

Fungicidal Effect of UV-C Light on Fungi Causing Root Rot/Wilt of Sage (*Salvia officinalis* L.)

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Abstract

One of the most significant ornamental and medicinal plants in the world is sage (*Salvia officinalis* L.). It is susceptible to a wide range of diseases, including root rot brought on by pathogens including *Macrophomina phaseoli*, *Rhizoctonia solani*, *Fusarium solani*, and others that have been identified by multiple studies. Due to the nontraditional nature of the crop, root rot is a serious disease that results in significant loss and little is known about the relationship between the pathogen(s) and its management. Therefore, the current inquiry was conducted to identify the pathogen(s) responsible for the root rot/wilt symptoms and to test if UV-C application may be used to treat this disease. It has been shown that the disease is spread throughout all trial fields in the several districts of the Minia governorate. Twenty-three isolates of fungi, belonging to seven different genera, were obtained from naturally infected samples of sage. The most dominant fungi were *Fusarium solani*, *M. phaseolina*, *Rhizoctonia solani*, which have proved their ability to infect sage plants inducing root rot/ wilt symptoms. The mycelial growth of these fungi was reduced gradually when exposed to UV-C radiation, the highest inhibition was recorded when mycelium was exposed to UV-C radiation for 120 seconds. Immersed cuttings of sage in pre-treated mycelium of these fungi with UV-C led to reduce the percentages of disease incidence and severity. Also, when cuttings of sage were exposed to UV-C for different periods of exposure, the disease incidence and severity were reduced more than mycelial treatment.

Key words: Sage, *Salvia officinalis*, wilt/root rot, UV-C radiation.

Introduction

Sage (*Salvia officinalis* L.) is an ornamental and medicinal herb plant belonging to the family *Lamiaceae*, native of Mediterranean countries and cultivated in Northern Africa and in the temperate zones of Europe (Skoufogianni et al., 2017). It is subjected to infection with many different fungal pathogens such as *Oidium erysiphoides*, *Ascochyta vicina* and *Phoma salvia*, *Peronospora lamii*, *P. swinglei*. Seedling damping off, root rot and wilt are considered as serious malady diseases that cause considerable losses in production and quality of sage in many world areas. These diseases were reported induced by several soilborne pathogens such as *Fusarium oxysporum*, *F. solani*, *Fusarium culmorum*, *F. moniliforme*, *Rhizoctonia solani* and *Alternaria alternata*. Root rot/ wilt diseases represent one of the main reasons for yield loss in different crops, especially root rots caused by *Fusarium solani*, *Rhizoctonia solani* and *Macrophomina phaseolina*, frequently observed in sage either alone or in combination. Solar ultraviolet (UV) radiation comprises three classes: UV-A (320-400 nm), UV-B (280-320nm) and UV-C (200-280 nm). The most harmful effect of UV on the living organisms increases toward shorter wavelengths (Barta et al., 2004). The radiation of ultraviolet (UV) directly affects both green plants and pathogenic agents, and the species interaction between them. The distinct bands of UV radiation (A, B and C) have different effects on the hosts and their associated pathogens. Generally, ultraviolet A and C mainly affect phototropism and morphogenesis, but UV-B and UV-C strongly trigger secondary metabolite compounds production. The UV-B and C radiation short waves (200-320 nm) negatively affect plant pathogens either direct or indirect. DNA damage, protein polymerization, enzyme inactivation and increment cell membrane permeability are considered as direct effects. UV-C is the most energetic radiation and is thus more effective at lower doses to kill microorganisms, but also

often causes plant damage (Vanhaelewyn et al., 2020). While indirect effects can be due to UV-B and UV-C up-regulated reactive oxygen species (ROS) and secondary metabolite production such as phenolic compounds accumulation and ultraviolet-B specific pathways such as the UVR8-dependent up-regulated defense responses in plants. Chloro-fluoro-carbons compounds, which used in many cooling instruments, i.e., refrigerators, air-conditions, deep freezers, and others, have the potential to cause a depletion of ozone layers, which is responsible for the attenuation of solar UV radiation reaching the surface of the earth and living organisms, has been proposed more than forty-five years ago (Molina and Rowland, 1974). Ozone layer depletion is closely related to an increase in UV-B radiation on surface of the earth (Kerr and McElroy, 1993). Changes of environmental factors, including UV radiation, may affect metabolic and pathological changes in plants (Salama et al., 2011). UV-C (245 nm) radiation is considered as a germicide and plays a major role in selecting for fungi that dominate the mycobiota of drying crops. Valero et al., (2007) reported that the UV-C irradiation of harvested grapes prevented germination of fungal contamination during storage or further dehydration, i.e. germination of *Aspergillus carbonarius* and *Alternaria alternata* conidial spores was reduced by 25%, whereas germination of *A. niger*, *Cladosporium herbarum* and *Penicillium janthinellum* spores was reduced greater than 70% when exposed for 10 seconds to UV-C. Significant reduction in root infecting fungi, *Macrophomina phaseolina* (Tassi) Goid, *Rhizoctonia solani* Kühn and *Fusarium* spp were recorded when groundnut (*Arachis hypogaea* L.) and mung bean [(*Vigna radiata* (L.) Wilczek] seeds were exposed to UV-C irradiation for 5-60 minutes times (Siddiqui et al, 2011). Özçelik (2007) reported that exposure of some fungal and bacterial species to UV light (254 nm) for 45 min was enough to inactive of yeast

like fungi and bacteria. Under the same conditions, fungicidal effects against molds were observed in 75 min in all surfaces used; microscope slide, aluminum foil, coin and solid medium, except for the medium surface. Increased doses of UV-B or UV-C irradiation decreased the colony counts of *Trichophyton rubrum* and *T. mentagrophytes*, but the size of colonies was dependent on the irradiation type and dose (Nematollahi et al., 2015). Conidial germination of *G. citricarpa* and formation of appressoria were affected by exposure to UV-C irradiation for different doses (Canale et al. 2011). The vegetable characters and yield of onion transplants (Giza 20 cv.) exposed for 15 min. to UV-B (280-320 nm) and cultivated under salt stress conditions were improved and enhanced plant tolerance to salinity by increasing antioxidants, oxidative enzymes and organic osmolytes, i.e., free amino acids, reducing sugars and total sugars (Abdellatif and Al-Senosi, 2018).

During 2018-2019 winter season, many seedlings and plants of common sage (*S. officinalis* L.) were observed sever from infection with damping off and root rot/wilt in many commercial private farms at different countries belonging to Minia governorate (Middle Egypt). This study aimed to isolate and identify the pathogens associated with these symptoms, to evaluate the *in vitro* effects of UV-C irradiation on these pathogens' development and on the control of sage root rot/wilt.

Materials and Methods

1- Survey of sage root rot/wilt different districts of Minia

Root rot/ wilt survey of sage was carried out in major sage cultivation villages in Maghagha (Abbad Sharouna) and Deyr Mawas (Dalga) districts, Minia Governorate during 2018–2019 winter season. Surveyed plants were 3 years old. Based on the farmer information, in each

village, two fields were surveyed at random. In each field, 100 plants were selected at five locations, four corners and one at the bottom.

The root rot/wilt incidence and severity of diseased plants were recorded. Disease incidence (%) was calculated according to the following formula: Percent disease incidence (PDI)= (Number of infected plants / Total number of plants) X 100

Disease severity percent (DS, %) of root rot/wilt was assessed according to Rowe (1980) using a rating scale of 0 to 5 on the basis of root discoloration and foliar symptoms as follows: 0, neither root discoloration nor leaf yellowing; 1, 1~25% root discoloration or one leaf yellowed; 2, 26~50% root discoloration or more than one leaf yellowed; 3, 51~75% root discoloration plus one-two leaves wilted; 4, up to 76% root discoloration or more than three leaves wilted; and 5, completely dead plants. Disease severity index (DSI) was calculated as described by Liu et al., (1995) as follows: $DSI = (\sum d / d_{max} \times N) \times 100$

where “d” is the disease rating possible, “d max” is the maximum disease rating, and “N” is the total number of plants examined in each replicate.

Natural infected sage plants showing root rot and wilt symptoms were collected from private commercial farms in Maghagha, Matai, Mallawi, Deir Mawas and Minia districts, Minia governorate, Egypt, for 2018-2019 winter season. Isolated pathogens were purified and identified on the basis of their morphological, cultural and microscopically characteristics according to Gilman (1957), Booth (1971), Barnett and Hunter (1972), Domsch et al. (1980), Ismail et al. (2015) and Koşar et al. (2021) and was further verified by Division of Fungal Taxonomy, Plant Pathology Research Institute, ARC., Giza. Egypt. and by Division of fungal classification, Faculty of Science, Assiut University, Egypt.

2–Pathogenicity tests

Pathogenicity trials with the isolated fungi were carried out in the greenhouse of Plant

Pathology Department, Faculty of Agriculture, Minia University, using cuttings of common sage (*S. officinalis*), 2 years old, obtained kindly from Ornamental Plants Division, Horticulture Department, Faculty of Agriculture, Minia University. Surface sterilized cuttings of sage were planted in clay pots (30 cm. in diameter) containing sterilized Nile Loamy-clay soil (about 4 kg/pot) infested with individual different fungi. Pots and soil sterilization was carried out (15 days before soil infestation) by autoclaving the soil for two hours at 2 kg/cm³ pressure and dipping the pots in 5% formalin solution for 5 minutes, then soil was serrated, and pots was aerated for 15 days before being used. The basal portions of sage cuttings were surface sterilized (to exclude the probability of accidental infection with saprophytic rotted organisms) by dipping in mercuric chloride (0.2%) solution for two minutes then washed several times by sterilized water before planting them in the prepared pots. Inocula of the isolated fungi were prepared, separately, on sterilized barley grains (150 g of grains + 200 ml water/ 1000-ml Erlenmeyer flask). Inoculated flasks were kept at 25±2°C for 15 days then used for soil infestation. Soil infestation was applied one week before planting, by thoroughly mixing 2% of the inoculum, representing a barley culture of one fungus, with the soil in each pot. The infested soil was irrigated daily till planting. Three replicates, each consisting of three pots and 4 cuttings of sage were grown per pot, were used in each treatment. Sterilized and uninoculated barley medium was used in the check treatment. The pots were watered when necessary. Regularly, plants were examined for disease symptoms, but final results were recorded 30 and 90 days after planting by recording the number of diseased plants then the percentages of infected plants (DI%) and DS%) were calculated as described before in survey experiment. Re-isolation was carried out from diseased cuttings to satisfy Koch's postulates. Isolates Fs3, Rs9 or Mp11 of *Fusarium solani*,

Rhizoctonia solani and *Macrophomina phaseolina*, respectively, which proved the highest sage root rot/wilt incidence and severity in pathogenicity tests, were chosen to complete other experiments.

3- Effect of UV-C radiation on fungal growth *in vitro*

Mycelia of *Fusarium solani*, *Rhizoctonia solani* or *Macrophomina phaseolina* were collected from seven-days-old cultures by flooding the surface of PDA cultures with sterile water at room temperature. The suspensions were gently swirled to dislodge the mycelia, which were transferred to sterile 50 ml Falcon tubes. The fungal mycelia were diluted using sterile distilled water to a concentration of 2x10⁵ CFU ml⁻¹ and aseptically transferred to sterile Petri plates. The fungal mycelia suspensions in the Petri plates (without the lids to prevent shielding) was subjected to UV light using a Philips TUV 15W G158T8 UV-C long life, Holland, lamp, which was placed at a distance of 25–30 cm from treated fungal mycelia. The applied distance is usually used in commercial fields, *i.e.* sterilization dental tools, scissors, spatula etc. All UV irradiations were performed in a custom-built UV chamber at a wavelength of 254 nm for different time intervals (0, 30, 60, 90 and 120 seconds). For each time interval, only three plates (a replicate) with fresh diluted fungal suspension were placed in the chamber for the required irradiation time; this was done to prevent any light from reaching the plates during transfer. Treated fungal mycelia were kept in the dark during and after UV treatment for at least one hour to prevent photo-reactivation. Following the exposure, UV-treated fungal mycelia were serially diluted and plated on PDA plates containing 0.05% (w/v) Tween 80 as a colony restriction factor, which caused the fungus to grow in small colonies (García-Cela *et al*, 2015). Surviving fungal mycelia developed into small colonies that were picked, transferred to PDA plates and incubated at 25°C. The experimental design was completely

randomized with three replications (one plate/set). UV untreated fungi were used as control. The linear growth was determined after 7 days, and the percentages of the growth inhibition were calculated.

4- Effect of UV–C radiation on Root rot/ wilt disease *in vivo*

4-1–The ability of previously UV-C treated fungi to induce root rot/wilt on artificial inoculated sage cuttings

The effect of UV-C treatment on the pathological ability of previously exposed fungi to induce root rot/wilt disease was studied. Healthy cuttings of sage, obtained from one year old plants, were used in these tests. Pot experiments were laid out in the open field of Experimental Farm of Plant Pathology Department, Faculty of Agriculture, Minia University, during the two successive growing seasons of 2019 and 2020. Sterilized clay pots, 30 cm in diameter, were filled with disinfected Nile Loamy soil and cuttings were surface sterilized by using sodium hypochlorite (2%) and then washed several times with sterilized distilled water.

Previously UV-C treated *F. solani* (isolate Fs3), *R. solani* (isolate Rs9) and *M. phaseolina* (isolate Mp11) mycelia were harvested, cleaned from the substrate with a sterilized spatula and distilled water. Mycelium was diluted using distilled sterilized water, adjusted to 2×10^5 CFU ml^{-1} by microscopic examination with a cell-counting hemacytometer. Surface sterilized cuttings of sage were immersed in fungal suspensions for one hour, then immediately cultivated in disinfected soil. A completely randomized design was applied with three replications, and three pots each consisting of 5 cuttings grown in each pot, were used in each treatment. Sterilized and untreated cuttings were used in the check treatment. The pots were watered when necessary. Percentages of disease incidence and severity were recorded, 30 and 90 days after planting as described in pathogenicity tests.

4-2–Using UV-C radiation as an alternative tool to control root rot/wilt of sage

This experiment was carried out in 2021 and 2022 seasons. Cuttings of sage were divided into three groups; the first and second ones were exposed to UV-C radiation (254 nm) from an artificial source (lamp of TUV 15W G158T8 UV-C long life, Holland Philips special) for 90 and 120 seconds which was situated at 25-30 cm over the cuttings. The treated cuttings were stored in complete darkness for 24 h to minimize any photoreaction processes. The other third number of the cuttings was subjected to normal light tubes 60W, was used as control treatment (without UV-C supplement). Sterilized pots, 30 cm in diameter, were filled with disinfected Nile Loamy soil, were artificially inoculated with *F. solani* (isolate Fs3), *R. solani* (isolate Rs9) or *M. phaseolina* (isolate Mp11) as described before in pathogenicity tests. The pots were irrigated with tap water for one week, then, the UV-C treated cuttings and non UV-C treated ones were sown in the previous three main groups of pots. Four cuttings were planted per pot. Disease incidence and DS were calculated, 30 and 90 days after sowing as described before. The experiment was repeated in two successive winter seasons, 2021 and 2022. Completely randomized design was applied with three replicates, and three pots each consisting of 5 (4) cuttings, were used in each treatment.

Statistical analysis

Data of all treatments were arranged and presented as means from three replicates. The experimental designs of all experiments were completely randomized. Data were statistically analyzed for significance in Statistix (8th edition, Analytical Software, USA, **Steel et al., 1997**) using analysis of variance (ANOVA). Significance between means was compared by Duncan's multiple range test at $p < 0.05$ probability according to the method of **Gomez and Gomez (1994)**.

Results

1. Survey for the incidence and severity of root rot/wilt of sage in Minia governorate, Egypt.

Root rot/ wilt survey of sage was carried out in major sage cultivation villages in Maghagha (Abbad Sharouna) and Deyr Mawas (Dalga) districts, Minia governorate during 2018–2019 winter season.

Data presented in Table (1) show that sage root rot/wilt is distributed in different fields under experimental areas (with average of 13.15% DI and 7.8% DS, %). The percentages

of disease incidence ranged between 7.0 and 17.8%. The highest DI% was shown in Dagla village (17.8%). The DS ranged between 3.44 and 9.44%. The highest severity of sage wilted plants (9.44%) was recorded in Dagla village, whereas the lowest one (3.44%) was observed in Abbad Sharouna village. Most of the diseased plants suffer from early wilt symptoms, including weakened growth and yellowing, whereas a few numbers of them, in of the fields which were visited, were severe from wilt symptoms.

Table (1): Root rot/wilt incidence and severity on 3 years old plants, in Minia Governorate, during 2018–2019 winter season.

Villages	Fields	No. of Inf. Pl.	% infected plants	% DS
Abbad	1	35 ^{*)}	7.00	3.44
Sharouna	2	42	8.4	4.92
	Mean	38.5	7.7	4.18
Dalga	1	76	15.8	8.52
	2	89	17.8	9.44
	Mean	82.5	16.5	8.98
Average		59	13.15	7.8

*) Each reading is an average of 500 samples.

2–Isolation and frequency of fungi associated with root rot/wilt of sage

Twenty-three isolates of fungi, belonging to eight different species, viz. 7 isolates of *Fusarium solani*, 3 isolates of *Rhizoctonia solani*, 4 isolates of *Macrophomina phaseolina*, *Sclerotium* sp. (3 isolates), two isolates of each *Aspergillus flavus* and *Rhizopus stolonifer*, and one isolate of either *Alternaria alternata* or *Mucor* sp. (Table 2) were isolated from naturally infected samples of sage. The most dominant fungus was *Fusarium solani* which presented the highest frequency (30.44%), followed by *M. phaseolina* (17.4%). Whereas each of *Rhizoctonia solani* and *Sclerotium* sp. presented 13.05%. The highest frequency of fungi was isolated from Abbad Sharona village (34.80%), followed by Farm of Fac. Agric., Minia University (30.43 %) and Dalga village (21.23%), whereas the lowest frequency (4.45%) was recorded in Matai district (Table 3). *Fusarium solani* and *R. solani* were isolated from infected plants collected from Abbad

Sharona and Farm of Fac. Agric. (Maghagha and Minia districts, respectively). *Macrophomina phaseolina* was isolated from samples obtained from Mallawi (Farm of Research Station, ARC), Abbad Sharouna and Dalga, whereas *Sclerotium* sp. was isolated from Abbad Sharouna and Dalga.

Pathogenicity tests

Data in Table (4) indicated that the highest disease incidence and severity, 30 days after sowing, were recorded when soil was infested with *R. solani* (33.33; DI% and 25.18%; DS%), followed by *F. solani* (27.5 and 19.67%) and *M. phaseolina* (28.47 and 18.06). Whereas the lowest percentages of DI% and DS% were recorded with *Mucor* sp. (2.78 and 1.11%, respectively). After 90 days from sowing, *R. solani* induced 68.52% DI, and 48.78% DS, followed by *F. solani* which caused 61.90 and 36.19% then *M. phaseolina* (59.72 and 37.78%). *Aspergillus flavus*, *A. alternata*, *Sclerotium* sp., *R. stolonifer* and *Mucor* sp. caused DS ranged between 1.11 and 5.0%.

Rhizoctonia solani caused infection on sage 44.44–91.67% DI% and 35.56–74.44% DS%, While *Fusarium solani* induced infection ranged between 44.44–94.44% DI and 22.78 and 67.22% DS%, followed by *M. phaseolina* which caused 44.44–86.11%, DI% and 16.67–38.89% DS. Isolates Fs3, Rs9 and Mp11 of *Fusarium solani* (isolate Fs3) and *Rhizoctonia solani* (isolate Rs9) and *M. phaseolina*, which were highly aggressive and induced the highest percentages of sage infection either 30 or 90 days after sowing, ranged between 86.11–

94.44 DI% and 67.78–74.44% DS%, 90 days after sowing, were chosen to carry out the other experiment.

The lowest percentages of surviving plants, 90 days after sowing, were infected with *R. solani*, *F. solani* and then *M. phaseolina* (31.48, 38.10 and 40.25%, respectively). The percentages of surviving plants ranged between 88.89 and 97.22% in plants inoculated with the rest of pathogens.

Table (2): Locality and frequency of isolated fungi, from sage diseased plants.

Fungi	No. of isolates	Code of isolates	Locality of sample		Frequency %
			District	Village	
<i>F. solani</i>	5	Fs1-Fs5	Minia	Farm of Fac. Agric.	21.74 ⁽¹⁾
<i>F. solani</i>	2	Fs6-Fs7	Maghagha	Abbad Sharona	8.70
<i>R. solani</i>	2	Rs8 & Rs9	Maghagha	Abbad Sharona	8.70
<i>R. solani</i>	1	Rs10	Minia	Farm of Fac. Agric.	4.35
<i>M. phaseolina</i>	1	Mp11	Maghagha	Abbad Sharona	4.35
<i>M. phaseolina</i>	2	Mp12 & Mp13	Mallawi	Farm of Research Station, ARC.	8.70
<i>M. phaseolina</i>	1	Mp14	Dayr Mawas	Dalga	4.35
<i>Sclerotium</i> sp.	2	Ss15 & Ss16	Maghagha	Abbad Sharona	8.70
<i>Sclerotium</i> sp	1	Ss17	Dayr Mawas	Dalga	4.35
<i>Aspergillus flavus</i>	1	Af18	Matai	Matai	4.35
<i>Aspergillus flavus</i>	1	Af119	Dayr Mawas	Dalga	4.35
<i>Alternaria alternata</i>	1	Aa20	Maghagha	Abbad Sharona	4.35
<i>Rhizopus stolonifer</i>	2	Rs21 & Rs22	Dayr Mawas	Dalga	8.70
<i>Mucor</i> sp.	1	Ms23	Minia	Farm of Fac. Agric.	4.35

(1) Each figure represents the percentage of isolates in relative to the whole isolated fungi.

Table (3): The numbers and percentages of isolated fungi according to locations

Location or Village	Number of different isolated fungi					Percentage s of isolated fungi
	<i>F. solar</i>	<i>R. solan</i>	<i>M. phaseoi</i>	<i>Sclerotium</i>	others	
Farm of Fac. Agric., Minia	5	1			1	30.43
Farm of Research Station, ARC at Mallawi			2			8.70
Abbad Sharouna	2	2	1	2	1	34.80
Dalga			1	1	3	21.23
Matai					1	4.35
Total	7	3	4	3	6	99.60%

Table (4): Pathogenicity test of sage root rot/wilt induced by isolated fungi.

Fungi	Isolate's code	30 days after transplanting		90 days after transplanting		Survival plants (%)
		DI (%)	DS (%)	DI (%)	DS (%)	
<i>F. solani</i>	Fs1	36.11bc*	28.89c	61.11cd	37.38b	38.89def
<i>F. solani</i>	Fs2	30.56cd	20.56de	72.22bc	39.44b	27.78fg
<i>F. solani</i>	Fs3	52.78a	49.44a	94.44a	67.22a	5.56h
<i>F. solani</i>	Fs4	19.44e-g	8.89g-j	52.78ef	30.00bc	47.24cd
<i>F. solani</i>	FS5	22.22d-f	11.11gh	52.78ef	22.78cd	47.22cd
<i>F. solani</i>	Fs6	16.67e-h	9.44ghi	55.56def	28.89bcd	44.44cde
<i>F. solani</i>	FS7	5.56ij	2.78igk	44.44f	27.22bcd	55.56c
	Mean	27.5	19.67	61.90	36.19	38.10
<i>R. solani</i>	Rs8	36.11bc	26.67cd	69.44cd	35.56bc	30.56ef
<i>R. solani</i>	Rs9	52.78a	43.89a	91.67a	74.44a	8.33h
<i>R. solani</i>	Rs10	13.89f-i	6.11g-k	44.44f	36.11b	55.56c
	Mean	33.33	25.18	68.52	48.7	31.48
<i>M. phaseolina</i>	Mp11	44.44ab	36.11b	86.11ab	67.78a	13.89gh
<i>M. phaseolina</i>	Mp12	30.56cd	18.33ef	58.33c-f	38.89b	41.67c-f
<i>M. phaseolina</i>	Mp13	25.00de	12.22fg	50.00ef	27.78bcd	50.0cd
<i>M. phaseolina</i>	Mp14	19.44e-g	9.44ghi	44.44f	16.67de	55.56c
	Mean	28.47	18.06	59.72	37.78	40.28
<i>Sclerotium</i> sp.	Ss15	11.11g-j	5.56g-k	11.11gh	3.33f	88.89ab
<i>Sclerotium</i> sp.	Ss16	8.33h-j	3.89igk	11.11gh	5.00ef	88.89ab
<i>Sclerotium</i> sp.	Ss17	13.89f-i	11.11gh	19.44g	4.44ef	80.56b
	Mean	12.96	7.04	13.89	4.26	86.11
<i>Aspergillus flavus</i>	Af18	5.56ij	2.78igk	5.56gh	1.67f	93.44ab
<i>Aspergillus flavus</i>	Af119	8.33h-j	3.33igk	8.33gh	3.33f	91.67ab
	Mean	6.95	3.06	6.94	2.50	92.55
<i>Alternaria alternata</i>	Aa20	8.33h-j	5.00h-k	8.33gh	1.67f	91.67ab
<i>Rhizopus stolonifera</i>	Rs21	5.56ij	3.33igk	5.56gh	1.11f	94.44ab
<i>Rhizopus stolonifera</i>	Rs22	5.56ij	2.22jk	5.56gh	2.22f	94.44ab
	Mean	5.56	2.78	5.56	1.67	94.44
<i>Mucor</i> sp.	Ms23	2.78j	1.11k	2.78h	1.11f	97.22a
LSD 5%		9.9606	7.0448	14.753	13.313	14.753

Each figure represents the average of three replicates, each consisting of 12 cuttings.

(*) Columns with the same letters in the same category are not significantly different from each other.

3–Effect of UV-C radiation on mycelial growth of the pathogens isolated from sage root rot/ wilt

Data resented in Figure (1) indicated that mycelial growth of different three fungi tested; *Fusarium solani* (isolate Fs3), *Rhizoctonia solani* (isolate Rs9) or *Macrophomina phaseolina* (isolate Mp11), which were exposed to different periods of exposure to UV-C, was affected with UV radiation comparing with the control. The inhibition of growth ranged between 5.22–17.1%, 42.22–58.5, 73.0–77.4 and 73.3–81.33% when fungi

were exposed to UV-C for 30, 60, 90 and 120 sec, respectively. The percentages of growth inhibition increased gradually with increasing the time of UV–C exposure, whereas the highest percentages of fungal growth inhibition were recorded when fungi were exposed for 120 sec. to UV radiation. Significant differences were recorded between the *R. solani* mycelial growth inhibition (81.33%) and that of *F. solani* (73.33%), but no significant differences were recorded for *M. phaseolina* (77.04%) after 120 sec of exposure.

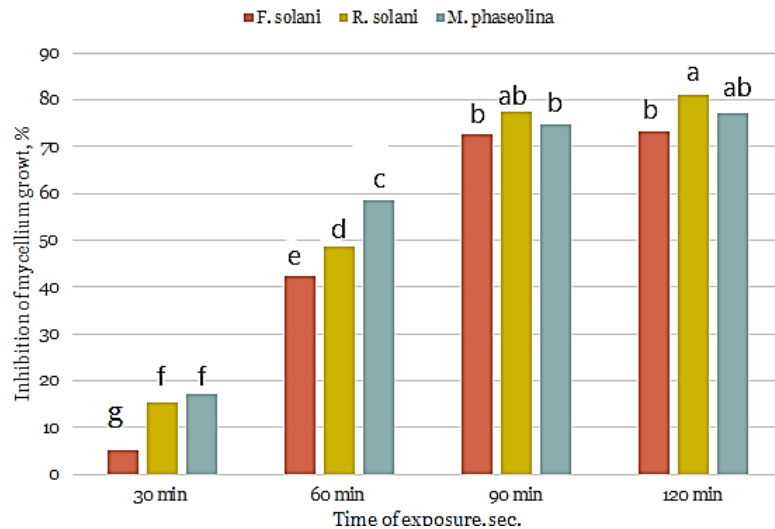


Figure (1): Effect of UV-C on *Fusarium solani* (Fs3), *Rhizoctonia solani* (Rs9) or *Macrophomina phaseolina* (Mp11), mycelial growth inhibition (%).

4–Effect of UV-C irradiation on root rot/ wilt occurrence

4-1–Using previously UV-C treated fungal mycelium

Immersed cuttings of sage in UV–pre-treated fungal suspensions of *F. solani* (isolate Fs3), *R. solani* (isolate Rs9) or *M. phaseolina* (isolate Mp11), were planted in disinfected soil. Fungi were previously exposed to UV-C for different times (0, 30, 60, 90, and 120 sec.). Disease incidence and DS were determined 30 and 90 days after sowing, at two successive seasons, 2019 and 2020. Data in Figures (2 and 3) indicated that all tested isolates of *F. solani*, *R. solani* and *M. phaseolina* induced root rot/wilt for sage plants by 91.67, 86.10 and 80.60%, 90 days after sowing, respectively. Except for *F. solani* after 30 days of sowing, exposure fungal mycelium of any tested fungi to UV-C significantly reduced both disease incidence and DS. The disease incidence of sage root rot/wilt induced by UV-C treated *F. solani* for 30 and 60 sec. was increased from 50.0% to 61.11 and 55.56%, respectively. Also, no significant differences were recorded when all tested fungi were exposed to UV-C for 30 and 60 sec. While significant decreases were shown when fungal mycelium was exposed to UV-C for 90 and 120 sec. (Tables 3 and 5). The disease incidence reduced to 41.67, 38.89 and

38.89% when the mycelium of *F. solani*, *R. solani* or *M. phaseolina*, respectively, were exposed to UV-C for 120 seconds. Whereas DS reduced from 53.61, 39.72 and 46.11% to 19.44, 16.11 and 17.22% after 120 seconds of exposure, when the treated mycelium of *F. solani*, *R. solani* and *M. phaseolina*, respectively was used (Figures 2 and 3). The data in the second season was like that of the first one.

4-2–Evaluation of UV-C to controlling sage root rot/wilt by cuttings treatment

Healthy UV-C radiated cuttings of sage (one year old) were planted in soil previously inoculated with *Fusarium solani* (isolate Fs3), *Rhizoctonia solani* (isolate Rs9) or *Macrophomina phaseolina* (isolate Mp11). Data in Figures (4 and 6) shown that application of UV-C radiation decreased the incidence and severity of sage root rot/wilt when compared with those non treated cuttings. The percentages of healthy growing plants gradually increased with increasing the time of exposure, and age of planting in comparing with the control.

Data clearly indicated that exposure of sage cuttings to UV-C radiation for 90 and 120 seconds reduced the disease incidence and severity caused by any of the tested fungi (Figures 4 and 6). The percentage of DS

decreased by 75.84% to 78.82% in case of sage cuttings exposed for 90 sec. The maximum reduction of DS was recorded after 120 seconds of exposure to UV-C radiation, which ranged between 83.48–91.72 %, 30 days after sowing, and between 92.96–98.42%, 90 days after sowing (Figures 5 and 7). When cuttings of sage were UV-C exposed for 90 seconds, the reduction in DS was high with *R. solani*,

followed by *M. phaseolina* (78.82 and 78.73%, respectively), while the less one was shown with *F. solani* (75.84%). The reduction of DS induced by *M. phaseolina* increased to 95.07 followed by *R. solani*, 90.95%, when cuttings were exposed to 120 seconds, while the DS induced by *F. solani* was 88.22%. The data of the second season was approximately like that of the first one (Figures 5 and 7).

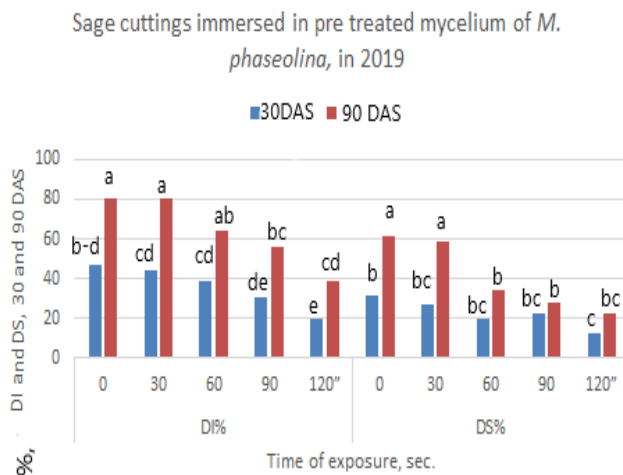
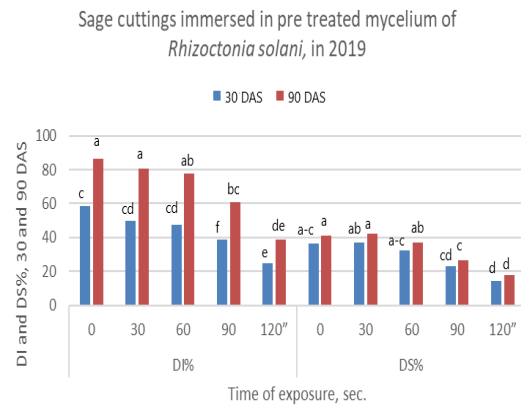
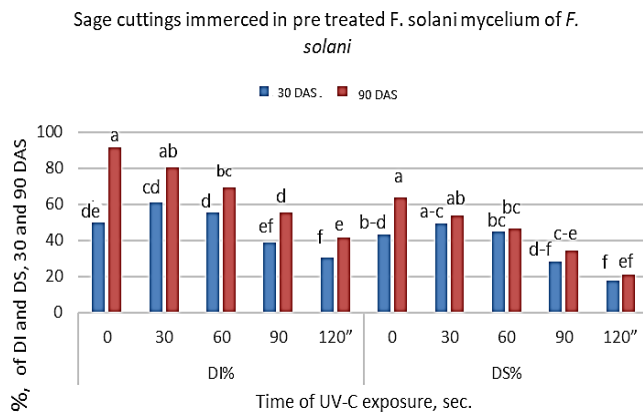


Figure (2): Effect of pre-treated mycelium (of *F. solani*, left *R. solani*, right and *M. phaseolina*, below) with UV-C on sage DI% and DS%, 30 and 90 days after sowing in 2019 season.

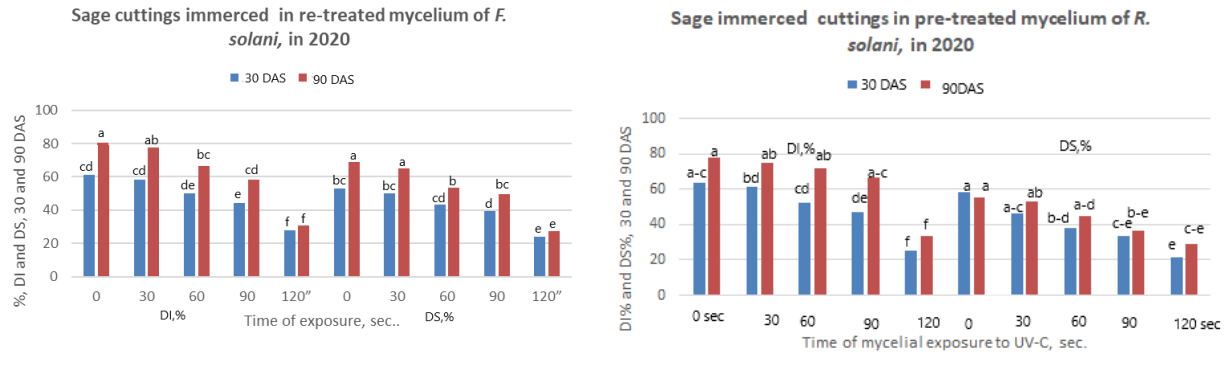


Figure (3): Effect of pre-treated mycelium (of *F. solani*, left *R. solani*, right and *M. phaseolina*, below) with UV-C on sage DI% and DS%, 30 and 90 days after sowing in 2020 season.

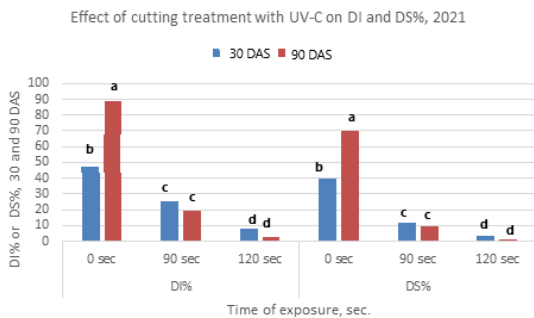
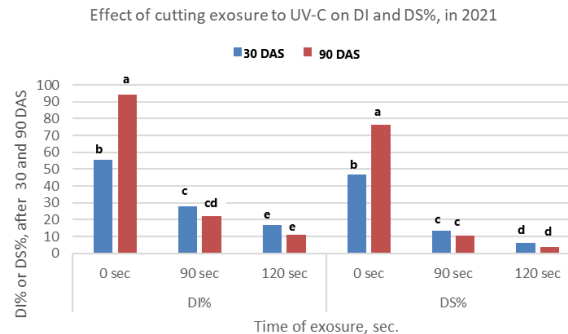
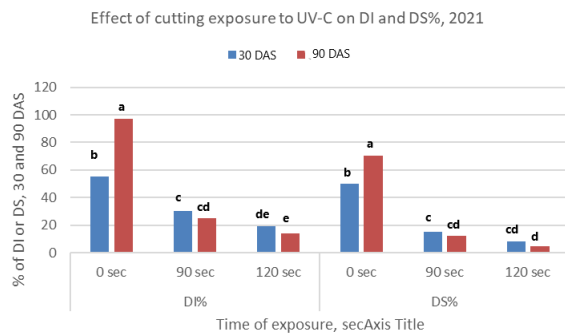
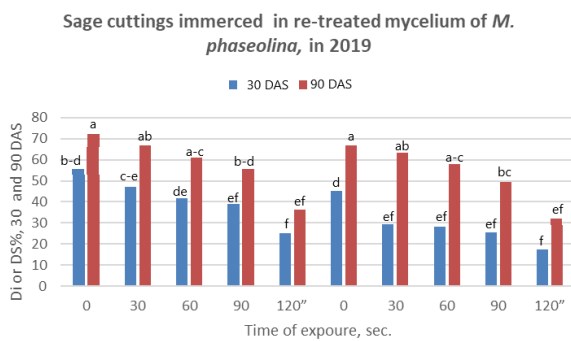


Figure (4): Effect of cutting treatments with UV-C on sage DI% and DS% induced by *F. solani*, left *R. solani*, right and *M. phaseolina*, below, 30 and 90 days after sowing in 2021 season.

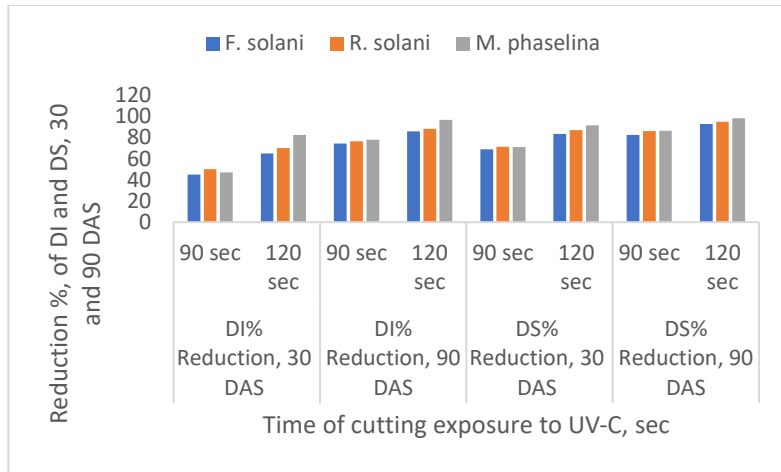


Fig. (5): Effect of cutting exposure to UV-C for 90 and 120 sec., on DI and DS% reduction, 30 and 90 days after sowing, in 1st season 2021.

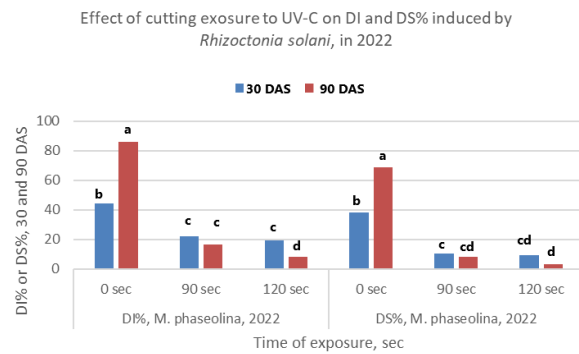
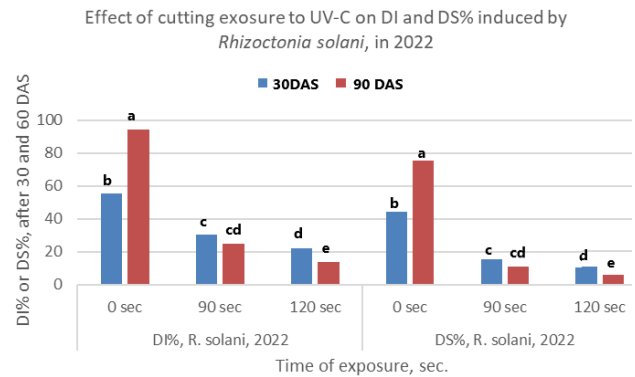
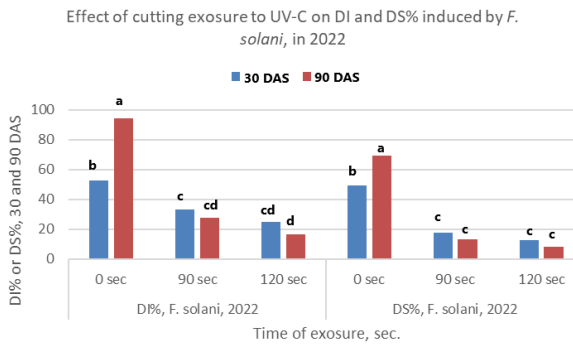


Figure 6): Effect of cutting treatments with UV-C on sage DI% and DS% induced by *F. solani*, left *R. solani*, right and *M. phaseolina*, below, 30 and 90 days after sowing in 2022 season.

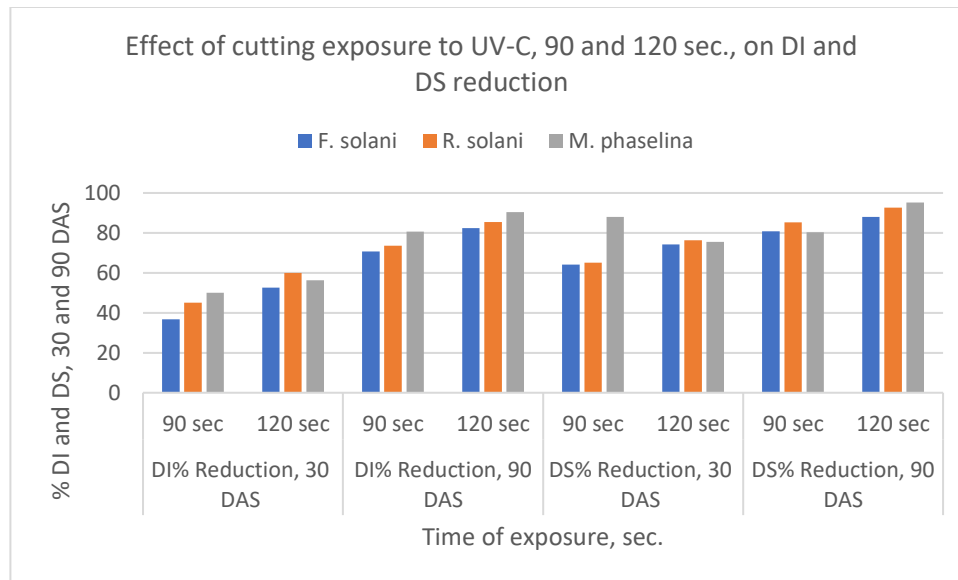


Fig. (7): Effect of cutting exposure to UV-C for 90 and 120 sec., on DI and DS% reduction, 30 and 90 days after sowing, in 2nd season 2022.

Discussion

Sage (*Salvia officinalis* L.) is one of the most commercially important species of plants belonging to *Lamiaceae* family (Avato, 2005). It is a perennial, evergreen subshrub, native to the Mediterranean region. It is cultivated in numerous countries (Raal et al., 2007). Sage has long enjoyed a reputation in traditional medicine for its healthy giving properties and for treating all kinds of ailments. Sage is used all over the world, and it has become a target for the search of the biologically active compounds and new drugs as it shows a broad range of medical activities. It cultivated in some regions in Minia governorate, Egypt, either for local consumers or for exportation. Our observations in sage farms in different districts of Minia governorate showed signs of root rot/wilting on two years old plants, in all their cultivation areas. The survey study indicated that sage root rot/wilt is distributed in different fields under experimental areas. The disease incidence ranged between 7.0–17.8%, while DS was 3.4–9.4%. Twenty-three isolates of fungi were isolated from naturally infected plants. *Fusarium solani* which presented the highest frequency (30.44%), followed by *M. phasiolina* (17.4%). *Rhizoctonia solani* and *Sclerotium* sp.

(13.05%) were the most dominant isolated fungi. Pathogenicity tests revealed that *R. solani* caused the highest disease incidence and severity, 30 and 90 days after sowing followed by *F. solani* and *M. phaseolina*, whereas *Aspergillus flavus*, *A. alternata*, *Sclerotium* sp., *R. stolonifer* and *Mucor* sp. caused the lowest root rot/wilt incidence and severity. These results are in agree with that obtained with several researchers who reported that sage is subjected to infect with different pathogens which attack roots or/and foliar systems. Anthracnosis; caused by *Colletotrichum dematium*, ascochitosis; caused by *Ascochyta sclarea*, and root rot caused by *Rhizoctonia solani* were reported as the most important diseases infect sage in European countries (Subbiah et al., 1996 and Voltolina, 2001). *R. solani*, isolated from the roots and stem base, can confirm the harmfulness of this fungus toward the enumerated parts of sage. This is indicated by the results of other studies on fungi infecting plants of thyme, sweet marjoram (*Origanum majoranum*L.), and rose in Egypt (Ishak et al., 2020 and Zakarya et al., 2016). Also, economically important pathogens in Italy and Spain include *Phomopsis sclarea*, *Phodosphaera inequalis*, *Erysiphe polygoni*

and *Sclerotinia sclerotiorum* (Subbiah *et al.*, 1996). Zimowska (2008) isolated 2743 isolates of different pathogenic fungi, belonging to 31 infectious fungal species from different parts of sage in Poland. Massive sage seedlings were died as a result of root rot and wilting of plants due to infection by *Fusarium oxysporum* in California, USA (Subbiah *et al.*, 1996). *Fusarium* wilt and *Phytophthora* foot rot were recorded in USA as new diseases infect sage plants (Farr *et al.*, 1989).

UV-C irradiation is known as an alternative method to supplement or replace the use of fungicides, especially because it promotes resistance against pathogens. On medical and ornamental crops, fungicides such as benzimidazol and imazalil may be used in the disease control (Fischer *et al.*, 2004), but the disease problem has worsened due to the development of fungicide resistance. The issue is complicated by the fact that demand for plants free of fungicide residues by costumers is on the rise (Cia *et al.*, 2007), hence alternative control strategies such as physical treatments are becoming attractive options. The results of this work indicated that the mycelial growth of *Fusarium solani* (isolate Fs3), *Rhizoctonia solani* (isolate Rs9) or *Macrophomina phaseolina* (isolate Mp11) was affected with UV radiation comparing with the control. The growth inhibition was increased, gradually, with increasing the time of UV-exposure, whereas the highest percentages of fungal growth inhibition was recorded when fungi exposed for 120 sec. to UV-C radiation. Non-significant differences in growth of *R. solani* and *M. phaseolina*, while the differences in the growth reduction was significant for *F. solani*. These findings were like that reported by García-Cela *et al.* (2016), who found intrinsic decrease in viability of six mycotoxigenic *Aspergillus* conidia, isolated from vineyards, over time when they were UV irradiated. While Canale *et al.* (2011) found that UV-C irradiation does not inhibit the mycelial growth of *Guignardia citricarpa*, the

causing agent of citrus black spot, *in vitro*, but it inhibited the conidial germination and appressoria formation. No effect on the formation of the sclerotia of *Sclerotinia sclerotiorum* when the fungal cultures were exposed to UV, however it had strong inhibitory effect on the mycelial growth (Nagy and Fischl, 2002). In particular, fungal spores in which the DNA is protected by a concentrated cytoplasm and pigmented cell wall need high doses of UV energy to be destroyed (Nematollahi *et al.*, 2015).

Numerous research pointed to the effectiveness of ultraviolet-C radiation (200-280 nm wavelength) in reducing fruits and vegetable deterioration, as a good sanitation method that does not leave residues on the product, additionally it is a low cost option that is easily applied (Rivera-Pastrana *et al.*, 2007). Our findings pointed to decrease root rot/ wilt of sage incidence and severity when cuttings of sage were immersed in pre-ultraviolet-C-treated mycelium and spores of *F. solani*, *R. solani* and *M. phaseolina*. Significant decreases were shown when the fungal mycelium was exposed to UV-C for 90 and 120 sec. The maximum decrease in DS was recorded when mycelial growth was exposed to UV-C radiation for 120 sec.

Results obtained during the present work have shown that the percentages of survival sage plants were gradually increased with increasing either the time of exposing the cuttings to UV-C radiation or the plant age after sowing. That may be due to the stimulated effects of the UV radiation which improve the healthy and physiological process of the growing plants. This result agrees with Ikehata and Ono, 2011, who reported that different UV treatments can induce distinct mutagenic consequences. Orth *et al.* (1990) reported that anthracnose was caused by *Colletotrichum lagenarium* or *Cladosporium cucumerinum*. development in cucumber because UV-B radiation varies

depending on cultivar, plant age, inoculation level, timing, and duration of UV-B exposure.

UV radiation interrupts the life cycle of several fungal pathogens (**Raviv and Antignus, 2004**). UV-C at low doses (0.25-8.0 kJ m⁻²) showed germicidal effect due to it generates harmful mutations on microorganisms and, additionally, the phenomenon of resistance induction in fruits and vegetable by UV-C has been frequently reported (**Wilson et al., 1994; Terry and Joyce, 2004 and Rivera-Pastrana et al., 2007**). Plants have multilayer tissues throughout their life cycle, fungi usually have mono-layered mycelia (with fruiting bodies as multi-layered exceptions), and bacteria exhibit significantly less complex organization, as single cells, or biofilms. Hence the effect and impact of UV radiation on these organisms will be fundamentally different, ranging from biomolecule damage to secondary metabolite production (**Vanhalewyn et al., 2020**). The germicidal effect on pathogens was also observed when *Botrytis cinerea* and *Monilinia fructigena* conidia were inactivated by UV-C (**Marquenie et al., 2002**). On plants treated with UV-C, the percentages of disease incidence and severity were significantly decreased when exposed to irradiation more than 60 sec. Also, UV-C irradiation was led to increase the accumulation of phytoalexins (scoparone and scopoletin) in orange fruits having an adverse effect on the fruit rot pathogen *Penicillium digitatum* (**Rodov et al., 1993; Ryalls et al., 1996**) and inducing phenylalanine ammonia-lyase and peroxidase activities in grapefruit (**Droby et al., 1993**).

Conclusion

To the best of our knowledge, this is the first report of root rot/wilt of sage in Egypt. Because sage is an important medicinal crop of Egypt, appropriate disease management practices should be developed and implemented.

Conflicts of Interest/ Competing interest

All authors declare that they have no conflicts of interest.

Data availability statement:

All data sets collected and analyzed during the current study are available from the corresponding author on reasonable request.

Abbreviations

ANOVA	Analysis of variance
DI	Disease incidence
DS	Disease severity
DSI	Disease severity index
ROS	Reactive oxygen species
UV	Ultraviolet

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تأثير الأشعة فوق البنفسجية المبيد للفطريات على الفطريات المسببة لعفن الجذور/الذبول للمريمية (*Salvia officinalis* L.)

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الملخص العربي

يعتبر نبات المريمية واحدا من أهم الأنواع النباتية الطبية والعطرية في جميع أنحاء العالم. وهو معرض للإصابة بالعديد من الأمراض النباتية، التي من أخطرها الإصابة بأعفان الجذور والتي تسبب خسائر كبيرة للمحصول. أجريت هذه الدراسة بغرض عمل حصر للمرض في مراكز محافظة المنيا، وعزل وتعريف المسببات المرضية المرتبطة بالأعراض المرضية ومحاولة مكافحة المرض بمعاملة العقل قبل الزراعة بواسطة الأشعة فوق البنفسجية (C).

وبينت الدراسة انتشار المرض في جميع الحقول التابعة لمراكز محافظة المنيا والتي تم فحصها، وعزلت 23 عزلة فطريات تنتمي إلى 7 أجناس فطرية من النباتات المصابة طبيعيا، كان أكثرها تكرارا فطريات *Fusarium solani*, *Macrophomina phaseolina*, *Rhizoctonia solani*. بينت دراسة القدرة الامراضية لهذه الفطريات قدرتها على إصابة العقل المزروعة بمرض عفن الجذور والذبول. كما أدى تعريض ميسيليوم هذه الفطريات للأشعة فوق البنفسجية (C) لفترات مختلفة تأثر نموها، حيث انخفض النمو نتيجة المعاملة.

أدى حقن عقل سليمة بالنمو الفطري (مادة الحقن) الذي سبق معاملته بالأشعة فوق البنفسجية (C) إلى خفض كل من نسبة حدوث المرض وشدته، كما أن معاملة العقل المعدة للزراعة بالأشعة فوق البنفسجية (C) لفترات مختلفة أدى أيضا إلى خفض نسبة حدوث المرض وشدته، ولكن بدرجة أعلي من المعاملة بتعريض النمو الفطري للأشعة.

الكلمات الافتتاحية: المريمية، اعفان وذبول الجذور، الاشعة فوق البنفسجية (C)