Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2018 15(2): 602-609. **OPEN ACCESS**

Occurrence of lettuce *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *lactucae* in Egypt and its management by using solarization, metam sodium and certain of bioproducts

Sayed F. El-Sayed [1]; Nour El-Houda A. Reyad[*2]; Abdel-Wahab A[1] and Amr M. Hanafy[1]

[1]Vegetable Crops Dept., Faculty of Agriculture, Cairo University, 12316, **Egypt**
[2]Plant Pathology Dept., Faculty of Agriculture, Cairo University, 12316, **Egypt**

.
***Correspondence:** nourelhouda__2009@hotmail.com **Accepted:** 03 Apr. 2018 **Published online: 3**0 Apr. 2018

During the last few years, lettuce wilt caused by *Fusarium oxysporum* f. sp. *lactucae* was observed in different Egyptian fields, causing a severe reduction in the yield. To solve this problem, three bioproducts (T34 biocontrol, ESRU biocontrol and algae extract) were evaluated in the greenhouse against the disease incidence. Consequently, a field experiment was performed during 2015 and 2016 growing season by using T34 biocontrol as the most effective one Also, solarization, metam sodium (MS) and Hattrick fungicide is considered. Our results indicate that solarization and MS were the most effective treatments where they induced the highest reduction in the percentages of the disease incidence and severity. Also, they have significantly affected the agronomic characters, especially during the second season. On the other hand, the residue of carbon disulfide as a degradation compound of MS in lettuce leaves was below the detection limit allowed by the Codex Alimentarius Commission. Thus, it is concluded that solarization and MS may be suitable for controlling lettuce Fusarium wilt in Egypt. Also, they may be safe in the agriculture.

Keywords: ***Lactuca sativa L.,*** **metam sodium, lettuce, Fusarium wilt, solarization, rDNA gene.**

INTRODUCTION

Lactuca sativa L, belongs to *Lactuca* genus that include more than 100 species and six types, is known as lettuce. It is the most important leafy vegetables in the Asteraceae family. Like other plants, lettuce is subjected to attack by many pathogens causing severe losses to the yield (Gilardi et al., 2017). One of the most destructive pathogen that attack lettuce plant in different countries is *Fusarium oxysporum* f. sp. *lactucae*. It causes a disease known as lettuce Fusarium wilt. This disease was discovered for the first time in Japan in 1955, then reported in other Asian and European countries (Matheron and Gullino, 2012). Many lettuce growers suffered from the severe shortage of the lettuce yield during the last few years. The control of this disease is extremely difficult, where the fungus can survive in soil for several years (Matheron and Porchas, 2010). However, different efforts have been evaluated to reduce its occurrence worldwide. Sanitation, cultural practices, solarization, fumigation, biocontrol and fungicide application examined have been in different countries (Gilardi et al., 2017). This work was throw light on the occurrence of lettuce Fusarium wilt in Egypt and its possible management by using solarization, metam sodium, and certain of bio products.

MATERIALS AND METHODS

Isolation of the pathogen.

Three isolates of *F. oxysporum* f. sp. *lactucae* (AUMC10895, AUMC10896 and AUMC10897) were isolated from infected Lettuce plant (cv. Aviram) showing wilt symptoms. These plants were collected from different fields located at Giza, Qalubiya, and Behera governorates, Egypt. The recovered isolates were subjected to pathogenicity test using lettuce plant seedlings (cv. Aviram). Due to its aggressiveness towards lettuce seedlings, *F. oxysporum* AUMC10895 isolate was selected as the main isolate throughout the present study.

Identification of the tested isolates Cultural and morphological identification.

The purified isolates were identified according to their morphological features by the Mycol. Center, Assiut Univ. Egypt where, the identified isolates were reserved under strain numbers AUMC10895, AUMC10896 and AUMC10897.

Molecular identification.

Molecular characterization of the fungal isolate (AUMC10895) was done by sequencing of rDNA gene with the help of Solgent Company, Daejeon South Korea. The ribosomal rDNA gene was amplified using the polymerase chain reaction (PCR) technique in which two universal fungal primers ITS1 and ITS4 were used. Sequences were analyzed using Basic Local Alignment Search Tool from the National Center for Biotechnology Information website. Also, the phylogenetic analysis of sequences was done with the help of MegAlign (DNA Star) software version 5.05.

Host range.

Many plant species belong to four different families as listed in Table (1) were evaluated against *F. oxysporum* AUMC10895 isolate. The reaction of the tested plants was indicated by (+) for the infected plants and (-) for the non-infected plants.

Effect of some bio products on the disease incidence in the greenhouse.

The greenhouse experiments were conducted in a completely randomized design with three different bio-formulations as listed in Table 2, each with 5 replicates. A replicate consisted of a sterilized plastic pot (25- cm diameter) containing formalin disinfested soil. Apparently, 25-day-old healthy lettuce seedlings (cv. Aviram) were dipped in each product for 30 min before planting. Extra 5 ml/pot of each formulation was added immediately after planting. 50 ml inocula (1×10^6 spore/ml) of *F. oxysporum* AUMC10895 isolate was added to the root zone three days after planting. Each replicated pot consisted of 3 seedlings (15 plants/treatment). The control treatment was only plants dipped in sterilized distilled water. The percentages of the disease incidence were estimated 25 days after planting according to (Hubbard and Gerik, 1993). The disease severity was calculated according to (Hong et al., 2008).

Field experiments.

Two experimental trials were carried out during 2015 and 2016 growing seasons in a field with a history of severe infection with lettuce Fusarium wilt. This field is located at Saft Al- Laban village, Giza governorate, Egypt. The experiments were conducted in a completely randomized block design with five treatments of solarization, Solasan 51% SL (metam sodium) obtained from Egyptian Group for development, El-Haram, Giza, Egypt. T34 biocontrol, Hattrick fungicide (Tebuconazole 6% FS) purchased from Shoura chemicals, and non-treated control each with three replicates. A replicate consisted of three rows of 3 m in length, (an area of 9 m^2). For solarization soil was covered with plastic sheets (50 µ thick) in 1st July 2015 and 2016 for 60 days. For fumigation soil was surface sprayed with metam sodium at the rate of 50 cm^3/m^2 according to the methods described by (Nelson et al., 2004), then covered with plastic sheets (50 µ thick) in 15th August 2015 and 10th August 2016 for 15 days. All agricultural practices were carried out according to the recommendation of Ministry of Agric., Egypt. The percentages of the disease incidence were estimated 25 days after planting according to (Hubbard and Gerik, 1993). The disease severity was calculated according to (Hong et al., 2008).

Effect of the test treatments on plant agronomic characters.

This experiment was performed at the harvest time (60 days after planting) in 2015 and 2016 growing seasons. The agronomic measurements included plant weight (g), head weight (g/plant), head diameter (cm)/plant, number of leaves/plant, number of leaves/head and total yield (ton/feddan).

Table 1. List of plant hosts evaluated against *F. oxysporum* AUMC10895

Family	Host	Cultivar	Source
Asteraceae	Iceberg	Aviram	Holland Agri Seeds B.V. Comp.
Cruciferous	Cabbage	O-S Cross	Takii Seed Co. Ltd
	Cabbage	Ruby King	Takii Seed Co. Ltd
	Cabbage	Cheers	Takii Seed Co. Ltd
	Cauliflower	White Magic	Sakata Seeds Comp.
	Broccoli	Heriklation	Sakata Seeds Comp.
Solanaceae	Pepper	Omega	Nongwoo Bio Co. Ltd
	Eggplant	Alabaster	Syngenta
	Tomato	023	Sakata Seeds Comp.
Cucurbitaceae	Squash	Azyad	Sakata Seeds Comp.
	Watermelon	Giza-1	Horticulture Research Institute, Egypt

Table 2. List of the tested bio formulations

The bio-product	Microorganisms	The recommended dose	Source
T34-Biocontrol	*Trichoderma asperellum* strain T34 ($1x10^9$spore/g)	3 g/l	Shoura Chemical Co.
ESRU-Bio control	Three strains of *Bacillus polymyxa*, two strains of *B.macerans*, one strain of *B. circulans* and one strain of *Enterobacter agglomerans*	The product was diluted with irrigation water at the rate of 3:10, v/v.	Environmental Studies and Research Unit (ESRU), Microbiology Department, Faculty of Agriculture, Cairo University, Giza, Egypt
Algae extract	Un-commercial blue-green algal extract in liquid phase entrapping *Anabaena flos aquae* and *Nostoc muscorum*	The solution was diluted with water at the rate of 1:1, v/v.	Soil, Water, and Environ. Res. Inst., Agric. Res. Center, Giza, Egypt

Determination of metam sodium residue in lettuce plants.

This work was carried out by the Ministry of Agriculture, Agriculture Research Center, and Central Laboratory of Residue. Analysis of pesticides and Heavy metals in Food. The quantitative determination of dithiocarbamate residues in lettuce plants was carried out according to procedures described by (Česnik and Gregorčič, 2006).

Statistical analysis.

The obtained data were subjected to statistical analysis using (MSTAT-C, 1991). The least significant difference (L.S.D., $p = 0.05$) for comparison between the means of treatments was used as mentioned by (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Identification of the tested isolates

Morphological identification.

The isolated fungi were identified depending upon the morphological features as *F. oxysporum* where the colonies are fast growing, reaching 8 cm diameter in 7 days at 28±1º C. on PDA medium. The aerial mycelium is white while the underside colonies are white to pink. They produce straight to slight macroconidia, with three septa, hook-like apical cell and measured 20-36 µ (average 28.8 µ) × 4-8 µ (average 6 µ). Oval microconidia measured 8-16 µ (average 11.2 µ) × 2-4 µ (average 3.8 µ) and single and terminal chlamydospores measured 4-12 µ (average 7.2 µ).

Molecular identification.

The *F. oxysporum* AUMC10895 isolate as the most virulence was identified using PCR amplification of ITS region. It was yielded a DNA fragment of 547 bp (Figure 1). The sequence of the fungus DNA was compared with the National Center for Biotechnology Information database using the BLAST network. The phylogenetic tree of 18S rRNA gene sequence of the tested isolate was aligned with the whole region using the CLUSTAL W program. The fungus was recorded 99% identity to different *F. oxysporum* (Figure 2).

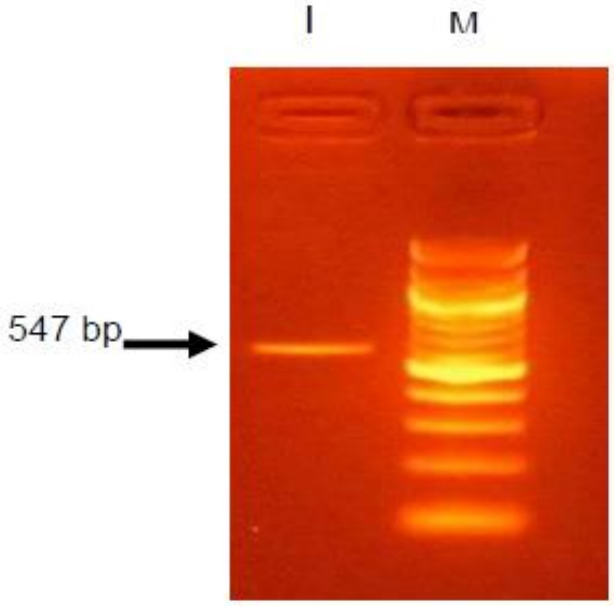

Figure 1. PCR product of *Fusarium oxysporum* amplified using ITS1 and ITS4. M= Marker; I = *F. oxysporum* AUMC10895

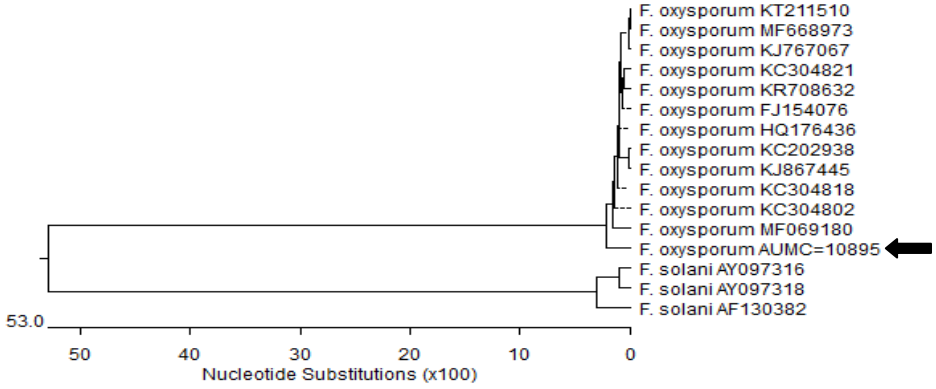

Figure 2. Phylogenetic tree of 18S rRNA gene sequences of *Fusarium oxysporum* AUMC10895 (arrowed)

Host range.

Many plant hosts were exposed to infection by *F. oxysporum* AUMC10895 isolate in the greenhouse. No symptoms were observed on the cruciferous, solanaceae and cucurbitaceae hosts. Meanwhile, a typical symptom of *Fusarium* wilt was detected on lettuce plant, cv. Aviram (Table 3).

Greenhouse experiments.

The effect of three bioproducts (T34 biocontrol, biocontrol ESRU, algae extract) on the percentages of the disease incidence and the disease severity caused by *Fusarium oxysporum* AUMC10895 isolate was examined in the greenhouse. T34 biocontrol was the superior treatment with the lowest percentage of disease incidence (26.67%) in comparison with the control (60%). Also, it was recorded the lowest percentage of disease severity (22.22%) in comparison with the control (64.44%). Algae extract was the lowest effective one with the lowest percentage of disease incidence (46.67%) and disease severity (44.44%) in comparison with

the control (Table 4).

Table 3. Host range of *F. oxysporum* AUMC10895

Host	Cultivar	Reaction
Iceberg	Aviram	+
Cabbage	O-S Cross	-
Cabbage	Ruby King	-
Cabbage	Cheers	-
Cauliflower	White Magic	-
Broccoli	Heriklation	-
Pepper	Omega	-
Eggplant	Alabaster	-
Tomato	023	-
Squash	Azyad	-
Watermelon	Giza-1	-

Table 4. Efficacies of some biological treatments for controlling lettuce wilt disease *F. oxysporum* AUMC10895 in the greenhouse

Treatments	Disease incidence (%)	Disease severity (%)
T34 Biocontrol	26.67	22.22
ESRU Biocontrol	33.33	37.78
Algae extract	46.67	44.44
Control	60.00	64.44
L.S.D 5%	29.14	28.85

Field experiments.

The effect of T34 biocontrol, solarization, metam sodium and Hattrick fungicide as a positive control was examined in the field naturally infested with *F. oxysporum* during 2015 and 2016 growing seasons. Data presented in (Table 5) show that during 2015 growing season, solarization was the most effective treatment with the lowest percentage of disease incidence (3.70%) in comparison with 98.15% for the control. Also, the lowest disease severity percentages were due to solarization and metam sodium application where they were recorded 6.66 and 11.11%, respectively without significant differences in comparison with 77.78% for the control. No significant difference was observed between T34 biocontrol and Hattrick fungicide. On the other hand, during the second season (2016 growing season), all treatments significantly reduced the disease incidence and severity in comparison with the control. The lowest disease incidence was due to solarization and metam sodium application being, 1.11 and 8.89%, respectively in comparison with the control (71.11%). Also, the lowest disease severity percentage was due to solarization and metam sodium application where they were recorded 2.22 and 4.40%, respectively in comparison with the control (77.78%). The unsatisfactory results of T34 biocontrol in the field May be contributed to the climatic variations, the biocontrol agent has poor competence and the product is unstable (Ruocco et al., 2011). Also, the poor effect of Hattrick fungicide in controlling the disease may be contributed to that this fungicide is unsuitable for controlling this disease. Concerning to the highest effect of solarization and MS application against the disease during the second season (2016 growing season) in comparison to the first season (2015 growing season), it may be contributed to that the experiment of the first season was conducted in a field previously planted with susceptible lettuce plant cultivars for several cycles, whereas the second experiment was conducted in another field has not been planted with lettuce plants for four consecutive years.

Table 5. Efficacy of some treatments for controlling lettuce wilts disease in the field naturally infested by *F. oxysporum* AUMC10895

Treatments	2015 growing season*		2016 growing season**	
	Disease incidence (%)	Disease severity (%)	Disease incidence (%)	Disease severity (%)
Solarization	3.70	6.66	1.11	2.22
Metam sodium	25.0	11.11	8.89	4.40
T34 biocontrol	63.89	53.33	44.44	51.11
Hattrick	59.26	44.45	43.33	44.44
Control	98.15	77.78	71.11	77.78
L.S.D 5%	12.58	21.78	5.64	16.86

*Three replicated plots (9 m^2), each with 36 plants, **Three replicated plots (9 m^2), each with 30 plants

Table 6. Effect of some treatments on the growth characteristics of lettuce plant (cv. Aviram) grown in soil naturally infested with *F. oxysporum* under field conditions

Treatments	2015 growing season						2016 growing season					
	PW	HW	HD	NP	NH	TY	PW	HW	HD	NP	NH	TY
Solarization	625.33	398.00	13.47	35.93	22.33	10.11	1040	720.0	14.80	40.40	26.60	14.38
Metam sodium	324.00	142.67	10.63	24.27	10.67	4.90	976.67	686.67	13.93	39.47	25.20	12.46
T34 Bio control	359.00	169.10	11.32	25.22	11.87	2.31	623.33	466.67	11.80	30.20	18.27	4.64
Hattrick	377.67	221.33	11.80	28.03	15.35	2.47	713.33	600.0	11.87	31.07	20.67	4.95
Control	200.00	80.00	6.00	13.33	6.00	0.09	523.33	220.00	9.83	25.47	12.60	2.12
L.S.D 5%	152.00	89.14	4.30	10.05	5.95	1.17	113.71	100.53	1.01	3.93	3.84	1.41

PW= plant weight (g); HD = head weight (g/plant); HD = head diameter (cm); NP = number of leaves/plant; NH = number of leaves/head and TY = total yield ton/feddan.

Effect of the tested treatments on plant agronomic characters.

The effect of the tested treatments (Solarization, metam sodium, T34 biocontrol, and Hattrick fungicide) on the growth parameters and yield of lettuce plants (cv. Aviram) was estimated at the end of the experiment (60 days after planting) during two successive growing seasons (2015 and 2016). Data presented in (Table 6) show that, during the first season (2015 growing season), solarization was the most effective treatment where it gave the highest increase in the plant weight (625.33 g/plant), head weight (398 g/plant), head diameter (13.47 cm), number of leaves per plant (35.93 leaf/plant), number of leaves per head (22.33 leaf/head) and the total yield (10.11 ton/feddan). Meanwhile, MS application was the lowest effective once where it gave 324 g/plant for the plant height, 142.67 g/plant for the head weight, 10.63 leaf/plant for the number of leaves per plant and 24.27 leaf/head for the number of leaves per head. But it was ranked second according to the total yield where it was recorded 4.90 ton/feddan. On the other hand, during the second season (2016 growing season), The highest significant increase in the plant weight was due to each of solarization and MS application being, 1040 and 976.67 g/plant, respectively without significant difference in comparison to the control being, 523.33 g/plant while the lowest effective treatment was T34 biocontrol where it was recorded 623.33 g/plant without significant difference with the control. The head weight was significantly affected by all treatments in comparison to the control. The highest significant increase was due to solarization and MS application where they were recorded 720 and 686.67 g/plant, respectively without significant difference followed by Hattrick fungicide (600 g/plant) which was statistically on par with metam sodium. The corresponding value in the control was 220 g/plant. On the other hand, each of number of leaves/plant, number of leaves/head and the total yield was significantly affected by the tested treatments in comparison with the control. Solarization and MS were the

most effective treatments without significant difference. Meanwhile, Hattrick fungicide and T34 bio control was the lowest effective treatment without significant difference in comparison with the control. The low efficacy of the MS during the first season contributed to the short period between soil fumigation and transplanting. This finding is in harmony with those obtained by (Desaeger et al., 2008).

Determination of MS residue in lettuce plants

The measurement uncertainty expressed as expanded uncertainty (at 95% confidence level) was within the range 28%. The limit of quantification of Dithiocarbamates was 0.05 mg CS2/kg sample. The residue of dithiocarbamate in lettuce recorded 0.08 mg CS_2/kg sample as reported in certificate number 624110 that issued by Ministry of Agriculture, Agriculture Research Center, Central Laboratory of Residue. Analysis of pesticides and Heavy metals in Food

CONCLUSION

The results of the entire work lead to conclude that lettuce Fusarium wilt caused by *F. oxysporum* f. sp. *lactucae* is occurred in Egypt. Also, it was confirmed that pre-plant treatment with solarization or metam sodium application may be a good method for controlling the disease in the Egyptian fields. Metam sodium may be safe where its residue in the plant tissue was less than the limit allowed by the Codex Alimentarius Commission

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest".

ACKNOWLEGEMENT

We thank Prof. Dr. Ahmed Moharram, Botany Department, Faculty of Science, Assiut University, Egypt for his assistance in the definition of the fungal isolates.

AUTHOR CONTRIBUTIONS

All authors contributed equally in all parts of this study. .

REFERENCES

Česnik, H.B. and Gregorčič, A. 2006. Validation of the method for the determination of dithiocarbamates and thiuram disulphide on apple, lettuce, potato, strawberry and tomato matrix. Acta Chimica Slovenica. 53: 100–104.

Desaeger, J.A., Seebold, K.W., Csinos, A., 2008. Effect of application timing and method on efficacy and phytotoxicity of 1,3-D, chloropicrin and metam-sodium combinations in squash plasticulture. Pest Manag Sci. 64:230–238.

Gilardi, G., Franco Ortega, S., van Rijswick, P.C.J., Ortu, G., Gullino, M.L., Garibaldi A., 2017. A new race of *Fusarium oxysporum* f. sp. *lactucae* of lettuce. Plant Pathology. 66:677–688.

Gomez, K.A. and Gomez, A.A., 1984. Comparison between treatment means. In Lviley A, editor. Statistical procedures for agricultural research. New York (NY): Interscience Publication: 187 pages.

Hong, C.F., Chang, P.F.L., Huang, J.L., Wan, Y.L., Huang, J.W., 2008. Identification of Physiological Races of *Fusarium oxysporum* f. sp. *lactucae* and screening of lettuce cultivars. Plant Pathology Bulletin. 17:233–242.

Hubbard, J.C. and Gerik, J.S., 1993. A New Wilt Disease of Lettuce Incited by *Fusarium oxysporum* f. sp. *lactucae* forma specialis nov. Plant Disease. 77:750–754.

Matheron, M.E. and Gullino, M.L., 2012. Fusarium wilts of lettuce and other salad crops. In: Gullino ML, Katan J, Garibaldi A, editors. Fusarium Wilts of Greenhouse Vegetable and Ornamental Crops, St Paul, MN: APS Press, pp. 175-183.

Matheron, M.E. and Porchas, M., 2010. Evaluation of soil solarization and flooding as management tools for fusarium wilt of lettuce. Plant Disease. 94:1323–1328.

MSTAT-C., 1991. A software program for the design, management and analysis of agronomic research experiments: 400 pages.

Nelson, S.D., Dickson, D.W., Ajwa, H.A., Sullivan, D.A., 2004. Efficacy of metam sodium under

drip and surface spray application in Florida tomato production. Subtropical Plant Science. 56: 16-20.

Ruocco, M., Woo, S., Vinale, F., Lanzuise, S., Lorito, M., 2011. "Identified difficulties and conditions for field success of biocontrol. 2. Technical aspects: factors of efficacy," in Classical and Augmentative Biological Control Against Diseases and Pests: Critical Status Analysis and Review Of Factors Influencing Their Success, ed. P. C. Nicot (IOBC-WPRS), 45–57.

Scott, J.C., Gordon, T.R., Shaw, T.V., 2010. Effect of temperature on severity of Fusarium wilt of lettuce caused by *Fusarium oxysporum* f. sp. *lactucae*. Plant Disease. 94:13-17.