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***Corresponding author**
Ayoob Obaid Alfalahi
ag.ayoob.obaid@uoanbar.edu.iq

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Antifungal Potential of *Sonchus oleraceus* and *Lactuca virosa Extracts Against Fusarium verticillioides*, the Causal Agent of Damping-Off in Sweet Corn in Iraq

Alyaa Abd Ali[1], Ayoob Obaid Alfalahi[1*], Aalaa Khudhair Hassan[2]
1Department of Plant Protection, College of Agriculture, University of Anbar.
2Department of Plant Protection, College of Agricultural and Engineering Sciences, University of Baghdad

ABSTRACT

A factorial experiment was conducted under laboratory conditions to evaluate the inhibitory effect of ethanomethanolic extracts of *Sonchus oleraceus* L. and *Lactuca virosa* L. against *Fusarium verticillioides* and *Fusarium oxysporum* causing root and crown rot disease in sweet corn. The GC-MS analysis indicated diverse phytochemicals in both plant types, however, *S. oleraceus* had more active components than *L. virosa*. In general, Tetracosane, Bis(2-ethylhexyl) phthalate and Nonadecane are the dominant active components. The results of pathogenicity revealed that the isolated fungi were pathogenic to sweet corn, as both led to significant increase ($p \leq 0.05$) in the percentage of infection. However, the *F. verticillioides* isolate had the highest infection percentage of 85.00% with significant differences ($p \leq 0.05$) compared to the control group. Results of the antagonistic effect showed that the ethanomethanolic extract of *S. oleraceus* had the highest significant inhibitory percentages ($p \leq 0.05$) against both *Fusarium* isolates. Furthermore, positive relationship between the investigated concentrations and the inhibition percentage towards the maximum concentration of 9 mg/mL that revealed 100% inhibition capacity. The concentration of 7% reported an inhibition rates against *F. verticillioides* of 87% and 81% for each of *S. oleraceus* and *L. virosa* extracts, respectively. Also, the 7 mg/mL concentration significantly reduced the radial growth of *F. oxysporum* fungi causing an inhibition rates of 90% and 78%, respectively. Further investigation would be valuable in precise characterization of active compounds and developing them into more sustainable fungicides.

1.Introduction

Sweet corn is an annual plant of great importance, belonging to the Poaceae family (Moon et al., 2023). Although sweet corn is not common in Iraq, it is the primary cereal crop after wheat and rice in different regions around the world (Ewaid et al., 2020, Mohammed, 2023). This crop is well-known for its high nutritional value due to high carbohydrate content making it a successful alternative for food, feed and also as a source of raw materials for various industrial products also an essential source of protein. Furthermore, sweet corn is a good source of the phenolic, flavonoid and antioxidants (Baranowska, 2023, Swapna et al., 2020). In contrast to traditional field corn, sweet corn has rapidly gained popularity due to contains an allelic mutation disrupting the synthesis of starch in the endosperm. This explains the three-fold increase in sugar content compared with traditional corn (Finegan et al., 2022). Like other crops, sweet corn is affected by many plant diseases, which lead to significant losses in the crop and its production. Recent studies have confirmed the exposure of the crop to more than 20 fungal diseases, 13 viral diseases, 4 nematode diseases, 3 bacterial diseases, and 7 diseases that affect the crop after harvest. The most important fungal pathogens that infect sweet corn plants and cause seed rot, seedling death and root rot are *Fusarium* spp., *Phytophthora* spp., *Rhizoctonia* spp. and *Pythium* spp. (Zhu et al., 2024). *F. verticillioides* is a common pathogen in maize fields globally, and it is also found in Iraq. This pathogen poses a significant threat to maize production and quality, mainly through fusarium ear rot (FER). However, recent reports have indicated that the fungus can cause a number of rotting diseases in different crops including maize, the most significant of which is seedling damping-off (Martín-Pinto et al., 2008, Maciel et al., 2017, Sun et al., 2020). Unfortunately, the Iraq's hot and arid climate meets the environmental requirements of *F. verticillioides* to thrive and thus cause diverse diseases, especially during warmer months when maize is cultivated.

Plant extracts is recently emerged as a valuable technique in controlling plant pathogens. Plant extracts can be obtained from different plant parts of a specific plant types using a wide range of chemical solvents (Ayaz et al., 2023). These extracts have a proven effect in reducing the pathogen's ability to spread and reducing the disease severity caused by a number of soil-borne pathogens. This is due to the anti-fungal activity of these extracts that lead to the inability of the fungi spores to stick to the surface of the plant parts or lead to inhibiting the fungus or killing it during the spore germination and growth, thus preventing the fungi from penetrating and invading the plant tissue (Abbas et al., 2022). *S. oleraceus* L. and *L. virosa* L. are widely growing in the local environment and commonly described as noxious weeds, invading fields as well as marginal lands. Several reports pointed to the high content of phenols and flavonoids, thus it can be used as a potential source for antioxidant and antimicrobial bioactive compounds (Yin et al., 2007, El Gendy et al., 2024).

Based on the foregoing and the importance of sweet corn root and crown rot disease, as well as the associated losses, the current study aims to evaluate the antifungal potential of *S. oleraceus* and *L. virosa* ethanomethanolic extracts on the incidence of damping-off fungi under laboratory conditions.

2.Materials and Methods

2.1. Isolation and identification of pathogenic fungi

Seedlings of sweet corn showing stunting, discoloration, damping off, and root rot were collected from Anbar governorate (32.5598° N, 41.9196° E) during the Spring growing season of 2023-2024. The infected plants were kept separately in polyethylene bags containing all the necessary details, and directly transferred to Postgraduate Lab. at the Department of Plant Protection/ College of Agriculture/ University of Anbar. Plant samples were cleaned with tap water for 5 min to remove surface debris. Infected crown and root parts were carefully separated, cut into small pieces (0.5-1 cm), and superficially sterilized with sodium hypochlorite solution (1% free chlorine) for 2 minutes. Subsequently, pieces were washed with distilled water for 2 min, after that dried with sterile filter paper. Four pieces from each infected plant were cultured in 9 cm plates containing autoclaved PDA and supplemented with tetracycline antibiotic at 200 mg/L. After three days of incubation at 25 ± 1°C, the fungal isolates were further purified by transferring a 0.5cm mycelia plugs to a new PDA containing plates. The incubation step was repeated for 5 days at 25 ± 1°C. Finally, *F. verticillioides* and *F. oxysporum* were identified based on colony appearance and microscopic examination using a Light Compound Microscope (LCM), conidiophores and spores shapes according to (Infantino et al., 2023). Two isolates were also prepared and stored in a refrigerator at 4 °C.

2.2. Molecular diagnosis of companion pathogenic fungi

The DNA was extracted from pure cultures of the two fungal isolates at Aims Center (Baghdad, Iraq) using specialized kit ZR Fungal/Yeast/Bacterial DNA MiniPrep (ZYMO, USA). The extraction steps were conducted as the supplier instructed.

2.2.1. Yield and purity of extracted DNA

After performing the DNA extraction, the extracted DNA quantity and quality were estimated with the aid of Nanodrop spectrophotometer at two wavelengths (260/280) nm according to the following equation [White *et al.*,1990]:

$$DNA\ purity\ ratio = O.D.260/O.D.280$$

Then dilutions were made to get a final work concentration of 50 ng/µL.

2.2.2. Polymerase chain reaction (PCR)

Polymerase chain reaction (PCR) was used to amplify the ITS encoding region found in all eukaryote as a conservative descriptive region using a couple of specific primers; ITS1 (5′-TCCGTAGGTGAACCTGCGG -3′) as forward and ITS4 (5′ TCCTCCGCTTATTGATATGC-3′) as reverse purchased from Integrated DNA Technologies company (Canada). The PCR reaction was performed in a total volume of 25µl containing 2µl DNA, 5 µl Taq PCR PreMix (Intron, Korea), 1µl of each forward and revers primers (10 pmol) then distilled water was used to reach volume of 25µl. Thermal Cycler PCR system 9700 (Applied Biosystem, USA) was programmed to apply the following thermal profile: Denaturation at 95 °C for 3 min, followed by 35 cycles of 95 °C for 30s, 55°C for 30s and 72 °C for 1 min with final incubation at 72 °C for 5 min. The amplified products were separated using 2% agarose gel and visualized on 302nm ultraviolet light after red staining (Intron, South Korea), then photographed and documented. The PCR products then sent to the Macrogen Inc. company (Seoul, South Korea) to analyze the sequences of the amplified ITS fragments. Sanger sequencing method was adopted to sequence the gel extracted DNA in the National Instrumentation Center for Environmental Management-NICEM(http://nicem.snu.ac.kr/main/?en_skin=index.html), Biotechnology lab using 3730XL Genetic Analyzer machine (Applied Biosystems, USA). Homology comparisons of the query ITS sequences were conducted using Basic Local Alignment Search Tool (BLAST) software at the National Center Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov).

2.3. Pathogenicity of the isolated fungi

Pathogenicity of both fungal isolates *F. verticillioides,* and *F. oxysporum* was conducted according to Bolkan and Butler (1974). The water agar medium (2%) was prepared by mixing 20 g of agar in 1 L distilled water and then autoclaved for 20 min at 121ºC. The autoclaved mix was poured into 9 cm plates. After solidify, each plate was inoculated in the middle with a 0.5 cm disc from each fungal culture colony and incubated for 3 days at 25 ± 1°C. Seeds of local variety of sweet corn was superficially sterilized with sodium hypochlorite (1% free chlorine) for 2 min and raised with sterile water for 2 min before being placed in new plates in a circle manner at a rate of 10 seeds per plate. All the applied treatments including the control group were repeated in four replicates, and incubated for 7 days at 25 ± 1°C.

The following equation was applied to estimate infection percentage (%).

$$Infection\ (\%) = \frac{No.\ infected\ seeds}{No.\ total\ seeds} \times 100$$

2.4. Collection of plant material

Two plant extracts Common sowthistle (*S. oleraceus* L.) and Bitter lettuce (*L. virosa* L.) of Asteraceae family were selected to test their antagonistic effect against the isolated pathogenic fungi. To ensure higher level of bioactive components, fresh plant samples were collected at full-bloom stage from marginal lands in Ramadi city, Anbar governorate (32.5598° N, 41.9196° E). The collected samples were washed with water to get rid of dust and suspended impurities, then the plant samples were dried by spreading them in a well-ventilated room on a piece of cardboard and in a thin layer with continuous stirring of the samples to prevent rotting and speed up their drying. After complete drying, the samples were ground by an electric mill, and the fine powder of each sample was placed in polyethylene bags to be used for extraction.

2.4.1. Preparation of plant extracts

The ethanomethanolic extraction solution was prepared by adding 750 ml of methyl alcohol (absolute), to 150 ml of ethyl alcohol (absolute) mixed with 10 ml of distilled water. A total weight of 200 g of dried plant powder were mixed well in 1000 ml flask of the previously prepared ethanomethanolic solution using electric shaker (Alfalahi et al., 2024). For further homogenization, the extract was placed in Ultrasonic Bath Sonicator (50 kHz) at 30°C for four hours. Then, the extract was filtered with Whatman No.1 filter paper using Buchner funnel and vacuum pump at 40 C°. The final concentrated product was weighed and preserved in ambient bottle at -20 °C up to use.

2.4.2. Phytochemical analysis of plant extracts

A total weight of 50 mg of the dried samples were placed in a laboratory tube and sent for identification active components using Gas Chromatography-Mass Spectrometry (GC-MS) technology with aid a GC-MS-QP2010 (Shimadzu, Kyoto, Japan). The resulting spectrum of the active components was compared with the data of the National Institute of Standards and Technology (NIST14) and Wiley 10th/NIST 2014 Mass Spectral Library (W10N14) and matching results were adopted in identifying the active components.

2.5. Antifungal activity of plant extracts using poisoned food technique

The efficacy of plant extracts in inhibiting the fungal growth of *F. verticillioides* and *F. oxysporum* isolates was tested under laboratory conditions. Working solutions were prepared as reported by Seema et al. (2011) with minor modifications. A total weight of 360, 600, 840 and 1080 mg of each crude plant extract was dissolved individually in 12 mL of dimethyl sulfoxide (DMSO). Then, 2 mL from each previously prepared concentration was added separately to Petri dish containing 18 mL of the nutrient PDA medium to obtain a final concentrations of 3, 5, 7 and 9 mg/mL, respectively. Each treatment was represented by three replicates for each fungal isolate in addition to negative control treatment, where only DMSO was added. Positive control for each fungal isolate was included Uniform fungicide (322 g/L Azoxystrobin+ 124 g/L Metalaxyl-M), (Syngenta, Australia) at a rate of 1 mg/mL. After solidifying, the medium was inoculated with a single 0.5 cm disc of the isolate taken from actively growing cultures at 7 days of age, and then incubated at 25±2°C for 7 days and checked periodically. The radial growth of the tested fungal colony was recorded over 7 consecutive days, and the results were analyzed based on the growth of fungal colony negative control.

The growth inhibition percentage of the tested fungi was calculated as follows:

$$\text{Inhibition (\%)} = \frac{n - \mathrm{x}}{n} \times 100$$

Where:

n= Diameter of the fungal colony in the negative control.

x= Diameter of the fungal colony per treatment.

2.6. Statistical analysis

The statistical analysis was conducted using the GenStat (Version 10.3.0.0) statistical program, and factorial arrangement with completely randomized design (CRD) was used in implementing the laboratory experiments, and the averages were compared according to the least significant difference LSD at the probability level (0.05).

3.Results

3.1. Morphologic and molecular identification companion pathogenic fungi

The morphological characteristics of fungal colony in addition to microscopic aspects indicated that the previously isolated fungi from sweet corn seedlings are *F. verticillioides* and *F. oxysporum* (Figure 1). Of these, straight to slightly curved and thin- walled macroconidia were noted with curved apical cell and foot shaped basal cell varied in size 28-45µm x 3-5µm with three septa. However, after 2-3 weeks abundant chlamydospore structures were formed in single and/or pairs, with some exceptions as clusters were identified (Leslie and Summerell, 2008, Infantino et al., 2023), (Figure 1).

The PCR results confirmed the morphological characterization of the damping-off associated fungi on sweet corn to be *F. verticillioides* and *F. oxysporum* (Figure 1). The two isolates were registered in the GenBank under the deposition numbers of LC807020 for *F. verticillioides* and LC807030 for *F. oxysporum*.

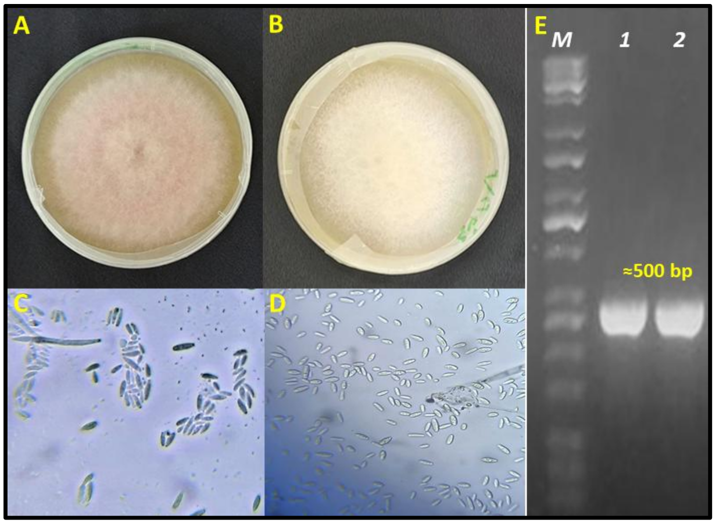

Figure 1: Colony morphology of *F. verticillioides* (A) and *F. oxysporum* (B) after 7 days on PDA, Macro and micro conidia of *F. verticillioides* (C) and *F. oxysporum* (D) under 40X magnification. E: PCR products of ITS region of *F. verticillioides* (1) and *F. oxysporum* (2) alongside 1kb ladder (M) electrophorsed on 2% agaros gel.

3.2. Pathogenicity of companion pathogenic fungi on Water Agar (WA)

The pathogenicity test revealed that the two isolates of *F. verticillioides* and *F. oxysporum* could significantly decrease the germination percentages ($p \leq 0.05$) compared to the control treatment that was 100% (Table 1). The isolate *F. verticillioides* and *F. oxysporum* decreased seed germination to 85% and 65 % respectively, compared to the control treatment (without pathogenic fungi) that exhibited 100% germination percentage. The reason for the low germination percentage in the presence of pathogen may be due to the ability of theses pathogens to secrete enzymes that decompose pectin and cellulose at the early stages of plant's life. This will be of great importance in penetrating the host, such as pectinase, pectin methyl esterase, pectinlyase and cellulase. In addition, the pathogen can produce some other substances that have a toxic effect on plant cells, like polygalactoronase enzyme in addition to fusaric acid, lycomarasmine and dehydrofusaric acid. Furthermore, fungal mycelium may impose physical effect by blocking the plant vascular system leading to plant starvation and death (Srinivas et al., 2019).

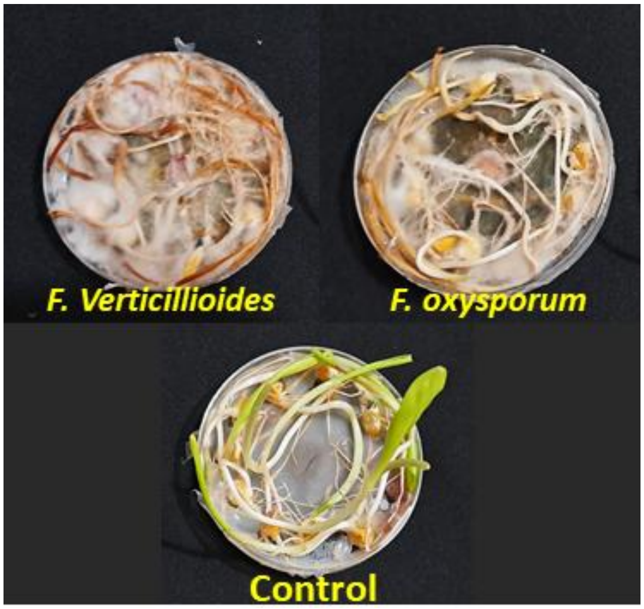

Figure 2: Pathogenicity of *Fusarium* spp. isolates against sweet corn seeds under in vitro conditions.

Table 1: Pathogenicity test of *Fusarium* spp. isolates

under in vitro conditions.

Treatments	Infection (%)
F. verticillioides	85.00
F. oxysporum	65.00
Control	0.00
$LSD_{0.05}$	10.66

*** Each number represents the average of 4 replicates**

3.3. Phytochemical analysis of plant extracts

Phytochemical analysis of plant ethanomethanolic extracts of *S. oleraceus* and *L. virosa* was conducted using GC-MS technology. The GC-MS profile (Figure 3, Table 2) indicated a wide range of chemical constituents in each plant type that can be classified into different categories based on their phytochemical group. Twenty six and 14 phytochemicals were detected in *S. oleraceus* and *L. virosa*, respectively. The obtained phytochemicals were distributed onto sterols, glycosides, phenolics, alkaloids, terpenoids, flavonoids and fatty acids along with saponins. For *S. oleraceus* (Figure 3), Tetracosane a high alkane hydrocarbon was the dominant compound with a total area of 38.52%. The other major component was Bis(2-ethylhexyl) phthalate with 7.78% area. Previous reports pointed to significant antifungal and antibacterial activity exhibited by this component (Lotfy et al., 2018, Kdimy et al., 2022), in addition to potential pharmaceutical applications (Javed et al., 2022).

L. virosa was less diverse in terms of chemical composition of ethanomethanolic extract, where only 14 active compounds were detected (Figure 3, Table 2). Nevertheless, some common major components were detected like Bis(2-ethylhexyl) phthalate but in greater area that was of 31.31%. Also, Nonadecane was found in high concentration reached 26.08%. This alkane was marked with a potential antifungal and antimicrobial activity according to El-Shahir et al. (2022).

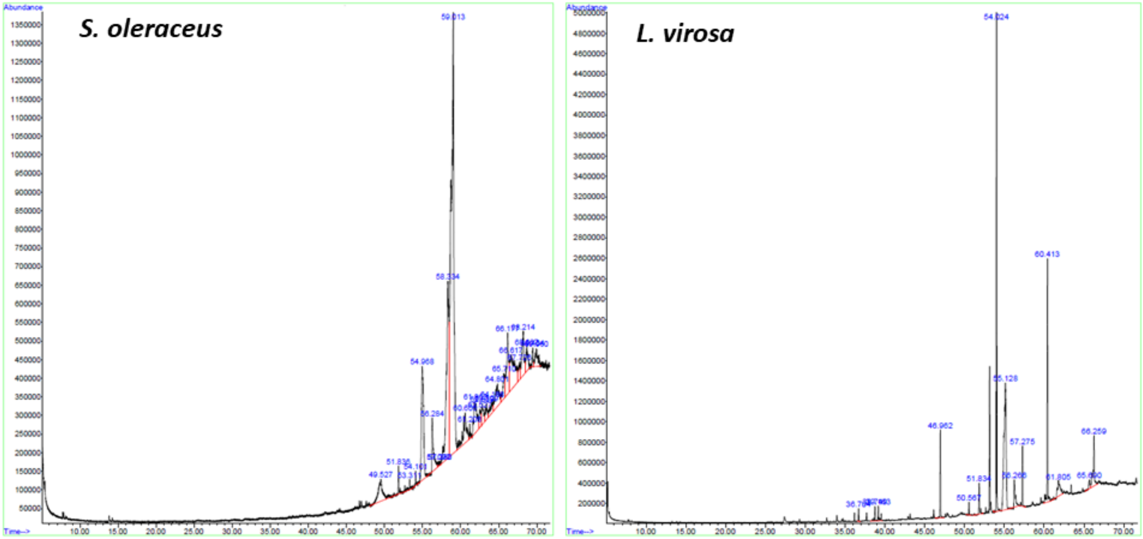

Figure 3: GC-MS profile of ethanomethanolic extract of *S. oleraceus* and *L. virosa*.

Table 2: Phytochemicals , Retention Time (RT) and Area (%) of GC-MS profile of ethanomethanolic extract of *S. oleraceus* and *L. virosa*.

	S. oleraceus			***L. virosa***		
Ref.	**Phytochemical**	**RT**	**Area %**	**Phytochemical**	**RT**	**Area %**
1	Heneicosane	49.53	2.85	Azulene, 1,2,3,4,5,6,7,8-octahydro	36.71	0.57
2	Neophytadiene	51.84	0.79	1H-Cyclopenta[1,3]cyclopropa[1,2]benzene	38.75	1.11

3	1-[trans-4-(Formylmethyl)cyclohexyl]-trans-4-pentylcyclohexane	53.31	0.42	pentadecane	39.16	1.45
4	2-Heptadecanone	54.10	0.31	Heptadecane	46.96	4.27
5	Bis(2-ethylhexyl) phthalate	54.97	7.78	Octadecane	50.56	0.55
6	n-Hexadecanoic acid	56.29	2.47	Neophytadiene	51.83	2.18
7	Oleyl alcohol, trifluoroacetate	57.09	0.15	Nonadecane	54.02	26.08
8	Z-(13,14-Epoxy)tetradec-11-en-1-olacetate	57.28	0.09	Bis(2-ethylhexyl) phthalate	55.13	31.31
9	Tetracosane	58.33	14.74	n-Hexadecanoic acid	56.27	3.67
10	Tetracosane	59.01	38.52	Eicosane	57.27	3.14
11	1-Bromo-11-iodoundecane	60.61	3.11	Heneicosane	60.41	13.32
12	9-Octadecenoic acid, methyl ester,	61.21	0.35	7-Pentadecyne	61.81	4.72
13	9-Octadecenoic acid (Z)-	61.96	3.2	9-Octadecenoic acid (Z)-	65.69	1.22
14	5-Formyl-5'-(4-N,N-dimethylaminophenyl)-2,2'-bithiophene	62.53	0.81	TRICOSANE	66.26	6.4
15	1,3-Dioxane, 2,2,4,5-tetramethyl-6-(1-methyloctadecyl)-	62.84	1.34			
16	CYCLOICOSANE	63.40	0.86			
17	Oxirane, tetradecyl-	64.19	1			
18	(22-Z)-DEHYDROCHOLESTEROL-1-ETHER	64.80	2.59			
19	(+-)-1-Ethoxy-r-1-phenyl-c-2-fluorobenzocycloheptane	65.71	1.66			
20	(22-Z)-DEHYDROCHOLESTEROL-1-ETHER	66.18	3.96			
21	(22-Z)-DEHYDROCHOLESTEROL-1-ETHER	66.62	5.43			
22	(22-Z)-DEHYDROCHOLESTEROL-1-ETHER	67.73	0.94			
23	1,3-Dioxane, 2,2,4,5-tetramethyl-6-(1-methyloctadecyl)-	68.21	3.13			
24	Cyclohexane, 1-(cyclohexylmethyl)-	68.67	1.48			
25	Docosanoic acid	69.47	0.52			
26	STENAZIDINEDIONE	69.96	1.49			

3.4. Antifungal activity of ethanomethanolic extracts of S. oleraceus and L. virosa against companion pathogenic fungi

The two investigated plant extracts varied significantly in their antagonistic effect on mycelial growth and colony diameter ($p \le 0.05$) of both tested fungi. In the same context, the *S. oleraceus* extract was very effective in controlling the development of *F. verticillioides* resulting in the minimum diameter of fungal colony with 3.26 cm (Tables 3 and Figure 4) compared to *L. virosa* extract that reflected the higher mean of colony diameter of 3.81 cm. Accordingly, *S. oleraceus* extract achieved the maximum significant inhibitory effect of 63.77% compared to *L. virosa* extract that exhibited the least percentage of 54.72% (Table 3). The inhibitory effect was significantly increased in response to the increased concentration of both plant extracts. Notably, the applied concentrations had a significant effect in respect of mycelium growth and inhibition (Table 3). In general, higher concentration of each of the applied extracts significantly reduced the colony diameter ($p \leq 0.05$), and hence higher inhibition was achieved, however the highest concentration (9 mg/mL) of both extracts completely inhibited the fungal growth of *F. verticillioides* with 0.00 cm of colony diameter, thus 100% inhibition percentage compared to negative control treatment that exhibited full growth (9.00 cm) and 0.00% inhibition percentage. However, the highest concentartion of 9 mg/ mL didn't differ significantly ($p > 0.05$) compared to the positive control where fungicide was added exhibiting 0.00 cm of *F. verticillioides* colony radial growth.

Table 3: Antifungal activity of *S. oleraceus* and *L. virosa* ethanomethanolic extracts against *F. verticillioides* and *F. oxysporum.*

Plant extract	Concentrations (mg/mL)	Inhibition(100%)	
		F. verticillioides	*F. oxysporum*
S. oleraceus	3	31.14	30.00
	5	63.7	64.07
	7	87.77	90.37
	9	100	100.00
	Control-	0	00.00
	Contro+	100	100.00
	Mean	63.77	64.07
L. virosa	3	2.74	19.26
	5	44.44	39.96
	7	81.11	78.89
	9	100	100.00
	Control-	0	00.00
	Contro+	100	100.00
	Mean	54.72	56.35
L.S.D$_{0.05}$			
PE		2.58	3.25
C		4.08	5.13
PE*C		5.76	7.26

PE= Plant extract, C= Concentration, PE*C= Plant extract * Concentration

The result presented in Table 3 and Figure 4 clearly pointed to significant difference in the antifungal activity ($p \leq 0.05$) of the used plant extracts against *F. oxysporum*, however, *S. oleraceus* extract was the most effective in controlling the development of *F. oxysporum* resulting in the minimum diameter of fungal colony of 3.23 cm compared to *L. virosa* extract that reflected the higher mean of colony diameter of 3.93 cm. All tested concentrations continued inducing significant inhibition zone against tested fungus compared to negative control. In this regard, *S. oleraceus* extract achieved the maximum significant inhibitory effect of 64.07 % compared to *L. virosa* extract that exhibited least percentage of inhibition of 56.35 % (Table 3, Figure 4). Obviously, the tested pathogenic fungus differs in its response to the different concentrations higher concentration of each of the applied extracts significantly reduced the colony diameter and hence higher inhibition was achieved, however the highest concentration (9 mg/mL) of both extracts completely inhibited the fungal growth of *F. oxysporum* with 0.00 cm of colony diameter, thus 100% inhibition percentage compared to negative control treatment that exhibited full growth (9.00 cm) and 0.00% inhibition percentage. The positive control group had no significant differences against the highest concentration where 0.00 cm of colony diameter, and hence 100% inhibition percentage was reported. Also, the positive relationship between the applied concentration and the antagonistic effect reflects the efficiency of theses extracts especially in their higher doses.

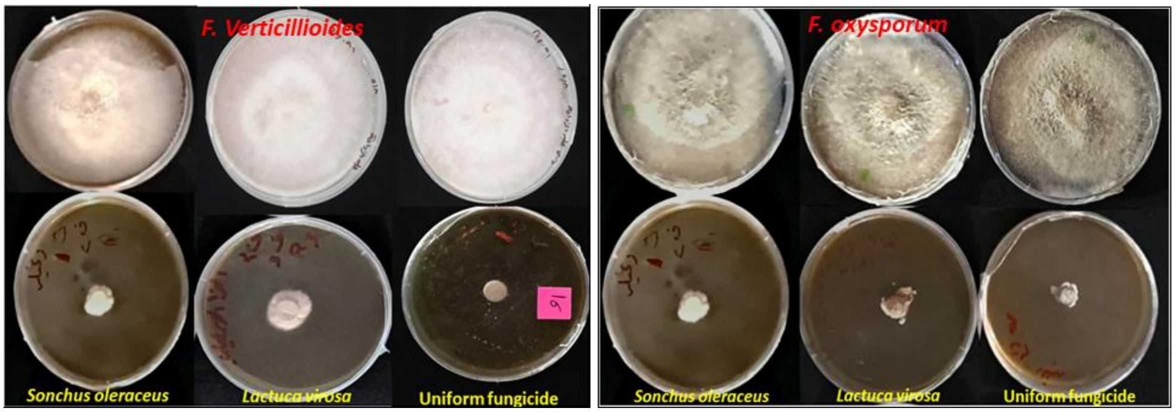

Figure 4: The inhibitory effect of *S. oleraceus, L. virosa* (9 mg/mL) and Uniform fungicide (1 mg/mL) against *F. verticillioides* and *F. oxysporum* grown on the PDA culture medium.

4.Discussion

The varied effects of the plant extracts were expected, given the differences in genetic backgrounds, morphological traits, and diverse phytochemicals, as confirmed by the GC-MS profile (Table 2, Figures 3). Several active components were marked with important antifungal activity like Tetracosane (Jusoh et al., 2013, Shirani et al., 2017) and Bis(2-ethylhexyl) phthalate in *S. oleraceus* (Lotfy et al., 2018, Kdimy et al., 2022); Bis(2-ethylhexyl) and Nonadecane in *L. virosa* (El-Shahir et al., 2022).

In general, higher doses of plant extracts had greater antifungal capability than lower doses.

These results agreed with some other previous results investigating the antifungal potential of different plant leaves extracts against phytopathogenic fungi. Shirani et al. (2017) found that the higher alkenes such as tetracosane had antioxidant and antifungal properties, particularly against fungal spores and germination. The used extracts exhibited a wide range of antifungal activity under laboratory conditions against fusarium isolates associated with tomato damping-off (Osman and Mohamed, 2017). In the same context the chemical analyses by GC-MS revealed the main components of different plant species exhibited great efficiency in inhibiting the growth of *F. verticillioides* and *F. oxysporum* isolates.

The studies reported that the extracts can hinder the growth and advancement of fungal pathogens by disrupting their cellular processes and structures (Waqas et al., 2024).

The applied plant extracts may had a crucial role in reducing the production of phytotoxins and decomposition materials such as fusaric acid occasionally produced by *F. verticillioides* and *F. oxysporum* pathogenic isolates. In addition, the antifungal activity of these plant extracts could effectively contribute to the depletion of cytoplasmic content in the hyphae, resulting in the death of the fungus. Also, direct action of these compounds could be obtained via the inhibition of fungal sporulation, germination of spores, and reduction of hyphae growth. The result indicated that the *S. oleraceus* leaf extract exposed higher efficiency in controlling *F. oxysporum* besides other fungal species (Javaid et al., 2023). There is evidence from earlier studies that water extract of leaves and roots parts of *S. oleraceus* were characterized by antifungal properties, and achieved the maximum significant inhibitory effect of 100% at 15 % concentration (Alkooranee et al., 2020).

Banaras et al. (2020) reported that GC-MS analysis for *S. oleraceus* methanolic extract indicated the presence of 14 different compounds, however, Hexadecanoic acid and methyl ester were the most dominant compounds. The antifungal potential of plant extract was verified against *Macrophomina phaseolina*.

Recently reported results confirmed the antifungal potential of two wildly growing plant types in Anbar, Iraq (*Launaea mucronata* (Forssk.) Muschl and *Launaea nudicaulis* (L.) Hook.fil.). the GC-MS profile of the extracted plant materials revealed different categories of phytochemicals, however, the antifungal activity of plant extracts was confirmed against *Fusarium* ssp. via *in-vitro* and *in-silico* investigations (Alfalahi et al., 2024).

5.Conclusion

The morphological and the molecular identification revealed the incidence of *F. verticillioides* in infected sweet corn plants along with *F. oxysporum*. However, *F. verticillioides* was more responsible for disease symptoms compared to *F. oxysporum*. According to the pathogenicity assessment, *F. verticillioides* is associated with damping-off disease in sweet corn. The natural plant extracts were effective in the control of tested plant pathogens, however, the *S. oleraceus* extract had the most potent inhibitory effect on mycelial growth of *F. verticillioides and F. oxysporum*. The plant extracts may be considered as a promising source of antifungal substances and for developing plant-derived compounds for the effective and more sustainable management of plant diseases.

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