Egypt. J. Microbiol. **Vol. 54,** pp. 117-135 (2019)

EgyptianJournalofMicrobiology
http://ejm.journals.ekb.eg/

Fungi as Potential Biocontrol Agents Against *Convolvulus arvensis* and *Portulaca oleracea* Infesting the Agroecosystems of Egypt

Nevin A. Ibrahim[(1)#], Merhan M. Tawfik[(2)]
[(1)]Microbiology Department, Faculty of Science, Ain Shams University, Cairo, Egypt;
[(2)]Plant Protection Department, Desert Research Center, El-Matariya, Cairo, Egypt.

FUNGAL biocontrol of weed is a promising eco-friendly alternative solution to reduce risks of synthetic herbicides. A field survey conducted in Wadi El-Natroun and North Sinai, Egypt, resulted in the isolation of 36 fungal isolates. Culture filtrates of the obtained isolates were screened for their ability to inhibit the seed germination and seedling development of *Portulaca oleracea* and *Convolvulus arvensis*. Filtrates crude extracts of *Albifimbria verrucaria* MN094460.1 and *Cladosporium cladosporioides* MN094461.1 gave the highest herbicidal activity against both tested weeds. *A. verrucaria* caused high reductions in seed germination, shoot and root length, of both weeds. Also, its crude extract significantly reduced seedling fresh weight of *P. oleracea* (85%) and *C. arvensis* (59%). On the other hand, *C. cladosporioides* completely inhibited seed germination, shoot and root length of *P. oleracea* while its extract caused 78% reduction in seedling fresh weight. In greenhouse, foliar application of 5×10^7 conidia/ml of *A. verrucaria* with 0.2% silwet-L-77 significantly reducted *P. oleracea* chlorophyll *a* (74%) and fresh weight (82%) also, it reduced *C. arvensis* chlorophyll *b* (57%). Whereas, conidial sprays of *C. cladosporioides* (5×10^7 conidia/ml with 0.2% silwet-L-77) caused significant reductions in *P. oleracea* chlorophyll *a* (73%) and fresh weight (74%). LC-MS/MS analysis revealed the presence of 12 compounds in *C. cladosporioides* extract and 7 compounds in *A. verrucaria* extract. This study concluded that *A. verrucaria* MN094460.1 and *C. cladosporioides* MN094461.1 are potentially effective biocontrol agents against *P. oleracea* and *C. arvensis*. Besides, the herbicidal activity of *C. cladosporioides* against *P. oleracea* was reported for the first time.

Keywords: Fungal weed control, Bioherbicides, Noxious weeds, Field bindweed, Common purslane.

Introduction

Weeds are ever-present and increasingly significant constraint to agricultural production worldwide. Current production levels can only be maintained through the regular and wholesale application of pesticides, particularly of chemical herbicides. Chemical weed control is always restricted by the fact that effective weed herbicides may affect many other plants, in addition to serious public concern regarding environmental problems associated with chemical pesticide usage (Defago et al., 2001; Chutia et al., 2007). FAO report published in 2011 showed that losses caused by weeds in crop yields were equivalent to $95 billion in 2009. Multiple control methods are used for weeds with chemical ones are the most used, however, many species of weeds were reported to acquire resistance against commercially available chemical herbicides. Vencill et al. (2012) mentioned that there are 372 known biotypes of herbicide-resistant weed species in the world. Such an increasing ratio of

[#]Corresponding author email: nevinibrahim@sci.asu.edu.eg
Received 28/9/2019; Accepted 30/10/2019
DOI: 10.21608/ejm.2019.17443.1117

herbicide-resistant weeds in addition to the public concern of their harmful effects continues to be the driving force for investment in researches on biocontrol agents (Harding & Raizada, 2015).

Many microbes have bio-herbicidal activity, and several phytopathogenic fungi and bacteria have been patented as weed-control agents (Hoagland et al., 2007a). Fungi are among the richest microorganisms for the production of bioactive compounds used in many disciplines. Thus, the use of fungal bioherbicides has been stimulated as part of the search for alternatives to chemical control, as the use of these eco-friendly formulations minimizes hazards resulting from herbicide residue to both human and animal health, and to the environment (Patel & Patel, 2015). Proposed benefits to this biocontrol strategy include reduced environmental impact, increased target specificity, reduced development costs compared to conventional herbicides and the identification of novel herbicidal mechanisms (Harding & Raizada, 2015).

Field bindweed (*Convolvulus arvensis*) also known as small morning glory is a highly competitive and invasive weed which can persist in all soils. It is a serious problem for many growers and has been described as the twelfth worst weed in the world and the seventh most important in Europe. It is the most difficult invasive weeds to control and nearly impossible to control with chemicals alone (Tóth & Cagáň, 2005; Jacobs, 2007). Field bindweed and Common purslane are two of the most frequently occurred weeds in Egypt (Mashaly & Awad, 2003; Balah, 2019). Therefore, this study was designed to develop biocontrol agents having the ability to control and manage field bindweed and common purslane. Soil and rhizosphere samples from Wadi El-Natroun and North Sinai, Egypt, have been screened-out to obtain potent fungal bioagents. The effectiveness of the obtained fungi for the control of the two targeted weeds both *in vitro* and in the greenhouse was evaluated. Also, preliminary screening and characterization of bioactive compounds in crude extracts from selected fungal culture filtrates were assessed using LC-MS/MS.

Materials and Methods

Field survey

Thirteen soil samples were collected both from rhizosphere and non-rhizosphere (bulk soil) associated with nine types of weeds. Also, 3 samples of infected weeds were collected. The surveyed weeds were obtained from 5 field sites in Wadi El-Natroun and North Sinai, Egypt. The collected samples were transferred to the laboratory and stored at 4°C until investigation. The GPS data of each surveyed region and types of weeds involved in sampling collection is shown

TABLE 1. Types of weeds involved in sampling collection and GPS coordinates of the surveyed regions.

	Region number	Region name	GPS				Weed type
Wadi El-Natroun	(1)	Sugar beet farm	N	30	29	1.8	- *Convolvulus arvensis* - *Cyperus rotundus* - *Cynodon* sp. - *Melilotus indica*
			E	30	0.5	17.6	
	(2)	El-hammra lake	N	30	23	52.6	- *Typha* sp.
			E	30	19	15.8	
	(3)	Natural vegetation	N	30	25	16.6	- *Chenopodium* sp. - *Malva* sp.
			E	30	18	46.6	
	(4)	Grapes farm, west of El Alamein	N	30	29	19.4	- *Cortaderia selloana*
			E	30	9	26.2	
North Sinai	(5)	Balouza, North Sinai	N	30	52	48	- *Aristida* sp.
			E	32	34	52	

in Table 1.

Isolation of fungi from infected weeds, soil and rhizosphere

Fungi were isolated from the collected infected weeds according to the method described by Sinclair & Dhingra (1995). Sections of diseased leaf tissues (1cm^2) were surface sterilized by 70% ethanol for one minute and then rinsed in sterile distilled water three times, then placed onto plates of potato dextrose agar (PDA; Difco, Sparks, MD, USA). Isolation from soil and rhizosphere samples was carried out using serial dilution method (Johnson & Booth, 1983). Dilutions 10^{-3}, 10^{-4} and 10^{-5} were prepared from soil samples and 0.2ml aliquots from each dilution were inoculated onto PDA plates. All PDA plates were incubated in at 28ºC for seven days. The obtained fungal colonies were purified before maintaining on PDA slants at 4ºC for further studies.

Conditioning of weed seeds and bioassay

To test the ability of fungal culture filtrates to inhibit the seed germination and seedling development of *Convolvulus arvensis* and *Portulaca oleracea*, seeds were surface sterilized by soaking in 0.5% sodium hypochlorite (NaOCl) for 2 min followed by rinsing thoroughly in sterile distilled water and blotted on filter paper then dried under aseptic conditions. Fungal culture filtrates used in bioassay were prepared according to Buchwaldt & Green (1992) and Smedsgaard (1997) as following: 250ml Erlenmeyer's flasks containing 100ml of potato dextrose broth (PDB; Difco, Sparks, MD, USA) were inoculated with discs (1cm diameter) of seven days-old fungal colonies (3 discs/ flask). The flasks were incubated at 28ºC in shaking incubator (Daihan scientific WIS-30) at 120rpm for one week, then the obtained broth was filtered through a Whatman filter paper no. 1 acetate (Sigma, St. Louis, MO, USA). The supernatants were re-filtered using 0.22µm syringe filters then, stored at 4ºC until use. The previously sterilized seeds (5 seeds per dish) were sprinkled in sterile 9cm diameter Petri dishes containing sterile filter paper and 5ml of cultural filtrate was added under aseptic conditions. Petri dishes were sealed with parafilm and incubated in darkness at 25±2°C. Three replicates were used for each treatment. The germination and seedling (root and shoot length) responses were determined after 7 days of incubation.

To determine the effect of fungal crude extracts on seedlings development, culture filtrates of the selected active isolates were adjusted to pH 3.8 before extraction by an equal volume of ethyl acetate (Sigma, St. Louis, MO, USA), for three times. The obtained extracts were dried to give crude oily residues, then re-dissolved in 10% ethanol from which a series of concentrations (5, 10, 20, 30, 40mg/ml) was prepared. The prepared concentrations were added into tissue culture tubes containing 3ml of liquid Murashige & Skoog medium (MS medium, DuchefaBiochemie, Netherland) where the roots of seven days old seedlings were submerged (one seedling per tube). After incubation at 25°C for seven days, the seedlings were removed, biomass fresh weights were measured and the reduction percentage (R%) was calculated. Three replicates were used for each concentration (Balah, 2014).

Identification of the highly active fungal isolates

The selected fungal isolates, with characteristic high activity against weeds, were identified based on their macroscopic and microscopic characteristics using the universal taxonomic key of Ellis (1971), Klich (2002) and Bensch et al. (2012). The morphological identification of the two most potent isolates was confirmed using molecular typing as following: DNA was extracted from a 5 days old culture grown on PDA media using DNeasy extraction kit (Qiagen, Germany) according to the manufacturer`s instructions. Primers for amplification of the internal transcribed spacer (ITS) region were ITS1 (*F*: 5'- TCCGTAGGTGAACCTGCGG -3') and ITS4 (*R*: 5´- TCCTCCGCTTATTGATATGC -3´). PCR reaction was performed in a total volume of 50µl with 30ng genomic DNA, 1X reaction buffer, 1.5mM MgCl2, 1U *Taq* DNA polymerase (Promega), 2.5mM dNTPs, and 30pmol of each primer.

Thermocycling was performed on an applied biosystem thermocycler. The cycling protocol was as follows: 5 min at 94ºC denaturating step followed by 35 cycles of 1min at 94ºC, 1min at 55ºC and 1min at 72ºC were followed by a final extension step for 7min at 72ºC. PCR products were run on 1.5% agarose gels and the purified PCR products were subjected to sequence analysis using an automatic sequencer ABI PRISM 3730XL Analyzer and Big Dye TM Terminator Cycle Sequencing Kits (ABI Applied Biosystems) following the manufacturer`s instructions. The obtained nucleotide sequences were compared

with the accessible sequences in the NCBI databases using the BLAST homology search. A phylogenetic tree based on the ITS sequence data for the studied isolates against the reference strains was constructed using the neighbor-joining method (Saitou & Nei, 1987). The tree is drawn to scale. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2004). Evolutionary analyses were conducted in MEGA X (Kumar et al., 2018). The nucleotide sequence data reported for the selected isolates were deposited the NCBI GenBank nucleotide sequence database.

Pathogenicity of selected fungal isolates to weeds in pot trials

Seeds of *C. arvensis* and *P. oleracea* were placed on the surfaces of moistened commercial soil mixtures and then covered with a thin layer of the soil mixture till the weed seedlings reached four-leaf growth stage in the first true-leaf growth, *C. arvensis* and *P. oleracea* weeds were inoculated by spraying suspensions containing 2.0×10^7 conidia/ml and 0.2% Silwet L-77 surfactant in a Treatment (1) and 5.0×10^7 conidia/ml of and 0.2% Silwet L-77 surfactant in a Treatment (2) until the foliage was fully wetted. Control plants received 0.2% Silwet L-77 surfactant only. Greenhouse temperatures were set at 28-32°C with 60-90% relative humidity. Fresh weight and percentage of chlorophyll *a*, chlorophyll *b* and carotenoid were determined after 14 days of inoculation. Experimental units consisted of groups of 10 pots, each containing five plants and treatments were replicated three times whereas each experiment was conducted twice. All experiments in these studies were arranged in randomized complete block designs and means were separated using Fisher's LSD (0.05).

LC-MS/MS analyses of crude extracts from selected fungal culture filtrates

The most active microbial crude extracts were analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) to separate and identify their active compounds. Analyses were carried out by using a 6420 triple quadrupole LCMS Agilent Technologies system. An Agilent Poroshell Zorbax SB-C18 column (4.6 × 150mm, 5µm) was used for the separation of samples and the flow rate was maintained at 0.5ml/min at 30°C. The mobile phase was composed of 5mM ammonium formate (A) and acetonitrile (B) using a gradient elution of 40–60% (B) at 0–10min, 10–90% (B) at 10–20min, 10–90% (B) at 20-30min, and 90-10% at 30-35min. The sample was dissolved in methanol and the volume injected was set at 5µL.

The LC-MS measurements were performed using Triple-quadrupole-Mass spectrometer and an electrospray ion sourcein positive-ionization mode, equipped with an Agilent HPLC system (Agilent, USA serial 6420). Mass fragments were monitored via multiple reaction monitoring (MRM) to obtain full scan mass spectra in a range of m/z 100–1000. The molecular ion (parent ion) was identified and the molecular weight of the compounds was calculated. Information about substructures was attained from the integration of the fragmentation patterns of each compound. Data analysis through Agilent LC-MS/MS Software (Agilent, USA) was used to process the obtained mass spectra.

Statistical analyses

Data of Randomized Complete Block Design experiments were statistically analyzed by one way ANOVA using IBM SPSS statistical software package (V. 20.0, IBM Corp., USA, 2016) according to Snedecor & Cochran (1990) and treatment means were compared by LSD test at 5% level of probability. The effective concentration EC_{50} values (half-maximum effective concentration) for each growth parameter were calculated by plotting concentration on a log scale (X) and the response (Y) on probit scale mathematically transformed, the data appear linear and sign the point in a semi-log graph paper (Ritz et al., 2015).

Results

Biological assay of fungal isolates

Culture filtrates of thirty-six fungal isolates were screened for the ability to reduce germination of seeds of *Convolvulus arvensis* and *Portulaca oleracea*. Such screening led to a selection of four main active fungal culture filtrates. This selection was based on the results obtained regarding the reduction percentage of seed germination, shoot length, and root length. The selected four highly active fungal isolates were morphologically identified as *Aspergillus flavus* (Link.), *Aspergillus terreus* (Samson & Gams.), *Cladosporium cladosporioides* (Fres.) de Vries and *Albifimbria verrucaria* (Alb. & Schwein.) Lombard & Crous which was previously named *Myrothecium*

verrucaria (Alb. & Schwein.) Ditmar, Deutschl. The source and isolation site of the selected fungi, in addition to their effect on *Convolvulus arvensis* and *Portulaca oleracea* seed germination, shoot and root length were listed in Table 2. The highest activity against *Portulaca oleracea* seed germination, shoot length, and root length was recorded by *C. cladosporioides* culture filtrate resulting in 100% reduction. Culture filtrate of *A. verrucaria* was less active but caused a significant reduction in seed germination, shoot length and root length of *Portulaca oleracea* by ratios of 75%, 93% and 80%, respectively. Shoot length of *Portulaca oleracea* was highly reduced (80%) by *A. terreus* culture filtrate, while it caused a lower reduction in seed germination and root length with values reached 67% and 73%, respectively. *A. flavus* culture filtrate caused a significant reduction only in shoot length (77%) and root length (65%) of *Portulaca oleracea* while causing a non-significant reduction in seed germination. Results recorded by culture filtrate assay on *Convolvulus arvensis* showed a different activity pattern. Filtrates of *A. flavus* and *A. terreus* cultures gave the highest reduction percentages. *A. flavus* culture filtrate resulted in 73%, 70% and 82% significant reduction in seed germination, shoot length and root length, respectively. While, *A. terreus* culture filtrate caused 81%, 63% and 76% reduction using the same tested parameters. Culture filtrate of *A. verrucaria* caused a significant reduction in both shoot length (65%) and root length (63%) without significant effect on seed germination.

Bioassay with ethyl acetate crude extracts for the four selected fungal isolates on weed seedlings showed that two of them (*A. verrucaria* and *C. cladosporioides)* gave the highest reduction recorded in total biomass fresh weight. Accordingly, the crude extracts from these two candidates were considered the most active against the two target weeds. The crude extract of *A. verrucaria* affected both *Convolvulus arvensis* and *Portulaca oleracea*. At concentrations of 5, 10, 20, 30 and 40mg/ml of *A. verrucaria* organic crude extract, the reduction percentage in total biomass fresh weight ranged from 48.67% to 84.92% for *Portulaca oleracea* and from 15.53% to 58.79% for *Convolvulus arvensis* (Tables 3, 4). On the other hand, the crude organic extract of *C. cladosporioides* affected only *Portulaca oleracea* with a recoded reduction percentage in *Portulaca oleracea* total biomass fresh weight reached 24.19, 52.69, 67.74, 74.19 and 77.96% at 5, 10, 20, 30 and 40mg/ml, respectively, no significant differences were detected between the last four concentrations (Table 4).

Quantitative assessment of effective concentration (EC_{50})

The Half maximum effective concentration (EC_{50}) was determined from probit curves by generating the linear curve. As shown in Table 5, *A. verrucaria* organic crude extract showed the lowest EC_{50} values against both *Portulaca oleracea* and *Convolvulus arvensis* by 0.76 and 1.43mg/ml, respectively. Whereas, *C. cladosporioides* organic extract showed an EC_{50} value of 0.92mg/ml against *Portulaca oleracea.* The dose-response curve of *A. verrucaria* and *C. cladosporioides* was displayed in Fig. 1, 2.

TABLE 2. Sources and isolation sites of the active fungal isolates, and their effect on *Convolvulus arvensis* and *Portulaca oleracea* seed germination, shoot and root length.

Fungal isolate	Source of isolation	Location	Reduction % on *C. arvensis*			Reduction % on *P. oleracea*		
			G	SL	RL	G	SL	RL
A. terreus	Soil of: *Cortaderia selloana*	Sugar beet farm	81	63	76	67	80	73
A. flavus	Soil of: *Convolvulus arvensis*	Grapes farm	73	70	82	38	77	65
C. cladosporioides	Soil of: *Typha* sp.	El-Hamra lake	43	41	20	100	100	100
A. verrucaria	Rhizosphere of : *Cynodon* sp.	Sugar beet farm	33	65	63	75	93	80

G= Germination, SL= Shoot length, RL= Root length.

TABLE 3. Effect of ethyl acetate crude extracts on total biomass fresh weight of *Convolvulus arvensis*.

Fungal isolate		Biomass fresh weight (mg)						LSD (0.05)
		Control	‘5	‘10	‘20	‘30	‘40	
C. cladosporioides	Mean ± SD	238^{a}± 8	229^{a}± 2	206^{b}± 8	187^{c}± 1	158^{d}± 12	151^{d}± 8	13.55
	R%	0.00	3.79	13.47	21.26	33.47	36.63	
A. verrucaria	Mean ± SD	125^{a}± 6	105^{b}±5	92^{c}± 1	88^{c}± 2	71^{d}± 1	51^{e}±2	11.55
	R%	0.00	15.53	25.94	29.68	43.05	58.79	
A. flavus	Mean ± SD	219^{a}± 17	234^{a}± 11	196^{b}± 11	173^{c}± 6	156^{cd}± 7	147^{d}± 5	18.33
	R%	0.00	7.00*	10.35	21.00	28.77	32.73	
A. terreus	Mean ± SD	125^{a}± 6	139^{a}± 6	138^{a}± 15	107^{b}± 6	98^{b}± 2	80^{c}±16	17.61
	R%	0.00	11.76**	10.43**	14.17	21.39	35.83	

Mean± SD: Average value of the 3 trials after seven days ± standard deviation, R%: Reduction percentage in biomass fresh weight, LSD= Least significant difference, Values that do not share a letter are significantly different (P≤ 0.05), ‘: Concentration (mg/ml), **: Stimulation in growth.

TABLE 4. Effect of ethyl acetate crude extracts on total biomass fresh weight of *Portulaca oleracea*.

Fungal isolate		Biomass fresh weight (mg)						LSD (0.05)
		Control	‘5	‘10	‘20	‘30	‘40	
C. cladosporioides	Mean ± SD	18^{a}± 1	15^{a}± 1	9^{b}± 0	6^{b}± 1	6^{b}± 0	3^{b}± 0	1.64
	R%	0.00	24.19	52.69	67.74	74.19	77.96	
A. verrucaria	Mean ± SD	40^{a}± 6	21^{b}± 1	19^{b}± 2	16^{bc}± 1	12^{c}± 2	6^{d}± 0	5.69
	R%	0.00	48.67	53.42	61.25	68.83	84.92	
A. flavus	Mean ± SD	22^{a}± 1	16^{b}± 1	15^{c}± 1	14^{d}± 0	12^{e}± 0	10^{f}± 0	0.75
	R%	0.00	26.57	31.05	36.42	46.72	53.88	
A. terreus	Mean ± SD	27^{a}± 2	33^{ab}± 1	31^{bc}± 4	25^{cd}± 1	23^{d}± 1	18^{e}± 0	4.06
	R%	0.00	20.73**	12.07**	8.78	17.56	32.93	

Mean± SD: Average value of the 3 trials after seven days ± standard deviation, R%: Reduction percentage in biomass fresh weight, LSD= Least significant difference, Values that do not share a letter are significantly different (P≤ 0.05), ‘: Concentration (mg/ml), **: Stimulation in growth.

TABLE 5. Effective concentration (EC_{50}) of microbial crude extracts on total biomass fresh weight of weed seedlings.

Fungal isolates	EC_{50} (mg/ml)	
	Convolvulus arvensis	*Portulaca oleracea*
C. cladosporioides	1.90	0.92
A. verrucaria	1.43	0.76
A. flavus	1.92	1.47
A. terreus	1.78	1.65

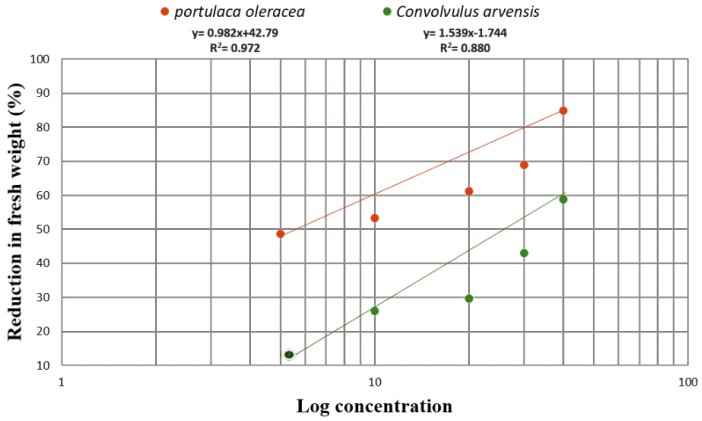

Fig. 1. Dose response curve of *A. verrucaria* on *Portulaca oleracea* and *Convolvulus arvensis.*

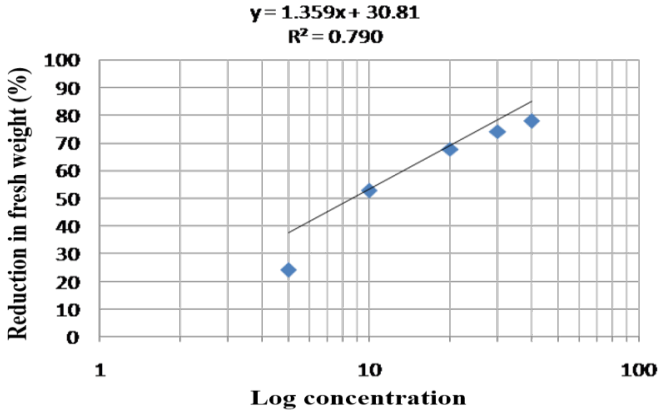

Fig. 2. Dose-response curve of *C. cladosporioides* on *Portulaca oleracea.*

Molecular Identification of the most active isolates

Molecular characterization of the two most potent isolates (*Albifimbria verrucaria* and *Cladosporium cladosporioides*) based on the DNA sequence data of the ITS1-5.8S-1TS2 region was used to confirm their identity. The nucleotide sequences of these isolates appeared in the NCBI GenBank under the following accession numbers: *A. verrucaria* (MN094460.1) and *C. cladosporioides* (MN094461.1). A phylogenetic tree was constructed using the sequence data of the studied fungal isolates in comparison to the reference strains in the GenBank. This tree revealed a clear similarity between the studied isolates and reference strains (Fig. 3). The tested isolate of *A. verrucaria* was 99.6% identical to the *A. verrucaria* KJ026703.1 reference strain. Whereas, the examined *C. cladosporioides* isolate was closely similar (99.0% identity) to the *C. cladosporioides* KR709050.1 reference strain.

Pathogenicity of the two selected fungal isolates to weeds in pot trials

The herbicidal effect of fungal conidia of *A. verrucaria* and *C. cladosporioides* on weeds were evaluated under greenhouse conditions. The treatment which contained a conidial concentration of 2.0×10^7 conidia/ml of *A. verrucaria* in 0.2% Silwet L-77 (Treatment 1) caused a reduction in chlorophyll *a* of *P. oleracea* by 40.36% and a significant one in chlorophyll b of *C. arvensis* by 36.15% as compared with control. While using 5.0×10^7 conidia/ml of *A. verrucaria* in 0.2% Silwet L-77 (Treatment 2) resulted in a significant reduction in chlorophyll *a* of *P. oleracea* by 74.09% while caused 57.47% reduction of *Convolvulus arvensis* chlorophyll *b*. Considering the effect of *A. verrucaria* on weed fresh weight, both treatments caused a highly significant reduction of *P. oleracea* fresh weight by 80.84% and 82.18%, respectively (Tables 6, 7). On the other hand, when 2.0×10^7 conidia/ml and 5.0×10^7 conidia/ml of *C. cladosporioides* in 0.2% Silwet L-77 was applied, a significant reduction in the percentage of chlorophyll a of *P. oleracea* reached 55.20% and 72.82%, respectively. Also, both treatments resulted in a reduction of *P. oleracea* fresh weight by 63.37% and 73.88%, respectively (Tables 8).

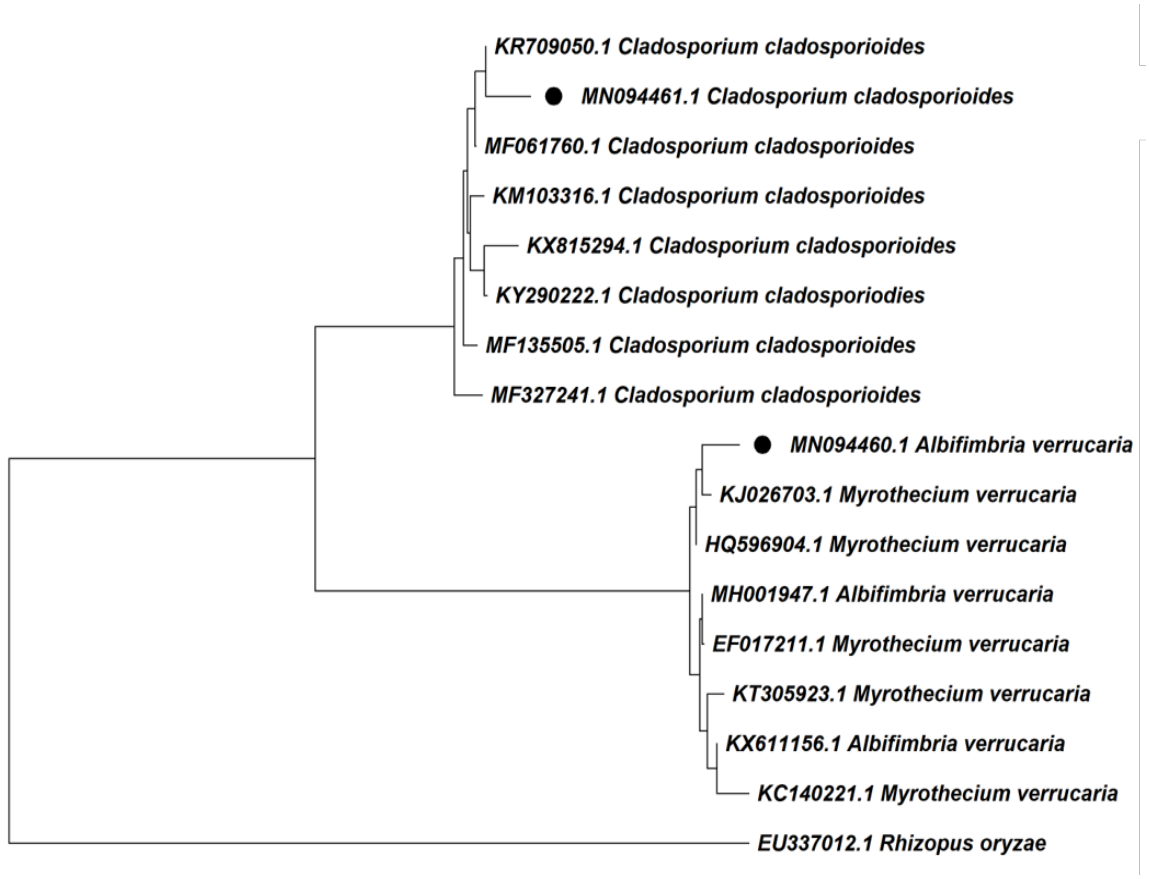

Fig. 3. Phylogenetic tree constructed using the Neighbor-Joining method based on ITS1-5.8S-ITS2 region sequences for the studied isolates and reference strains. This evolutionary analysis was conducted in MEGA X. The two studied isolates were *Cladosporium cladosporioides* MN094461.1 and *Albifimbria verrucaria* MN094460.1 (previously named *Myrothecium verrucaria)*. The genus *Myrocethecium* was re-evaluated recently leading to the introduction of 13 new genera including *Albifimbria* thus, some isolates appeared in this tree were named *M. verrucaria* because they were deposited in the NCBI database prior to such nomenclature re-evaluation. The tree was rooted with *Rhizopus* oryzae EU337012.1 (an outgroup taxon).

TABLE 6. Effect of *A. verrucaria* on chlorophyll and carotenoid contents and fresh weight of *Convolvulus arvensis* in greenhouse experiments.

Treatment	Pigment mg g^{-1} fresh weight						Fresh weight (mg)	
	Chlorophyll *a*		Chlorophyll *b*		Carotenoid			
	Mean±SD	*R%	Mean±SD	*R%	Mean±SD	*R%	Mean±SD	*R%
Control	1.06±0.05	0.00	0.30±0.03	0.00	0.38±0.03	0.00	337±6.0	0.00
**T1	0.73±0.23	31.66	0.19±0.01	36.15	0.35±0.04	8.52	295±0.6	12.26
**T2	0.69±0.11	35.11	0.13±0.01	57.47	0.31±0.02	18.63	214±2	36.39
LSD	0.30		0.04		***N.S		0.083	

*R%: Percent of reduction.

**Conidial concentrations were: T1= 2.0 x 10^7conidia ml^{-1}, T2= 5.0 x 10^7conidia ml^{-1}; Spray applications were made with hand-held aerosol sprayers; foliage was sprayed until fully wetted. Treatments were replicated three times.

***N.S: Not significant .

TABLE 7. Effect of *A. verrucaria* on chlorophyll and carotenoid contents and fresh weight of *Portulaca oleracea* in greenhouse experiments.

Treatment	Pigment mg g^{-1} fresh weight						Fresh weight (mg)	
	Chlorophyll *a*		Chlorophyll *b*		Carotenoid			
	Mean±SD	*R%	Mean ± SD	*R%	Mean ± SD	*R%	Mean ± SD	*R%
Control	0.55±0.07	0.00	0.44±0.08	0.00	0.110±0.01	0.00	275±17.0	0.00
**T1	0.33±0.05	40.36	0.39±0.05	12.78	0.113±0.00	2.68	52±1.0	80.84
**T2	0.14±0.03	74.09	0.38±0.03	15.04	0.082±0.01	25.29	49±0.5	82.18
LSD	0.12		***N.S		0.01		0.02	

*R%: Percent of reduction.

**Conidial concentrations were: T1= 2.0 x 10^7conidia ml^{-1}, T2= 5.0 x 10^7conidia ml^{-1}; Spray applications were made with hand-held aerosol sprayers; foliage was sprayed until fully wetted. Treatments were replicated three times.

***N.S: Not significant .

TABLE 8. Effect of *C. cladosporioides*on chlorophyll and carotenoid contents and fresh weight of *Portulaca oleracea* in greenhouse experiments.

Treatment	Pigment mg g^{-1} fresh weight						Fresh weight (mg)	
	Chlorophyll *a*		Chlorophyll *b*		Carotenoid			
	Mean ± SD	*R%	Mean ± SD	*R%	Mean ± SD	*R%	Mean ± SD	*R%
Control	0.474±0.01	0.00	0.401±0.01	0.00	0.103±0.00	0.00	314±1.0	0.00
**T1	0.211±0.01	55.20	0.359±0.02	10.47	0.096±0.01	1.16	115±0.5	63.37
**T2	0.128±0.01	72.98	0.354±0.01	11.72	0.074±0.00	28.15	82±0.3	73.88
LSD	0.016		0.036		0.0029		0.012	

*R%: Percent of reduction.

**Conidial concentrations were: T1= 2.0 x 10^7conidia ml^{-1}, T2= 5.0 x 10^7conidia ml^{-1}; Spray applications were made with hand-held aerosol sprayers; foliage was sprayed until fully wetted. Treatments were replicated three times.

LC-MS/MS analyses of crude extracts from the selected fungal culture filtrates

Organic extracts in ethyl acetate of the most active isolates (*A. verrucaria and C. cladosporioides*) were subjected to LC-MS/MS analysis to confirm the presence of different secondary metabolites. Seven compounds belonging to macrocyclic trichothecene were identified by comparison of the fragmentation pattern, obtained from the analysis of *A. verrucaria* organic extract, with reference standards and/or by spectral library searches. The seven identified macrocyclic trichothecene were as following: Verrucarin-L-acetate, Verrucarin M, Verrucarin A, Myrothecol, Roridin E, Roridin A, and Verrucarol. In the same context, the fragmentation pattern of *C. cladosporioides* crude organic extract revealed the presence of twelve compounds in major amount which included eleven phenolic compounds (P-methyl benzoic acid, P-hydroxy benzoic acid, Cladosporin, Diacetylcladosporin, Cladosporinone, Cladosporol, Cladosporol C, 3-7 dimethyl 8-hydroxyl 6-methoxy isochroman, Cinnamic acid, Chlorogenic acid, and Viriditoxin) and one polyketide compound (Mollicellin A). The patterns of metabolites recorded by LCMS/MS analysis were displayed in Fig. 4, 5 (a-c).

Discussion

Traditional weed control methods have become unsuccessful for several reasons. Herbicide-resistant weed are the main problem in weed control because of the number of weed biotypes resistant to herbicides that are constantly increasing due to the continuous use of the same products for years. There is a need to develop alternative weed control methods to help reduce dependence on chemical herbicide (Singh & Pandey, 2019). Biological control is highly specific and is represent an innovative long term solution to control herbicide-resistant weed using the natural bio-herbicidal power produced by some microorganisms (Abouzeid & El-Tarabily, 2010). Fungi and fungal metabolites had been used widely for the production of bioherbicides (Khattak et al., 2017). The safety of fungal biocontrol agents towards human and non-target organisms is an important criterion for consideration. However, current research suggests that risks should be

assessed compared to the benefits of microbial bio-agents that always outweigh those of chemical pesticides, due to the numerous adverse health effects of chemical pesticides that include carcinogenicity and many types of delayed toxicity such as reproductive defects, fetal damage, delayed neurologic manifestations, possible respiratory and immunologic disorders, etc. (Pell et al., 2001; Singh & Pandey, 2019).

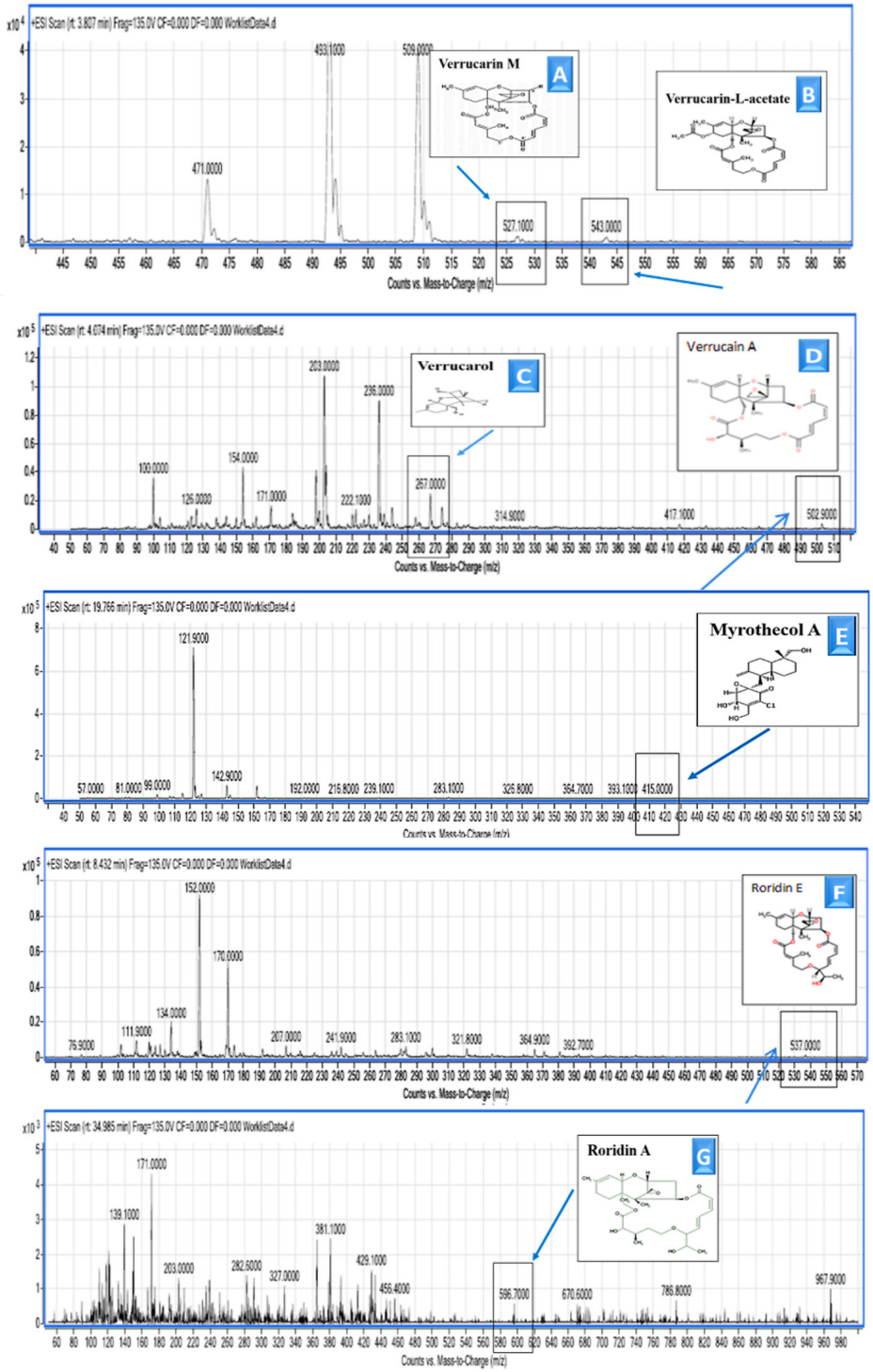

Fig. 4. LC-MS/MS fragmentation pattern of *A. verrucaria* organic extract showing peaks coresponding to (A) Verrucarin-L-acetate (m/z 527); (B) Verrucarin M (m/z 543); (C) Verrucarol (m/z 267); (D) Verrucarin A (m/z 502); (E) Myrothecol (m/z 415); (F) Roridin E (m/z 537); (G) Roridin A (m/z 596).

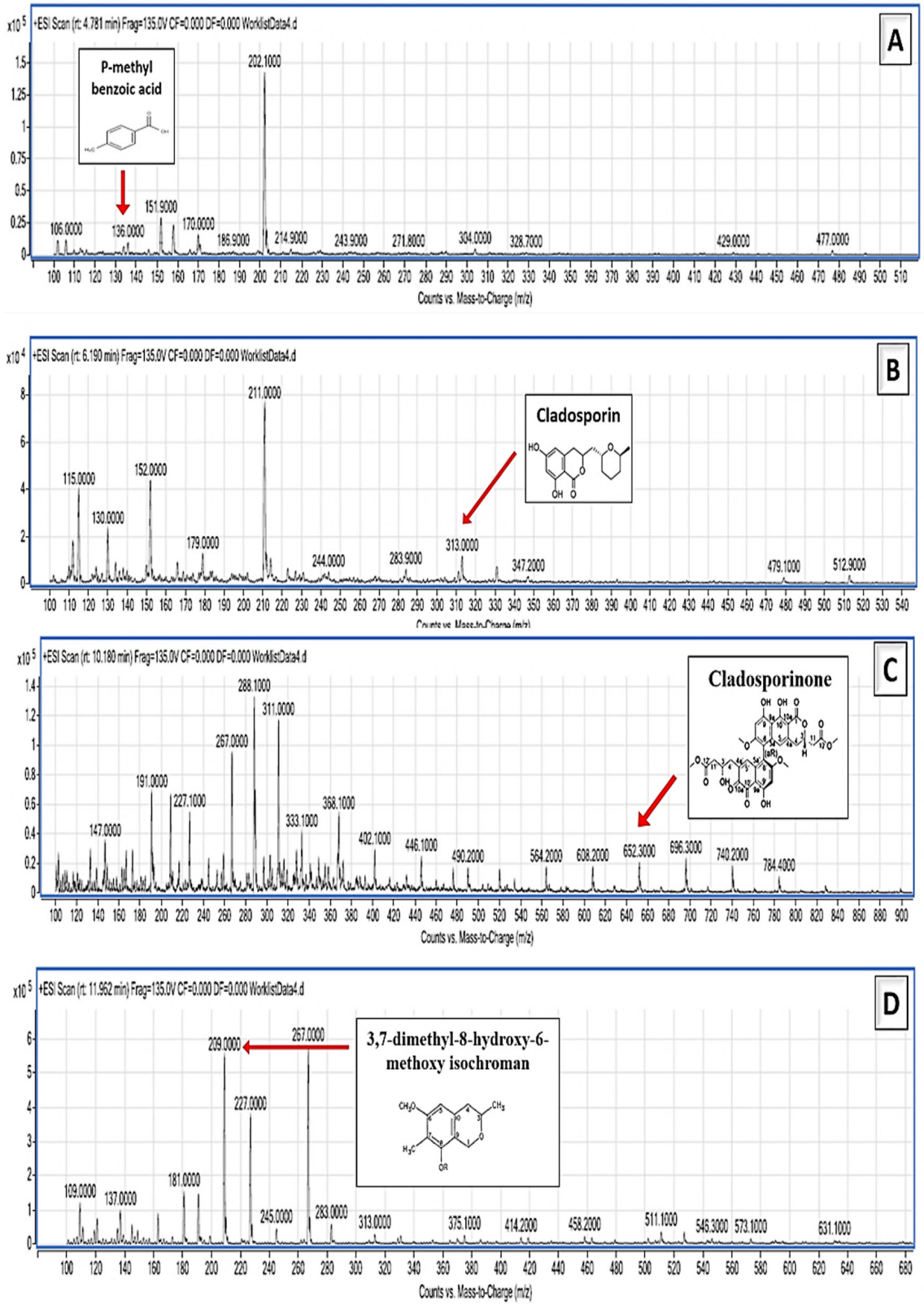

Fig. 5a. LC-MS/MS fragmentation pattern of *C. cladosporioides* organic extract showing peaks coresponding to (A) P-methyl benzoic acid (m/z 136); (B) Cladosporin (m/z 313); (C) Cladosporinone (m/z 652); (D) 3,7-dimethyl-8-hydroxy-6-methoxy isochroman (m/z 209).

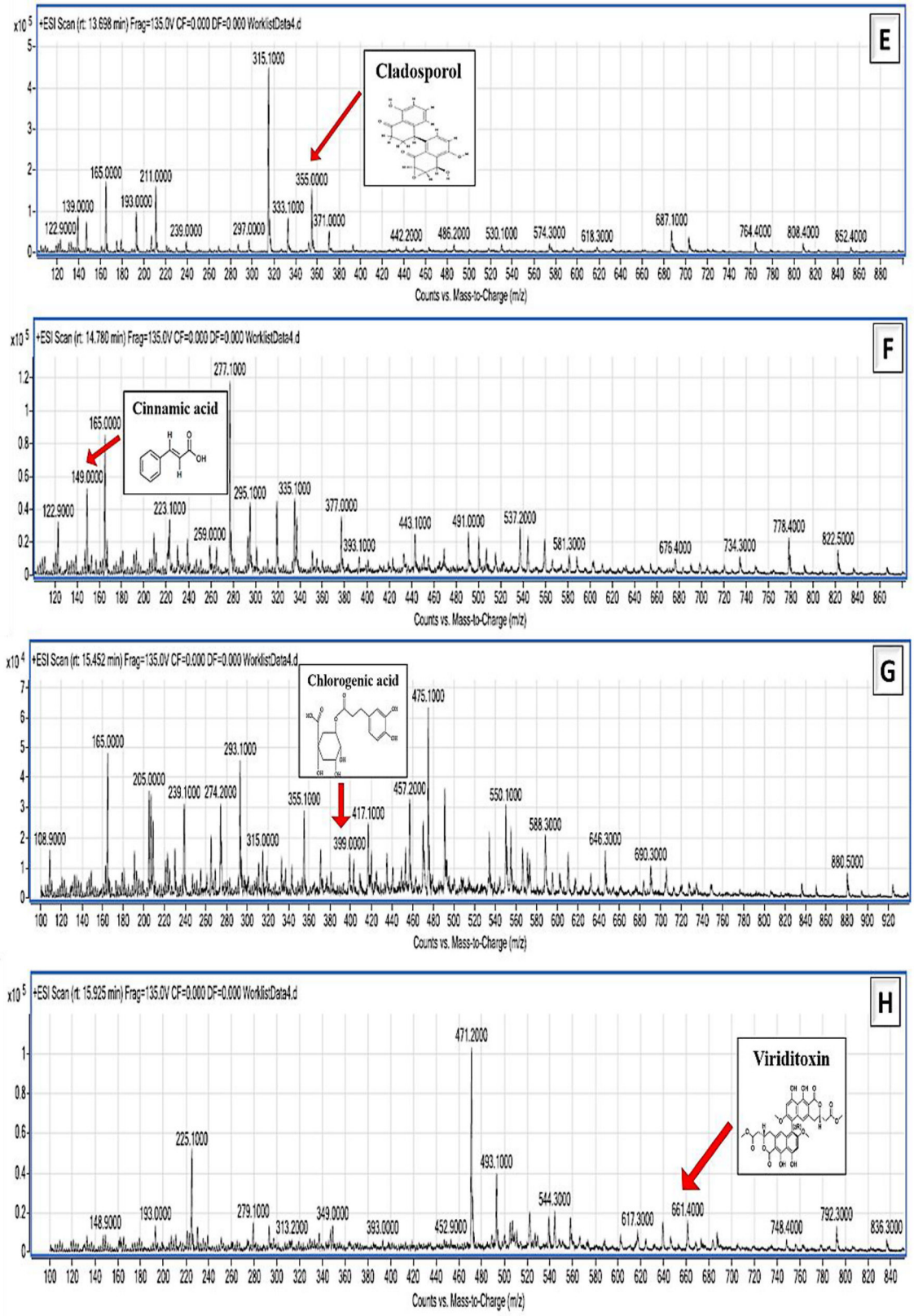

Fig. 5b. LC-MS/MS fragmentation pattern of *C. cladosporioides* organic extract showing peaks coresponding to (E) Cladosporol (m/z 355); (F) Cinnamic acid (m/z 149); (G) Chlorogenic acid (m/z 399); (H) Viriditoxin (m/z 661).

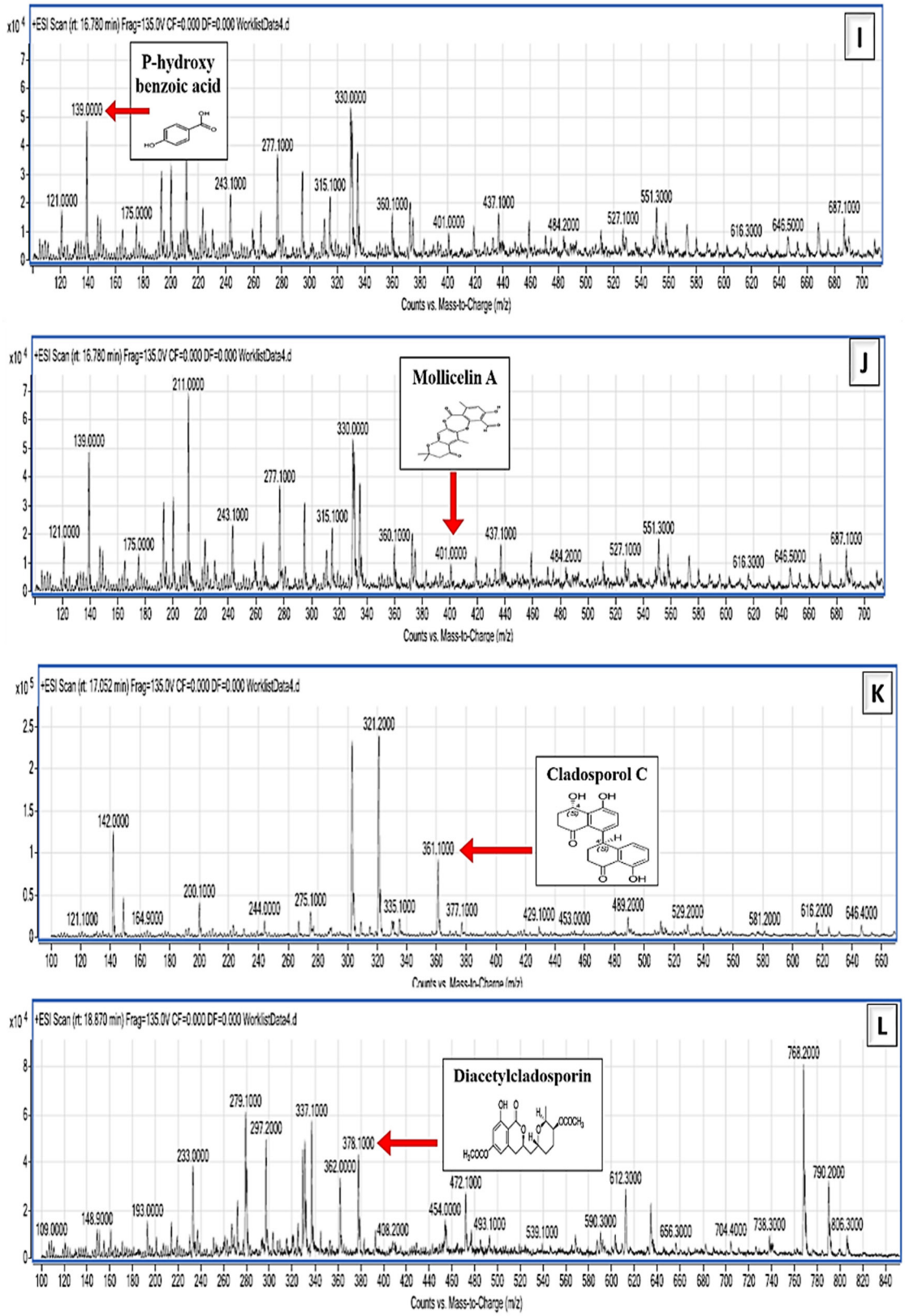

Fig. 5c. LC-MS/MSfragmentation pattern of *C. cladosporioides* organic extract showing peaks coresponding to (I) P-hydroxy benzoic acid (m/z 139); (J) Mollicelin A (m/z 401); (K) Cladosporol C (m/z 361); (L) Diacetylcladosporin (m/z 378).

Aspergillus is one of the most common fungi known to produce numerous secondary metabolites. Several studies demonstrated the herbicidal activity of various *Aspergillus* sp. against different weeds (Javaid et al., 2014; Khattak et al., 2014). In this study, a high reduction in seed germination and growth of *Portulaca oleracea* and *Convolvulus arvensis* were obtained by *A. flavus* culture filtrate. While *A. terreus* showed a selective herbicidal activity which reduced *Convolvulus arvensis* seed germination and growth without affecting *Portulaca oleracea*. On the other hand, both *Aspergillus* species had less herbicidal activity against the seedling stage of *Portulaca oleracea* and *Convolvulus arvensis*. This finding was consistent with the studies conducted by Wolf & Peng (2011) and Boyette et al. (2014). They concluded that the efficacy of some mycoherbicides decreases as the plants begin to mature.

The potential use of *Cladosporium* sp. in weed control was recorded in several previous studies (Kumar et al., 2009; Waqas et al., 2013; Lu et al., 2016; Wang et al*.,* 2016). *C. cladosporioides* is a common airborne saprobe which wasn't recorded to cause any serious health problems. Whose spores may only trigger an allergic reaction in asthma patients like many other airborne fine particles including dust and pollen grains (Ogórek et al., 2012; Lin et al., 2016). Besides that *C. cladosporioides* wasn't previously reported to produce any mycotoxin of concern. On the contrary, the ability of this fungal species to degrade ochratoxin A was recorded (Abrunhosa et al., 2002; Chalfoun et al., 2009) and the biological pest control potential of *C. cladosporioides* has been widely studied. It was recorded as a bioactive agent against several pests including toxigenic fungi, weed and mites. Thus, it was considered as a promising candidate for biological pest control (Chalfoun et al*.,* 2009; Wang et al., 2016; Gámez-Guzmán et al., 2019).

In the present study, *C. cladosporioides* showed highly significant inhibitory effect against *Portulaca oleracea* seed germination and seedling growth causing a 100% reduction in seed germination and a reduction percentage in total seedling biomass fresh weight reached 77.96%. However, to our knowledge*, C. cladosporioides* has not been reported before for its herbicidal activity against *Portulaca oleracea.* The LC-MS/MS analysis of *C. cladosporioides* MN094461.1 crude organic extract revealed the presence of 11 active phenolic compounds mostly related to cladosporin, benzoic acid and viriditoxin derivatives. Cladosporin was first isolated and studied by Scott et al. (1971) and a recent study carried out by Wang et al. (2016) concluded that Cladosporin is a promising naturally occurring fungal metabolite mainly isolated from *C. cladosporioides*, which was proved to be a potent antibacterial, antifungal, insecticidal and anti-inflammatory agent. Besides, the authors reported also that cladosporin selectively inhibited the growth of *Agrostis* (bentgrass) and showed no activity against lettuce, which indicates its great potential as a selective bio-herbicide. Similarly, the present study revealed that the *C. cladosporioides* MN094461.1 organic crude extract (containing metabolites) significantly inhibited the seed germination and growth of *P. oleracea* without having any effect on *C. arvensis*, thus it can be used as a potent selective bioherbicide against *P. oleracea*. The presence of two benzoic acid derivatives including P-methyl benzoic acid and P-hydroxy benzoic acid were recorded in the present study. Likewise, Waqas et al. (2013) isolated benzoic acid from *C. cladosporioides* LWL5 culture and identified it for the first time as a plant growth inhibitory metabolite. A study conducted by Liu et al. (2016) isolated the viriditoxin derivative, cladosporinone, along with the known viriditoxin from *C. cladosporioides*. The authors reported that the viriditoxin had potent cytotoxic and antibacterial activity while cladosporinone possed only strong cytotoxicity against the murine lymphoma cell line.

Myrothecium sensu lato (belonging to family Stachybotryaceae) has a worldwide distribution. The genus was re-evaluated based on phylogenetic analyses using multi-locus gene sequences leading to the introduction of 13 new genera with Myrothecium-like morphology (Lombard et al., 2016). In this study, the most potent fungal isolate against the target weeds was molecularly identified based on the DNA sequence data of the ITS1-5.8S-1TS2 region as *Albifimbria verrucaria* MN094460.1, which was previously named *Myrothecium verrucaria*.

M. verrucaria is a fungal pathogen that has been extensively reported as a biocontrol agent for use against several weeds (Anderson et al., 2004; Boyette et al., 2007; Weaver et al., 2012; Boyette et al., 2014; Hoagland et al., 2016). In

this study, organic crude extract of *A. verrucaria* MN094460.1 was applied to control *Portulaca oleracea* (common purslane) obtaining high reduction in seed germination, shoot length, root length, and biomass fresh weight. It was also highly virulent when applied as conidial sprays in greenhouse causing significant reduction in the common purslane fresh weight (81-82%). Boyette et al. (2007) concluded that conidial spray of *M. verrucaria* showed an effective herbicidal activity (95% reduction in dry weight) against common purslane in greenhouse and field tests. Whereas, Lee et al. (2008) recorded that *M. roridum* F0252 organic crude extract had 95-100% herbicidal activity against *Calystegia japonica* (bindweed) which is a very similar weed to *Convolvulus arvensis* and belongs to the same family (Convolvulaceae). In this study, *A. verrucaria* MN094460.1 crude extract was significantly reduced the total biomass fresh weight of *Convolvulus arvensis* seedling (59%).

Although, *M. verrucaria* (now named *A. verrucaria*) possesses promising bio-herbicidal properties with high virulence and broad weed host range, production of undesirable macrocyclic trichothecene mycotoxins represent significant safety concerns (Hoagland et al., 2007b). Many successful approaches have been conducted to reduce or eliminate the hazards associated with macrocyclic trichothecenes production in order to develop safe *M. verrucaria* formulations (Daba, 2019). These approaches include: Preventing spore production during growth of the fungus via submerged fermentation to produce a bio-herbicidal mycelial formulation with mitigated mycotoxin (Boyette et al., 2008), influencing the types of carbon and nitrogen sources and their concentrations in the nutritional media to regulate mycotoxin production (Weaver et al., 2009), washing of spores to reduce macrocyclic trichothecene concentrations in the obtained fungal preparations with no significant effect on the bio-herbicidal efficacy (Weaver et al., 2012). Also, more recent research developed non-sporulating mutant strain of *M. verrucaria* with effective phytotoxicity to several weeds and lower levels of mycotoxins (Hoagland et al., 2016).

Conclusion

In conclusion, the present investigation revealed that *Albifimbria verrucaria* MN094460.1 is a highly effective bioherbicide against both *Portulaca oleracea* and *Convolvulus arvensis*. It is worthy to mention that this study represents the first report on the herbicidal activity of *Cladosporium cladosporioides* against *P. oleracea*. Also, the phenolic compounds (including cladosporin and benzoic acid derivatives) recorded in the organic extract of *C. cladosporioides* MN094461.1 are known to have low toxicity and bioherbicidal activity. Thus, it could be considered as a promising bioagent for use in weed control. However, further studies are needed to optimize the production of active compounds on a large scale. Also, future work is required to minimize or eliminate the risks associated with macrocyclic trichothecenes production to develop safe formulations of *A. verrucaria* MN094460.1. In addition, bioassay using non-target organisms is essential to safely apply the bioherbicide in the field without harming the environment. Also, finding a stable formulation of bioherbicide to increase shelf life of the living organisms to improve survival and efficacy will facilitate the successful application of these bioherbicides in agricultural fields.

Acknowledgment: The author would like to acknowledge the Analytical Chemistry Unit (ACAL), Faculty of Science, Assiut University for assisting in performing the LC-MS/MS analysis. Many thanks are due to Prof. Dr. Mohamed A. Balah, Plant Protection Department, Desert Research Center, for the interpretation of LC-MS/MS data and identification of the expected compounds. Many thanks and gratitude to Prof. Dr. Mohamed A. Abouzeid, Head of Microbiology Department, Faculty of Science, Ain Shams University, for whose helpful comments and suggestions on the experiments' design and the critical revision of the early draft of this manuscript.

References

Abouzeid, M.A., El-Tarabily, K.A. (2010) Suppression of germination and parasitic establishment of bean and hemp broomrapes by *Fusarium* isolates. *Phytopathologia Mediterranea*, **49**(2), 51-64. Doi: 10.14601/Phytopathol_Mediterr-3181

Abrunhosa, L., Serra, R., Venâncio, A. (2002) Biodegradation of ochratoxin A by fungi isolated from grapes. *Journal of Agricultural and Food Chemistry*, **50**(25), 7493-7496. Doi: 10.1021/jf025747i

Anderson, K.I., Hallett, S.G. (2004) Herbicidal spectrum and activity of *Myrothecium verrucaria*. *Weed Science*, **52**(4), 623-627. Doi: 10.1614/ws-03-101r1

Balah, M. (2014) Exploiting *Matricaria chamomilla* flowers allelopathic constituents for controrolling associated weeds to wheat crop. *Egyptian Journal of Desert Research*, **64**(1), 1-16. Doi: 10.21608/ejdr.2014.5807

Balah, M.A.A. (2019) Weeds flora demographic analysis of date palm orchards in El-Bahariya, El-Frafra and Siwa Oases. *Acta Ecologica Sinica*, **39** (1), 14-20. Doi:10.1016/j.chnaes.2018.07.002

Bensch, K., Braun, U., Groenewald, J., Crous, P. (2012) The Genus Cladosporium. *Studies in Mycology*, **72**, 1-401. Doi: 10.3114/sim0003

Boyette, C.D., Hoagland, R.E., Abbas, H.K. (2007) Evaluation of the bioherbicide *Myrothecium verrucaria* for weed control in tomato (*Lycopersicon esculentum*). *Biocontrol Science and Technology,* **17**(2), 171-178. Doi: 10.1080/09583150600937451

Boyette, C.D., Weaver, M.A., Hoagland, R.E., Stetina, K.C. (2008) Submerged culture of a mycelial formulation of A bioherbicidal strain of *Myrothecium verrucaria* with mitigated mycotoxin production. *World Journal of Microbiology and Biotechnology*, **24**(11), 2721-2726. Doi: 10.1007/s11274-008-9759-6

Boyette, C., Hoagland, R., Stetina, K. (2014) Biological control of the weed hemp sesbania (*Sesbania exaltata*) in rice (*Oryza sativa*) by the fungus *Myrothecium verrucaria*. *Agronomy*, **4**(1), 74-89. Doi: 10.3390/agronomy4010074

Buchwaldt, L., Green, H. (1992) Phytotoxicity of destruxin B and its possible role in the pathogenesis of *Alternaria brassicae*. *Plant Pathology*, **41**(1), 55-63. Doi: 10.1111/j.1365-3059.1992.tb02316.x

Chalfoun, S.M., Souza, L.P., Pereira, M.C., Pimenta, C.J., Botelho, D.M.S. (2009) Antifungal potential of *Cladosporium cladosporioides* (Fres.) de Vries Metabolites in reduction of coffee contamination by toxigenic *Aspergillus genera*. *BioMicroWorld, Lisboa,* **259**, 13-117.

Chutia, M., Mahanta, J.J., Bhattacharyya, N. , Bhuyan, M., Boruah, P., Sarma, T. (2007) Microbial Herbicides for weed management: Prospects, progress and constraints. *Plant Pathology Journal*, **6**(3), 210-218. Doi: 10.3923/ppj.2007.210.218

Daba, G. M. (2019) Myrothecium as promising model for biotechnological applications, potentials and challenges. *Biomedical Journal of Scientific & Technical Research*, **16**(3). Doi: 10.26717/bjstr.2019.16.002869

Defago, G., Ammon, H.U., Cagan, L., Draeger, B., Greaves, M.P, Guntli, D., Hoeke, D., Klimes, L., Lawrie, J., Moenne-Loccoz, Y., Nicolet, B., Pfirter, H.A., Tabacchi, R., Toth, P. (2001) Towards the biocontrol of bindweeds with a mycoherbicide. *BioControl,* **46**, 157-173. Doi:10.1023/A:1011441816615

Ellis, M.B. (1971) Dematiaceous Hyphomycetes, *Commonwealth Mycological Institute, Kew, England*.

FAO (2011) The Lurking Menace of weeds. In: Food and Agriculture Organization of the United Nations. 20.07.2011. Available from: <http://www.fao.org/news/story/0/item/29402/icode/en/>.

Gámez-Guzmán, A., Torres-Rojas, E., Gaigl, A. (2019) Potential of a *Cladosporium cladosporioides* strain for the control of *Tetranychus urticae* Koch (Acari: Tetranychidae) under Laboratory Conditions. *Agronomía Colombiana*, **37**(1), 84-89. Doi: 10.15446/agron.colomb.v37n1.73353

Harding, D.P., Raizada, M.N. (2015) Controlling weeds with fungi, bacteria and viruses: A review. *Frontiers in Plant Science,* **6**, 659. Doi: 10.3389/fpls.2015.00659

Hoagland, R.E., Boyette, C.D., Weaver, M.A., Abbas, H.K. (2007a) Bioherbicides: Research and risks. *Toxin Reviews*, **26**(4), 313-342. Doi: 10.1080/15569540701603991

Hoagland, R.E., Weaver, M.A., Boyette, C.D. (2007b) *Myrothecium verrucaria* fungus: A bioherbicide and strategies to reduce its non-target risks. *Allelopathy Journal,* **19**, 179-192.

Hoagland, R.E., Boyette, C.D., Stetina, K.C., Jordan, R.H. (2016) Bioherbicidal efficacy of a *Myrothecium verrucaria*-sector on several plant species. *American Journal of Plant Sciences*, **07**(16), 2376-2389. Doi: 10.4236/ajps.2016.716208

Jacobs, J. (2007) Ecology and management of field bindweed (*Convolvulus arvensis* L.). US Department of Agriculture, *Natural Resources Conservation Service,* **9***,* 1-9.

Javaid, A., Shoaib, A., Bashir, U., Akhtar, R. (2014) Screening of various species of *Aspergillus* for herbicidal activity against *Parthenium* weed. *Pakistan Journal of Weed Science Research,* **20**(2), 137-144.

Johnson, A., Booth, C. (1983) "*Plant Pathologist's Pocketbook*", 2nd ed. Commonwealth Agricultural Bureaux. *International Mycological Institute, Kew, England,* 269-274.

Khattak, S.U., Iqbal, Z., Lutfullah, G., Bacha, N., Khan, A.A., Saeed, M., Ali, M. (2014) Phytotoxic and herbicidal activities of *Aspergillus* and *Penicillium* species isolated from rhizosphere and Soil. *Pakistan Journal of Weed Science Research,* **20***,* 293-303.

Khattak, S.U., Lutfullah, G., Iqbal, Z., Rehman, I.U., Ahmad, J., Khan, A.A. (2017) Herbicidal activity of pure compound isolated from rhizosphere inhabiting *Aspergillus flavus*. *Natural Product Research*, **32**(10), 1212-1215. Doi: 10.1080/14786419.2017.1326038.

Klich, M.A. (2002) Identification of Common *Aspergillus* Species. *Centraalbureau voor schimmelcultures.*

Kumar, A., Verma, V.C., Gond, S.K., Kumar, V., Kharwar, R.N. (2009) Bio-control potential of *Cladosporium* sp. (MCPL-461), against a noxious weed *Parthenium hysterophorus* L. *Journal of Environmental Biology,* **30**(2), 307-312.

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K. (2018) MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, **35**(6), 1547-1549. Doi: 10.1093/molbev/msy096

Lee, H.-B., Kim, J.-C., Hong, K.-S., Kim, C.-J. (2008) Evaluation of a fungal strain, M*yrothecium roridum* F0252, as a bioherbicide agent. *The Plant Pathology Journal*, **24**(4), 453-460. Doi: 10.5423/ppj.2008.24.4.453

Lin, W.R., Chen, Y.H., Lee, M.F., Hsu, L.Y., Tien, C.J., Shih, F.M., Wang, P.H. (2016) Does spore count matter in fungal allergy?: The role of allergenic fungal species. *Allergy, Asthma & Immunology Research*, **8**(5), 404. Doi: 10.4168/aair.2016.8.5.404

Liu, Y., Kurtán, T., Wang, C.Y., Lin, W.H., Orfali, R., Müller, W.E., Proksch, P. (2016) Cladosporinone, a new viriditoxin derivative from the hypersaline Lake derived fungus *Cladosporium cladosporioides*. *The Journal of Antibiotics*, **69**(9), 702-706. Doi: 10.1038/ja.2016.11

Lombard, L., Houbraken, J., Decock, C., Samson. R.A., Meijer, M., Re-´blova.´M., Groenewald, J.Z., Crous, P.W. (2016) Generic hyper-diversity in Stachybotriaceae. *Persoonia,* **36**, 156-246. Doi: 10.3767/003158516X691582

Lu, Y.-H., Li, S., Shao, M.-W., Xiao, X.-H., Kong, L.-C., Jiang, D.-H., Zhang, Y.-L. (2016) Isolation, identification, derivatization and phytotoxic activity of secondary metabolites produced by *Cladosporium oxysporum* DH14, a Locust-associated fungus. *Journal of Integrative Agriculture*, **15**(4), 832-839. Doi: 10.1016/s2095-3119 (15) 61145-5

Mashaly, I.A., Awad, E.R. (2003) Weed flora of orchards in the Nile Delta, Egypt: Floristic features. *Asian Journal of Plant Sciences,* **2**(3), 314-324. Doi: 10.3923/ajps.2003.314.324

Ogórek, R., Lejman, A., Pusz, W., Miłuch, A. Miodyńska, P. (2012) Characteristics and taxonomy of *Cladosporium* fungi. *Mikologia Lekarska*, **19**(2), 80-85.

Patel, R., Patel, D.R. (2015) Biological control of weeds with pathogens: Current status and future trends. *International Journal of Pharmacy and Life Sciences,* **6**(6), 4531-4550.

Pell, J.K., Eilenberg, J., Hajek, A.E., Steinkraus, D.C. (2001) In: "*Fungi as Biocontrol Agents: Progress, Problems and Potential*", Butt, T.M., Jackson, C., Magan, N. (Eds.), pp. 71-153. CAB International, Wallingford.

Ritz, C., Baty, F., Streibig, J.C., Gerhard, D. (2015) Dose-response analysis using R. *PlosOne*, **10**(12). Doi: 10.1371/journal.pone.0146021

Saitou, N., Nei, M. (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*. **4,** 406-425. Doi: 10.1093/oxfordjournals.molbev.a040454

Scott, P.M., Walbeek, W.V., Maclean, W.M. (1971) Cladosporin, a new antifungal metabolite from *Cladosporium cladosporioides*. *The Journal of Antibiotics*, **24**(11), 747-755. Doi: 10.7164/antibiotics.24.747

Sinclair, J.B., Dhingra, O.D. (1995) "*Basic Plant Pathology Methods*". 2nd ed., CRC Press, USA.

Singh, A.K., Pandey, A.K. (2019) Fungal metabolites as a natural source of herbicide: A novel approach of weed management. *Journal of Applied and Natural Science*, **11**(1), 158-163. Doi: 10.31018/jans.v11i1.1994

Smedsgaard, J. (1997) Micro-scale extraction procedure for standardized screening of fungal metabolite production in cultures. *Journal of Chromatography A*, **760**(2), 264-270. Doi: 10.1016/s0021-9673(96)00803-5

Snedecor, G.W., Cochran, W.G. (1990) "*Statical Methods*". 8th ed., Iowa State Univ. Press, Ames. Iowa, USA.

Tamura, K., Nei, M., Kumar, S. (2004) Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences*, **101**(30), 11030-11035. Doi: 10.1073/pnas.0404206101

Tóth, P., Cagáň, Ľ. (2005) Organisms associated with the family Convolvulaceae and their potential for biological control of *Convolvulus arvensis. Biocontrol News and Information,* **26**(1), 17N-40N.

Vencill, W.K., Nichols, R.L., Webster, T.M., Soteres, J.K., Mallory-Smith, C., Burgos, N.R., Mcclelland, M.R. (2012) Herbicide resistance: Toward an understanding of resistance development and the impact of herbicide-resistant crops. *Weed Science*, **60**(SP1), 2-30. Doi: 10.1614/ws-d-11-00206.1

Wang, X., Wedge, D.E., Cutler, S.J. (2016) Chemical and biological study of cladosporin, an antimicrobial inhibitor: A review. *Natural Product Communications*, **11**(10). Doi: 10.1177/1934578x1601101039

Waqas, M., Khan, A., Ali, L., Kang, S.-M., Kim, Y.-H., Lee, I.-J. (2013) Seed germination-influencing bioactive secondary metabolites secreted by the endophyte *Cladosporium cladosporioides* LWL5. *Molecules*, **18**(12), 15519-15530. Doi: 10.3390/molecules181215519

Weaver, M.A., Boyette, C.D., Hoagland, R.E. (2012) Bioherbicidal activity from washed spores of *Myrothecium verrucaria*. *World Journal of Microbiology and Biotechnology*, **28**(5), 1941-1946. Doi: 10.1007/s11274-011-0996-8

Weaver, M., Hoagland, R., Boyette, C., Zablotowicz, R. (2009) Macrocyclic trichothecene production and sporulation by a biological control strain of *Myrothecium verrucaria* is regulated by cultural conditions. *World Mycotoxin Journal*, **2**(1), 35-43. Doi: 10.3920/wmj2008.1026.

Wolf, T.M., Peng, G. (2011) Improving bioherbicide spray deposition on vertical plant structures: The role of nozzle angle, boom height, travel speed and spray quality. *Pest Technology,* **5**(1), 67-72.

الفطريات كعوامل مكافحة حيوية محتملة ضد العليق و الرجلة التي تغزو النظم الإيكولوجية الزراعية لمصر

نيفين أحمد إبراهيم[1]، ميرهان محمد توفيق[2]

[1]قسم الميكروبيولوجي- كلية العلوم- جامعة عين شمس- القاهرة- مصر، [2]مركز بحوث الصحراء - قسم وقاية نبات- المطرية- القاهرة- مصر.

تعد المكافحة الحيوية الفطرية للأعشاب الضارة بديل صديق للبيئة للحد من مخاطر مبيدات الأعشاب الأصطناعية. أسفر المسح الميداني الذى أُجري في منطقتي وادي النطرون وشمال سيناء بمصر، عن عزل 36 عزلة فطرية. تم فحص مرشحات مزارع العزلات التي تم الحصول عليها لإختبار قدرتها على تثبيط إنبات بذور وتطور بادرات كلٍ من الرجلة والعليق، أبدت مستخلصات مرشحات المزارع الخام لكل من *Albifimbria verrucaria* MN094460.1 و *Cladosporium cladosporioides* MN094461.1 أعلى تأثير مبيد للأعشاب ضد كلتا العشبتين محل الدراسة. حيث قد أدى مرشح مزرعة فطرة *A. verrucaria* إلى خفض إنبات البذور بشكل ملحوظ، وخفض وأيضاً طول الساق وطول الجذر لكلتا العشبتين المختبرتين. كما أن مستخلصه الخام قلل بشكل كبير من الكتلة الحيوية للوزن الطازج للبادرات بنسب بلغت 85% في حالة نبات الرجلة و59% في حالة نبات العليق. ومن ناحية أخرى، فقد تبين أن مرشح مزرعة فطرة *C. cladosporioides* منع إنبات بذور نبات الرجلة تماماً، بينما تسبب مستخلصه الخام في إنخفاض بنسبة 78% في الكتلة الحيوية لوزن البادرات الطازج. هذا وقد تم إجراء اختبار لتأثير الفطرتين سالفتي الذكر على نمو بادرات الرجلة والعليق فى الصوبة الزراعية من خلال رش حشائش الرجلة والعليق بجراثيم الفطرتين كلٍ على حدا، حيث أدى تطبيق تركيز 5×10^7 جرثومة/مل من *A. verrucaria* بعد خلطها مع 0.2 silwet-L-77% على حشائش الرجلة إلى خفض في نسبة الكلوروفيل-أ بقيمة 74% كما قلل في الكتلة الحيوية للوزن الطازج بنسبة 82%. أما في حالة نبات العليق، فقد قلل نسبة الكلوروفيل-ب بنسبة 57%. وكذلك فقد أدى رش نفس التركيز ولكن من جراثيم فطرة *C. cladosporioides* (مع 0.2 silwet-L-77%) إلى تقليل نسبة الكلوروفيل-أ فى نبات الرجلة بقيمة 73% كما خفض الكتلة الحيوية للوزن الطازج بنسبة 74%. وكشف تحليل LC-MS/MS عن وجود 12 مركبة في المستخلص الخام لمرشح مزرعة فطرة *C. cladosporioides* و7 مركبات في المستخلص الخام لـ *A. verrucaria*. وخلصت هذه الدراسة إلى أن كلٍ من *A. Averrucaria* MN094460.1 و*C. cladosporioides* MN094461.1 تعتبر عوامل بيولوجية فعالة محتملة للمكافحة الحيوية لعشبتي الرجلة والعليق. بالإضافة إلى ذلك، فقد تم تسجيل النشاط المبيد للأعشاب لـ *C. cladosporioides* ضد عشبة الرجلة للمرة الأولى.