, the virus was diagnosed in the plants that showed symptoms using DAS-ELISA kit with polyclonal antibodies, which is supplied by Bio – Reba Swiss Company according to (Clark and Adams, 1977). The results were evaluated by the plate wells tests using UV-9200 Spectrophotometer at the wavelength 405 nanometer.

Fig. 2: The symptoms of mosaic and deformation in the tomato plants infected in the field with tobacco mosaic virus

Estimation of peroxidase activity:

Al-Jarrah's *et al*., (2011) method was employed to estimate the effectiveness of peroxidase enzyme. This method involves collecting samples of tomato and tobacco leaves treated with ultra violet ray for the two periods of exposure, which are inoculated with the virus after 12 days from the last treatment in addition to plants that are infected with tobacco mosaic virus but not treated with the radiation in addition to plants that are not inoculated and not treated with the radiation as two control groups. Leaves were thoroughly washed with distilled water and marked. Then, 0.5 g of the sample leaves were chopped to small pieces and crushed with a ceramic pounder with the presence of the regulating solution of (KH_2PO_4) with a calibration of 0.02 mol/l and a pH of 7.5. the extract was kept in sterile plastic tubes sterile plastic tubes. Samples were distributed in centrifuge tubes with a size of 1.5 ml. The mixture was shaken in a vortex mixer for one minute to achieve the uniformity of the contents and then centrifuged with a speed of 6000 rpm for five minutes. After

that, 3 ml of the reaction mixture consisting of (0.05 mole Guaiacol, 30% Hydrogen peroxide H_2O_2 0.02 mole, and Tris-Base 0.04 mole). The solutions are mixed with a ratio of 1:1:1 with seven parts of distilled water before use. After the reaction mixture is put in the cuvette tube of the spectrophotometer, 0.2 ml of the floating part of the plant extract is added and the sample absorption to the light was estimated by a spectrophotometer type GmbH232-HK at the wavelength 420 nanometer every 30 seconds (6 readings were obtained). The amount of change in absorption relative to the time was calculated according to the equation of (Whitaker and Berhard, 1972): peroxidase effectiveness = $\frac{\Delta A}{\Delta T}$ / g fresh weight (ΔA = change in absorbance, ΔT = change in time)

RESULTS AND DISCUSSION

Activity of ultra violet ray type C in terms of inducing the resistance of tobacco and tomato against tobacco mosaic virus:

Results showed that the causal agent in question was Tobacco mosaic virus according to the results of DAS-ELISA test using polyclonal antibodies. The wells containing the virus yielded a positive result compared to the (positive control infected tissue TMV) and supplied by the manufacturing company. Moreover, no positive reaction with the intact samples and also the extraction solution by the indication of the yellow color on the microtiter plates-Elisa. The results of the treatment of tobacco and tomato plants with UV-C showed that this exposure provided protection against the tobacco mosaic virus, compared to the infected plants which are not exposed to UV ray. As the treatment of exposing the tomato plants to the UV-C for 5 minutes 2 days before the infection resulted in a lower infection percentage (57%) compared to the control treatment with the presence of the virus only, which was (100%). On the other hand, the treatment of exposing to UV-C, two days before the infection, the percentage of infection was lower (66%) compared to the control treatment (Table 1).

When tobacco plants were exposed to UV-C two days before infected with the virus, the number of the local lesions was 13 per square centimeter, which is higher compared to the tobacco plants inoculated with tobacco mosaic virus or the plants unexposed to the radiation as the average number of the local lesions was 60 spots/cm^2 Fig. (3). However, no significant differences for the number of local lesions were observed during the two periods of exposure.

A B

Fig. 3: The effect of UV-C on the number of local lesions on tobacco leaves infected with tobacco mosaic virus. (A): A tobacco leaf infected with the virus and treated with UV. (B): A tobacco leaf infected with the virus, not treated with UV.

Table 1: Percentages of infection and the Peroxidase enzyme absorbance values in tomato plants exposed to the radiation.

Treatment	Infection %	Values of POX absorbance at 420 nm 14 days after infection
Exposing tomato plants to UV-C for 5 minutes and then inoculated with the virus after 2 days.	57	59.220
Exposing tomato plants to UV-C for 10 minutes and then inoculated with the virus after 2 days	66	41.219
The control treatment	100	28.016

Estimation of Peroxidase Enzyme:

The results of peroxidase enzyme effectiveness in the plants of tomato treated with UV-C and infected with tobacco mosaic virus (Table 1) that the level of peroxidase was superior in the treatment with UV-C and for exposure duration of 5 minutes over the treatment of the second exposure for 5 minutes and also over the control treatment. The change in light absorbance/minute/g fresh weight of tomato plants was 59.220 after 14 days of the infection. From the other hand, the change in the control treatment (virus + distilled water) was 28.016, while the change in peroxidase enzyme when treating the tomato with UV-C for ten minutes after 14 days of infection was 41.219 compared to the control treatment.

The result of this experiment indicates that UV-C has the capacity to induce certain counter-materials inside the plant that act against the pathogens that invade their tissues and also induce the plants to produce defensive compounds that fight the pathogen. Huckelhoven *et al.* (1999), Iriti and Faoro (2003) highlighted the role played by these activators in inducing the plant resistance against a wide spectrum of pathogens through various defensive mechanisms such as the oxidative burst or through the secondary metabolism products. Amongst these materials is the peroxidase enzyme as it is considered as one of the proteins related to the defense and it is called PR-9 (Vanloon and Vanstrin, 1999; Xu *et al*., 2016). From the other hand, (Ride, 1975; Kachroo and Robin, 2013) mentioned that the peroxidase contributes in the processes of the manufacture and the precipitation of lignin and hydrogen peroxide which, in turn, strengthen the cell walls against the invasion of the pathogen. Many studies indicated that the activity of this enzyme increases when adding induction factors including the biological and the chemical factors (Shehata and El-Borollosy, 2008; Sabr *et al*., 2013; Hameed and Noor, 2018).

The results confirm the observations indicated by Charles *et al*., (2008) and Windram *et al*., (2012), that shows that UV of either types B or C, under different durations of exposure can stimulate the plan defenses. Although only little is known about the mechanism of paths, the signals of assimilation and the hormone doses stimulated by the ultra violet ray (Urban *et al*., 2018), it can be assumed that the high energy levels provided by UV ray resulted in the production of types ROS either via the photosynthesis mechanism in the green plastids and also increasing the level of NADP (H) oxidase on the plasma membranes, or increasing the activity of the Zanthin oxidase in the peroxisomat and increasing the activity of NADP-malic in the mitochondria (Megeroy *et al*., 2010). Although the antioxidants, the anti-oxidant enzymes and the anti-oxidant systems that are present in all the parts of the cell are dealt with in full efficiency in general, but they can be in the essence of the oxidized signals and this leads to inducing of regulating the metabolic signals and paths to produce the secondary defense compounds (Jiang *et al*., 2012). UV-B and UV-C upregulated ROS accumulation, and secondary metabolite production such as phenolic compounds. In addition to that, the direct damage of fats or the oxidation by ROS resulting from the oxidations of Linolic acid products, that act as to create the Jasmine acid and it is a hormone playing important roles in plant responses to biotic stressors (Xu *et al*., 2016). Also, the hypothesis, which stipulates that producing reactive oxygen species (ROS), the photo receptors can contribute with it especially the proteins and URV8. The second type of protein has an evident and approved role in determining its path by the ultra violet ray. In addition, UV-C light was it was found, recently, to alleviate transcriptional gene silencing in *Arabidopsis* an indicator that UV-C light has

epigenetic (Conrath *et al*., 2015, Ramirez-Prado *et al*., 2018). Therefore, a considerable attention should be given in the future to such effects of the ultra violet ray taking into consideration that there are many evidences about the role epigenetic mechanisms play in controlling the immunity of the plants.

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استخدام الأشعة فوق البنفسجية نوع – سي لاستحثاث المقاومة ضد فايروس موزائيك التبغ

الملخص

يناقش البحث استخدام ومضات من الاشعة فوق البنفسجية نوع – سي في استحثاث المقاومة ضد المسببات المرضية الفايروسية وامكانية توظيفها في البيوت المغطاة او المشاتل البستنية، حيث تم تحت ظروف الزراعة المحمية تعريض عدد من نباتات الطماطة والتبغ نوع*Nicotiana tabacum* الى اشعة فوق بنفسجية نوع سي UV-C عند الاطوال الموجية 200−280 نانوميتر باستخدام جهاز مصباحي الكتروني LED ولمدتي 5 و10 دقيقة، تم تلقيح جميع النباتات بعد 48 ساعة من اخر تعريض للأشعة فوق البنفسجية. تم حساب عدد البقع الموضعية وحجمها على اوراق نباتات التبغ الملقحة، وكذلك مراقبة تطور الاعراض على نباتات الطماطة الملقحة بالفايروس لمدة 12 يوم من التلقيح وقدر تركيز الفايروس بالاعتماد على مقدار الامتصاصية عند 405 نانوميتر باختبار الاليزا. أشارت النتائج أن الاشعة فوق البنفسجية المستعملة عززت من مقاومة النبات تجاه الاصابة الفايروسية وذلك من خلال الزيادة الملحوظة بأنزيم البيروكسيديز، إذ بلغ في النباتات المعاملة (59220 نانوميتر) مقارنة بالنباتات المصابة وغير المعاملة والتي كانت (28,016 نانوميتر). وان التشعيع للدقائق الخمس الاولى كان أعلى تأثيرا من التشعيع الأطول الذي استمر لعشر دقائق، فضلا عن أن التشعيع لفترات متقطعة ولمدة قصيرة كان أفضل من التعريض المستمر لمرة واحدة. وجد أن الاوراق البعيدة عن منطقة التعريض الاشعاعي اكتسبت صفة إستحثاثية للمقاومة ضد المسبب المرضي.

الكلمات الدالة: فايروس موزائيك التبغ، الاشعة فوق البنفسجية نوع سي، مقاومة النباتات، فحص الاليزا، الانزيمات البيروكسيدية.