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Desertifilum tharense Methanol Extract Inhibits Vascular Wilt Caused by *Fusarium oxysporum* f. sp. *lycopersici* and Promotes Tomato Growth

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THE APPLICATION of cyanobacteria metabolites in agriculture is considered a promising alternative to chemical fungicide because they are rich in many bioactive metabolites. Antifungal potentiality of *Desertifilum tharense* PS 11 extracts with different solvents was tested in *vitro* against *Fusarium oxysporum* f. sp. *lycopersici*. The *D. tharense* PS 11 methanol extract showed the highest antifungal activity against *F. oxysporum* (19.11%). Then, it was analyzed by GC-MS and LC-ESI-MS to identify and quantify its bioactive compounds. GC-MS and LC-ESI-MS analysis of *D. tharense* methanol extract investigate that the extract was rich in several metabolites such as phenols, alkanes amino acids, peptides, flavonoids, fatty acids and phthalic acid derivatives. After that, pot experiment was performed to test the efficiency of *D. tharense* PS11 methanol extract in the control of tomato vascular wilt disease caused by *F. oxysporum* f. sp. *lycopersici* and growth promotion activity. *Correspondingly,* the results indicate *a 50% reduction* in disease incidence. Furthermore, *D. tharense* methanol extract exhibited a significant effect on plant growth and tomato yield. Therefore, this study clearly demonstrated that methanol extract from *D. tharense* PS 11 is a promising biochemical control agent against *F. oxysporum* f. sp. *lycopersici* and can enhance tomato growth.

Keywords: Antifungal activity, Cyanobacteria, *Desertifilum tharense*, *Fusarium oxysporum*, Vascular wilt.

Introduction

Fusarium oxysporum is one of the most common soil inhabiting fungi. *F. oxysporum* invades roots and causes vascular wilt disease in more than 150 hosts and thus reducing crop yield and economic loss. (Srinivas et al., 2019). Tomato is one of the vegetable crops cultivated all over the world (Brookie et al., 2018). It belongs to the Solanaceae family including more than 3000 species. As for the symptoms resulting from *F. oxysporum* f. sp. *Lycopersici* attack, the vascular wilt progressing into partial or total wilting of tomato plants, the stunting, vascular system browning, and finally the leaf chlorosis (Olivain & Alabouvette, 1999; Di et al., 2016). With regard to the plant damage caused by soil-borne pathogenic fungi, it has led to the focus of considerable effort on fighting plant pathogens by using natural product extracts rather than synthetic fungicides for being safe. This considers an eco-friendly method for protecting crops from soil-borne pathogenic fungi (Ibraheem et al., 2017).

Algae liquid extracts were applied in the form of hydroponic solution and with or without suitable carrier in soil (Renuka et al., 2018), on plants as foliar sprays (Abdel-Hafez et al., 2015; Grzesik et al., 2017), or seed or seedlings treatment (Kim & Kim, 2008) in order to control plant diseases caused by phytopathogenic fungi, bacteria and nematodes. In addition, due to

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the presence of bioactive compounds such as polyphenols, tocopherol, carbohydrates, proteins, oils, and pigments (Michalak & Chojnacka, 2015; Renuka et al., 2018), Algal extracts can inhibit the growth of phytopathogens or increase plant defense mechanism. Furthermore, they have a positive effect on plant health and growth, which led to changes in some metabolic functions such as cell division, elongation of shoot and root, and initiation of flowering due to presence of certain hormones such as cytokinin and minerals (Allen et al., 2001).

Chaudhary et al. (2012) evaluated that *Anabaena variabilis* RPAN59 can be considered as a bio-stimulant and biocontrol agent to control damping-off disease in tomatoes caused by fungal consortium; *Pythium debaryanum*, *Fusarium oxysporum lycopersici*, *Rhizoctonia solani* and *Fusarium moniliforme*. Extracts from *Nostoc commune* FA-103 have in *vitro* and in *vivo* suppression effect to *F. oxysorum* f. sp. *lycopersici* growth, spore sporulation, and infection to tomato seeds (Kim & Kim, 2008).

There is a gap in knowledge about the antifungal activity of *Desertifilum tharense*, so this motivated us to explore the antifungal potential of *D. tharesnse* to control vascular wilt diseases caused by *F. oxysorum* f. sp. *lycopersici*.

In general, the aim of this study is to investigate the antifungal activity of intracellular and extracellular metabolites of *D. tharense* against *F. oxysorum* f. sp. *lycopersici* either in *vitro* or in *vivo* for controlling vascular wilt disease and promoting tomato growth.

Materials and Methods

Phytopathogen isolation growth conditions

Fusarium oxysporum f. sp. *lycopersici* isolation was prepared through collectioning of naturally infested tomato root tissues. Small pieces (ca.≤ 9mm^2) from root samples were cut and placed on potato dextrose agar (PDA) medium in a Petri dish and incubated for 7 days at 28°C in the dark. The identification of *F. oxysporum* was based on morphological characteristics (Leslie & Summerell, 2008).

Sampling, isolation and identification of Desertifilum tharense PS 11

Desertifilum tharense PS 11 was isolated and purified from freshwater samples, collected from rainwater swamps at Port Said City under sterilized conditions. BG11 medium (Barsanti & Gualtieri, 2014) has been used for its isolation and propagation. The morphology of this strain was accessed by light microscopy as well as imaged using Optika 4083.B5 digital camera. The morphological identification was performed according to Dadheech et al. (2012)

The molecular identification was based on 16S rDNA sequence analysis. The genomic DNA of *D. tharense* PS 11 was extracted by phenol/chloroform technique and precipitated by isopropanol, followed by washing with ethanol (Ausubel et al., 1994). The extracted DNA was examined for purity by running on agarose gel electrophoresis (1%) in TAE buffer pH 8.0 (0.04M Tris-acetate and 0.001M EDTA); in addition, it was visualized by UV trans-illuminator after being stained with ethidium bromide. According to Weisburg et al. (1991), the universal prokaryotes primers, 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') were used for amplifying and sequencing of the 16S rRNA gene of cyanobacterial isolate. The amplified PCR products were sequenced by an automated sequencer (Macrogen, South Korea) using the same previous primers.

Basic local alignment search tool nucleotide (BLASTn) was performed for search alignment of the resulting 16S rDNA sequence in order to match the best similarities with other related sequences on database (Altschul et al., 1997). The best DNA sequence similarities with the resulting regions were obtained from the national center for biotechnology information (NCBI) GenBank and the aligned using CLUSTAL Omega. Unaligned terminal regions were excluded manually, but some sequences of the same species and unidentified organisms were discarded. Finally, phylogenetic tree analysis was viewed and analyzed through using the MEGA version 4 (Tamura et al., 2007).

Antifungal well diffusion assay (in-vitro)

Desertifilum tharense PS 11 was cultivated in 2L bottle media containing 1800mL media at 25^{0}C under 8:16 LD cycle with 36µmol m^{-2} s^{-1} with cool white fluorescent lamps and continuous aeration by vacuum pump (3.5L/min) to ensure that all the filaments are in contact with nutrients, CO_2 and light for optimum growth. Biomass and cultivation medium were harvested after 35 days

by centrifugation according to Mundt et al. (2014) the search for new antimicrobial substances is of increasing importance. Based on the hypothesis that microorganisms living in an aquatic environment produce secondary metabolites as chemical weapons to survive in their daily fight against cohabitants of the biotope, a screening of 133 microalgae (121 cyanobacteria, 12 eukaryotic microalgae. The biomass was washed with distilled water in order to remove salts and then was air-dried.

Four extraction solvent, hexane, chloroform, acetone, and methanol were used in the extraction process of definite amount of *D. tharense* powder by being macerated for 24h and continuous shaking at 200rpm. The percentage of each crude extract was determined as follows: (Abdel-Aal et al., 2015):

Extract % = (Weight of extract (g) / Weight of algal sample (g)) x 100

The crude extract was totally dissolved in dimethyl sulfoxide (DMSO), then sterilized by using micro filters (Millipore, 0.22µm). The concentration of each extract was calculated as follows: (Concentration= weight of extract (mg) / volume of solvent (mL)) (Moubayed et al., 2017).

The extracellular metabolites extraction was performed according to Bhore et al. (2010), while the cultivation medium was extracted by absolute ethyl acetate (1:3 v/v). Afterwards, the mixture had been shaken for 24h at 200rpm before being imparted in a separating funnel and left to settle until the appearance of two distinct layers; one of them is the upper layer which is ethyl acetate that was separated from aqueous layer. After separation, the extract was sterilized through micro filters (Millipore, 0.22µm) and stored in refrigerator for further use.

The antifungal activity of *D. tharense* extracts against *F. oxysporum* f. sp. *lycopersici* was carried out in *vitro* using a well-cut technique (Bodet et al., 1985). The disk of *F. oxysporum* f. sp. *lycopersici* was inoculated in the middle on solid potato dextrose agar medium (PDA medium). In addition, two wells, with 5mm in diameter, were punched in PDA media*;* one well was filled with 100µL of *D. tharense* extract, while the negative control well with 100µL of DMSO, in case of intracellular metabolites, and 100µL of ethyl acetate in case of extracellular metabolites. Moreover, positive control was performed by using miconazole fungicide at 0.03mg/mL.

After that, plates were incubated at 30°C and the results were recorded 5 days later. *Percentage* of mycelium growth inhibition (MGI %) was measured by using the following equation (De Corato et al., 2017):

MGI% = [(Control − Test)/Control] × 100

where, Control= fungus radial growth from the middle of fungal disc toward the negative control well, Test= fungus radial growth from the middle of fungal disc toward the extract well.

Determination of minimal inhibitory concentration (MIC)

MIC determination was achieved by using different concentrations of each crude extract (75, 50 and 25%). The MIC value was then taken as the lowest concentration of extract**,** which inhibit the phytopathogen growth (El Shafay et al., 2016). Overall, all experiments were performed in triplicate.

Metabolic profile of Desertifilum tharense PS 11

Methanol fraction of *D. tharense* exhibited the highest antifungal activity against *F. oxysporum*. As the methanol extract was analyzed using GC-MS and LC-ESI-MS to ensure the detection of all extract metabolites.

GC-MS analysis

Mass spectra were recorded by using Shimadzu GCMS-QP2010 (Koyoto, Japan) equipped with Rtx-5MS fused bonded column (30m x 0.25mm i.d. x 0.25µm film thickness) (Restek, USA), *which was, in turn,* equipped with a split–splitless injector. This analysis was carried out in the Faculty of Pharmacy, Ain Shams University. The initial column temperature was kept at 50^{O}C for 3min (isothermal), programmed to 200^{O}C at a rate of 15^{O}C/min at 200^{O}C for 5min (isothermal). Then, the temperature was programmed to 240^{O}C at a rate of 3^{O}C/min and kept constant at 240^{O}C for 10min (isothermal). Finally, the temperature was programmed to 300^{O}C at a rate of 4^{O}C/min and kept constant at 300^{O}C for 10min (isothermal). As for injector temperature, it had been set at 280^{O}C, while Helium carrier gas flow rate was 1.41mL/min. All the mass spectra were recorded applying the following condition: (equipment current) filament emission current, 60mA; ionization

voltage, 70eV; ion source, 220^{O}C. Diluted samples (1% v/v) were injected with split mode (split ratio1:5).

LC-ESI-MS

The sample (100µg/mL) solution was prepared using high performance liquid chromatography (HPLC); it is an analytical grade solvent of MeOH, filtered using a membrane disc filter (0.2µm), and then subjected to LC-ESI-MS analysis. This analysis was carried out in Faculty of Pharmacy, Ain Shams University. With regard to the samples injection volumes (10µL), they were injected into the UPLC instrument equipped with reverse phase C-18 column (ACQUITY UPLC - BEH C18 1.7µm particle size 2.1 × 50mm Column). Sample mobile phase was prepared by filtering, using a 0.2µm filter membrane disc and degassed by sonication before injection. Next, mobile phase elution was made with the flow rate of 0.2mL/min, using gradient mobile phase comprising two eluents: eluent A is H_2O acidified with 0.1% formic acid, and eluent B is MeOH acidified with 0.1% formic acid. The elution was performed using the above gradient. The parameters for analysis were carried out using negative ion mode as follows: source temperature 150^{O}C, cone voltage 30eV, capillary voltage 3kV, desolvation temperature 440^{O}C, cone gas flow 50L/h, and desolvation gas flow 900L/h. Mass spectra were detected in the ESI between m/z 100–1000. Both the spectra and peaks were processed using the Maslynx 4.1 software and tentatively identified by comparing its mass spectrum and retention time (Rt) with reported data.

Vascular wilt suppression potential and tomato growth-promoting ability

Methanol extract of *D. tharense* PS 11 was tested in pots experiment in order to control *F. oxysporum* f. sp. *Lycopersici*, the causal agent of vascular wilt of tomato plant. Next, plastic pots (25cm in diameter) were filled with 3kg of autoclave sterilized clay soil mixed with sandy soil by ratio1:3. The soil was then artificially infested with 3% (w/w) barley and sorghum inoculated with 5 X 10 ^{6} spores of *F. oxysporum* f. sp. *lycopersici* according to Soliman et al. (2018), while the pots that were free from the pathogen *were* treated with sterilized barley and sorghum grains only. After that, the pots were left for seven days with continuous irrigation before seed sowing in order to ensure the fungus dispersal in soil.

The seeds of *solanum lycopersicum* Alisa were first surface sterilized with 10% sodium hypochlorite (v/v) for five minutes, then washed by sterilized distilled water (Kim & Kim, 2008). Later, some of the tomato seeds were soaked in *D. tharense* methanol extract, mixed with Tween 20 (10%, v/v) for 15min according to Kim & Kim (2008). Afterwards, some of the coated tomato seeds with the extract were sown in the soil containing the pathogen and the others were sown in the soil free from the pathogen. Positive and negative controls were carried on during the experiment. In addition to the fact that each treatment had three replicates, the treated pots were kept in the greenhouse and watered every two days as required. The experiment results were recorded every two weeks until 113 days.

The percentage of seed germination was evaluated after seven days of seeds sowing, while the percentage of vascular wilt disease incidence was calculated every two weeks by measuring the number of dead plants. Growth parameters were also recorded such as total fresh weight, plant height, total yield, and root and shoot length. In addition, the concentration of Chlorophyll-a, Chlorophyll-b, total chlorophylls and carotenoids were determined according to Sumanta et al. (2014) and Lichtenthaler (1987) chloroplasts, thylakoid particles, and pigment proteins. The chapter focuses on the spectral characteristics and absorption coefficients of chlorophylls, pheophytins, and carotenoids, which are the basis for establishing equations to quantitatively determine them. Therefore, the specific absorption coefficients of the pigments are re-evaluated. This is achieved by using a two-beam spectrophotometer of the new generation, which allows programmed automatic recording and printing out of the proper wavelengths and absorbancy values. Several procedures have been developed for the separation of the photosynthetic pigments, including column (CC).

Statistical analysis

The results were expressed as a mean*s* of three replicates ± standard error. The data were statistically analyzed by using the analysis of variance (ANOVA) at $P< 0.05$ in order to assess the significance among treatments, and the means were separated using the least significant difference (LSD) (Gomez & Gomez, 1984). Moreover, the statistical analysis was done using IBM SPSS software version 25.

Results

Identification of the Desertifilum tharense isolate

Desertifilum tharense (Dadheech et al., 2012) is a blue green, unbranched, thin and long filament; it is solitary and can be categorized under loose bundles; it sometimes forms a dense mesh with intertwined trichome. Motility is implemented by gliding and oscillating motion. Meanwhile, the trichome is surrounded by a thin and colorless gelatinous sheath. Brownish aggregates can be sometimes found between elongated filaments. Generally, all cells in the filamentous are cylindrical except for the apical cells which are rounded and sometimes accompanied with extrusions. The cells are longer than wide and are 4.016 ± 0.108µm long and 2.61 ± 0.140µm wide (Fig. 1).

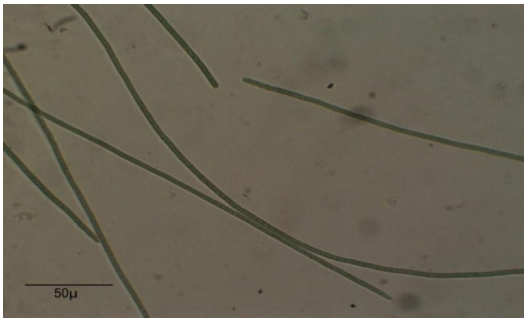

Fig. 1. Microscopic image of *Desertifilum tharense* PS 11

The 16S rDNA sequence of *Desertifilum tharense* PS 11 revealed 1366 bp which was submitted in the GenBank under accession number: MW411006. The sequence alignment of *D. tharense* PS 11 *is* isolate*d* with other related Cyanophyta genera using NCBI-Blastn exhibited the highest identity percentage with *Desertifilum tharense* strain PD2001/TDC4 and *Desertifilum salkalinema* strain CHAB7200 (99.61% and 99.41%, respectively). On the other hand, it showed lower similarity about 90% with *Nodularia sphaerocarpa*, *Schizothrix cf. calcicola*, *Calothrix* sp., *Aulosira laxa*, *Tolypothrix tenuis* and *Oscillatoria spongeliae* (Fig. 2).

Antifungal activity of D. tharense extracts against F. oxysporum (In- vitro assay)

Organic solvents have an obvious impact on extract concentration obtained from *D. tharense* by the extraction process of intracellular metabolites. In addition, the highest amount of extract was obtained by acetone (12.56) and methanol (7.692) respectively. By contrast, the lowest percentage of extract was obtained by chloroform (0.718) (Fig. 3).

The selection of effective solvent and concentration of *D. tharense* extracts were based on (MIC) assay, and it was noticeable that *Fusarium oxysporum* f. sp. *lycopersici* was resistant to chloroform and ethyl acetate extracts (Table 1). There is no significant difference between the effect of hexane and acetone extract against *F. oxysporum* (Table 2). Finally, the highest mycelium growth inhibition of *F. oxysporum* f. sp. *lycopersici* was observed by *D. tharense* methanol extract at 0.02mg/mL (19.11%) (Fig. 4).

Metabolic profile of Desertifilum tharense PS 11

Desertifilum tharense methanol fraction which exhibited the highest antifungal activity against *F. oxysporum* f. sp. *lycopersici* was analyzed by GC-MS and LC-ESI-MS for identification of bioactive compounds.

GC-MS analysis of D. tharense methanol extract

Four compounds were detected in *D. tharense* methanol extract by GC/MS (Table 3), *which* belong to different chemical classes; alkanes, acids, phenols, and phthalic acid derivatives (Fig. 5). *However*, the highest peak area in *D. tharense* extract was identified as di-n-octyl phthalate (Fig. 6).

LC-ESI-MS analysis of D. tharense methanol extract

The LC-ESI-MS was used to identify the polar compounds in *D. tharense* methanol extract in order to elucidate their chemical profile. Twenty one peaks were detected in the LC-ESI-MS of *D. tharense* methanol fraction (Table 4). Most of them were included in chemical classes, such as peptides (19.04%), phthalic acid derivatives (19.04%), fatty acid derivatives (14.28%), alkanes (9.52%), flavonoids (9.52%), amino acids (99.52%), and fatty acids (9.52%) (Fig. 7). Anyhow, the highest peak area was identified as trihydroxy dimethoxy flavone (Fig. 8).

Biological control of tomato vascular wilt by D. tharense methanol extract

Percentage of vascular wilt disease incidence

The percentage of disease incidence in negative control was 3.33% after 48 days, and the value was still constant until the end of the experiment (Table 5), while the highest percentage of disease incidence observed in positive control was (26.66%) (Fig. 9, C). *Nonetheless,* there was no significant difference in disease incidence between *D. tharense* treatments (Table 5).

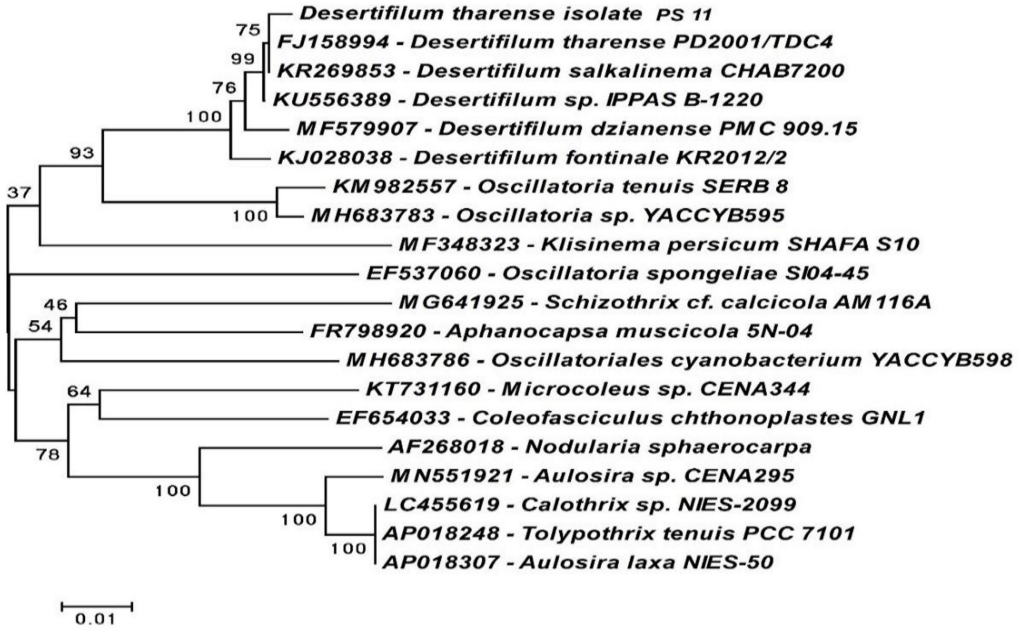

Fig. 2. Phylogenetic tree analysis based on 16S rRNA gene sequence alignment of *D. tharense* isolate PS11 (AC No.: MW411006) with some other related cyanophyceae genera

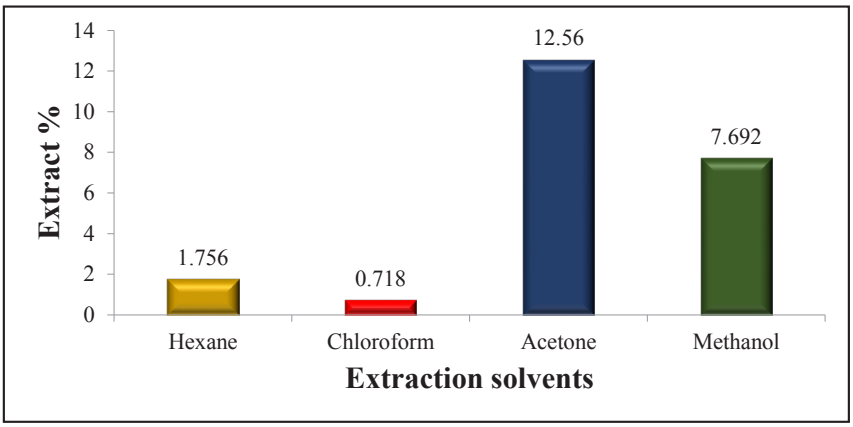

Fig. 3. Variation in crude extract percent of *D. tharense* with different organic solvents

TABLE 1. Determination of MIC for different extraction solvents of *D. tharense* against *F. oxysporum* f. sp. *lycopersici*

Hexane	Concentration (mg/mL)	**1.5**	**3**	**4.5**	**6**
	F. oxysporum f. sp. *lycopersici*	-	-	-	+
Chloroform	Concentration (mg/mL)	**0.4**	**0.6**	**0.8**	**1**
	F. oxysporum f. sp. *lycopersici*	-	-	-	-
Acetone	Concentration (mg/mL)	**0.005**	**0.01**	**0.015**	**0.02**
	F. oxysporum f. sp. *lycopersici*	-	-	-	+
Methanol	Concentration (mg/mL)	**0.005**	**0.01**	**0.015**	**0.02**
	F. oxysporum f. sp. *lycopersici*	-	-	-	+
Ethyl acetate	Concentration (v/v)	**5%**	**15%**	**25%**	**35%**
	F. oxysporum f. sp. *lycopersici*	-	-	-	-

(+) means there is an antifungal activity to extract and (–) means that no antifungal activity was detected to extract.

TABLE 2. Percent of mycelium growth inhibition of *F. oxysporum* f. *sp. lycopersici by different extraction solvents of D. tharense PS 11 resulting from MIC*

Treatments	Conc	*F. oxysporum* f. sp. *lycopersici* MGI %
Miconazol	0.03mg/mL	**24±1.76** [d]
Hexane	6mg/mL	**11.37±1.21** [a]
Chloroform	1.08mg/mL	0 [b]
Acetone	0.021mg/mL	**15.67±0.86** [a]
Methanol	0.02mg/mL	**19.11±0.18** [c]
Ethyl acetate	35% v/v	0 [b]
LSD at p ≤ 0.05		**2.91**

All values are mean (n=3) ± standard error.

Values with the same letters in the same column are not significantly different at P≤ 0.05

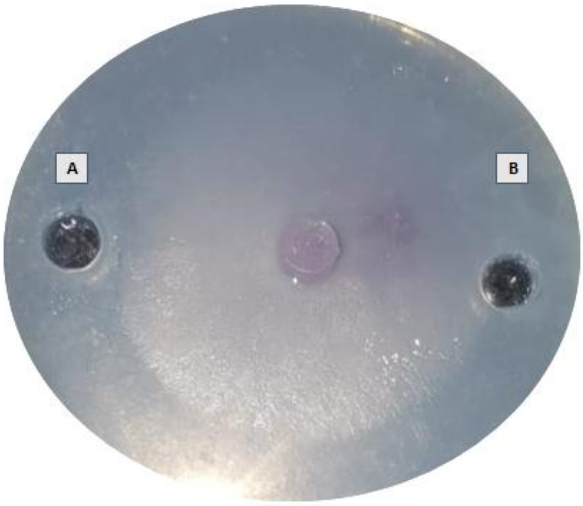

Fig. 4. Mycelial growth inhibition of *F. oxysporum* f. sp. *lycopersici by D. tharense* PS 11 methanol extract; A. refers to methanol extract dissolved in DMSO, and B. refers to DMSO (negative control).

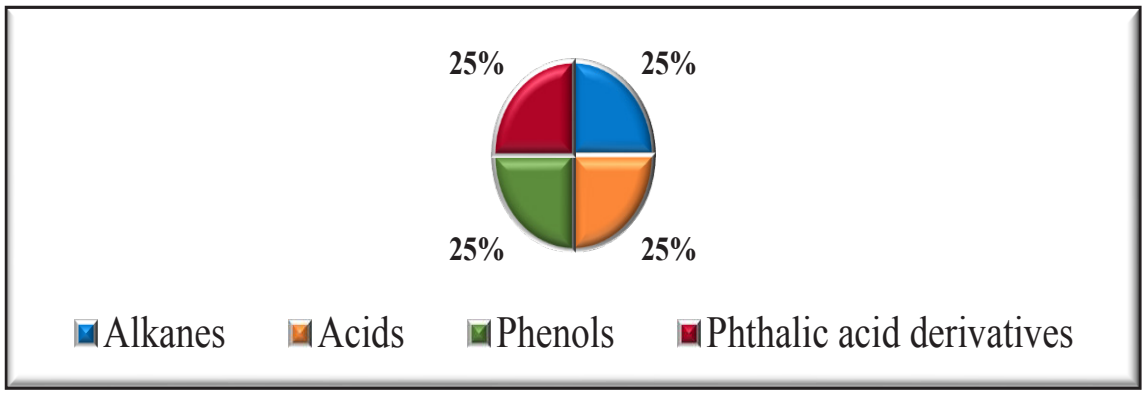

Fig. 5. Percentage of metabolites chemical groups identified by GC/MS in *D. tharense* PS 11 methanol extract.

TABLE 3. Identified compounds from *D. tharense* PS 11 methanol fraction by GC/MS

Peak No.	R_t	Metabolites	Molecular formula	Molecular weight	Chemical group	Base peak m/z	Peak area %	Biological activities	References
1	14.233	Oxalic acid, propyl tridecyl ester	$C_{18}H_{34}O_4$	314	Acid	43.05	15.72	-	-
2	15.947	1-Octadecyne	$C_{18}H_{34}$	250	Alkanes	43.05	6.60	-	-
3	29.604	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	$C_{23}H_{32}O_2$	340	Phenol	177.05	22.37	Antifungal, Antibacterial activity, germicidal	(Celis et al., 2011)
4	32.910	Di-n-octyl phthalate	$C_{24}H_{38}O_4$	390	Phthalic acid derivative	149.10	55.30	Antifungal activity	(Akhtar et al., 2020)
Total identified							**99.99**		

(-) Means no reported biological activity.

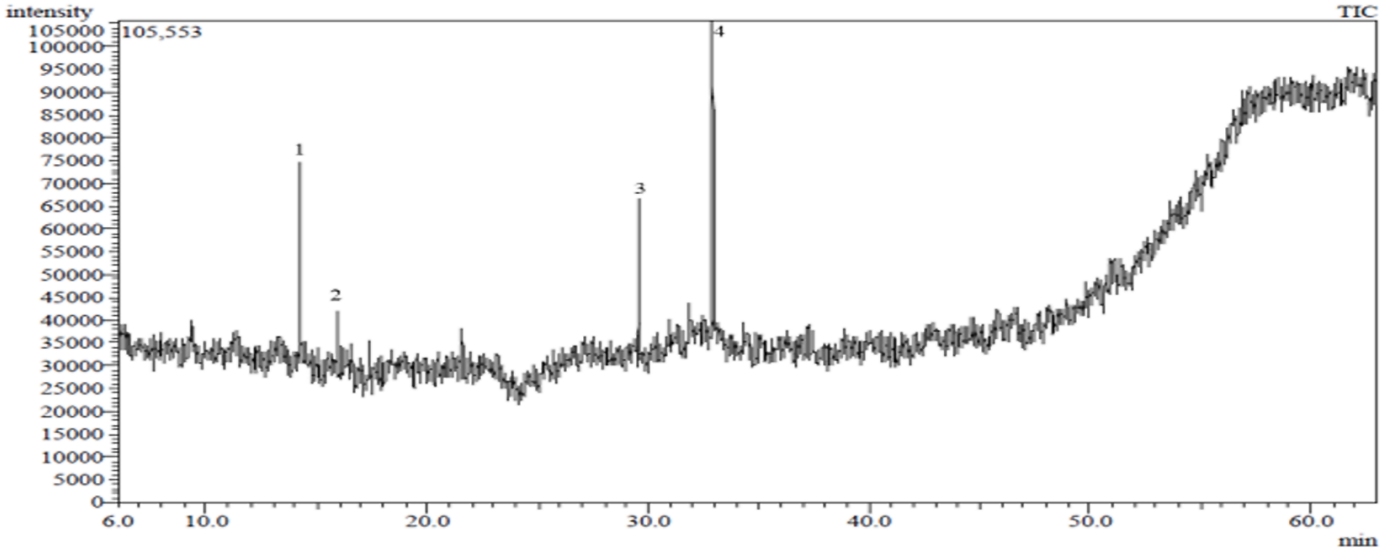

Fig. 6. Total ion chromatogram of GC-MS of *D. tharense* PS 11 methanol extract

TABLE 4. LC-ESI-MS analysis of *D. tharense* methanol fraction

No.	t_R (min)	$[M+H]^+$	*m/z* fragments $[Ms^2]$	Identified compounds	Chemical class	References
1	0.14	264.8113	221.0129 193.1423 188.9997 173.9912 159.9716 141.9163 118.1547	Phenylalanyl valine	Peptide	(Steigenberger et al., 2019)
2	0.68	266.9915	245.0604 226.9095 118.9663 91.0134	Phenylalanyl threonine	Peptide	(Steigenberger et al., 2019)
3	0.75	439.0763	365.0549 242.8575 226.9099 118.9526 104.0694	Threoninyl phenyl alanyl alanyl threonine	Peptide	(Huang et al., 2019)
4	8.67	178.0935 **$[M+Na]^+$**	156.0939 138.8969 121.0457	Histidine	Amino acid	(Thiele et al., 2008)
5	12.34	203.0933 **$[M+Na]^+$**	182.1311 181.1309 163.0132 102.0306	Tyrosine	Amino acid	(Thiele et al., 2008)
6	17.33	437.2108	275.3391 274.2493 243.0839	Octadecapentaenoic acid-*O*-hexoside	Fatty acid	(Napolitano et al., 2018)
7	17.66	378.3013	334.366 290.2510 230.2162	Alanyl alanyl serinyl methionine	Peptide	(Huang et al., 2019)
8	18.59	331.1773	304.3033 291.1902 236.0631	Trihydroxy octadecenoic acid	Fatty acid	(Napolitano et al., 2018)
9	19.31	579.3045 **$[2M+Na]^+$**	301.1281 $[M+Na]^+$ 279.1433 $[M+H]^+$ 167.0267 149.0074	Dibutyl phthalate (isomer I)	Phthalic acid derivative	(Net et al., 2015)
10	19.67	579.2447 **$[2M+Na]^+$**	301.1233 $[M+Na]^+$ 279.1912 $[M+H]^+$ 167.0267 205.0723	Dibutyl phthalate (isomer II)	Phthalic acid derivative	(Net et al., 2015)
11	20.64	333.3584	149.0004 293.1967 257.1171 403.1767	Trihydroxy octadecanoic acid	Fatty acid	(Napolitano et al., 2018)
12	21.33	425.1948 **$[M+Na]^+$**	361.1830 319.2301 279.2063 259.0570	Trihydroxy propanoyl hexadecanoic acid acetate	Fatty acid derivative	(Napolitano et al., 2018)

TABLE 4. Cont.

No.	t_R (min)	$[M+H]^+$	*m/z* fragments $[Ms^2]$	Identified compounds	Chemical class	References
13	21.84	543.1926	511.2912 390.3399 346.333 264.2025 235.1676 120.9929	Linoleic anhydride	Fatty acid derivative	(Napolitano et al., 2018)
14	23.66	678.5082	540.4272 539.3336 413.2144 355.3052 278.2034 263.2126 179.0856	Linoleic cholestanyl ester	Fatty acid derivative	(Honda et al., 2010)
15	24.37	515.3184	377.2301 331.2708 285.1425 239.1182 222.9518	Unknown		
16	24.89	353.2516 **$[M+Na]^+$**	331.2708 313.2649 254.9116 239.1774	Trihydroxy dimethoxy flavone (isomer I)	Flavonoid	(Marzouk et al., 2018)
17	25.20	353.2956 **$[M+Na]^+$**	331.2699 313.2627 254.9116 239.1870 109.0603	Trihydroxy dimethoxy flavone (isomer II)	Flavonoid	(Marzouk et al., 2018)
18	26.92	803.6048 **$[2M+Na]^+$**	413.2612 $[M+Na]^+$ 391.2621 $[M+H]^+$ 279.1419 167.0338 149.0007	Dioctyl phthalate (isomer I)	Phthalic acid derivative	(Net et al., 2015; Khaled et al., 2019)
19	27.03	803.5968 **$[2M+Na]^+$**	413.2561 $[M+Na]^+$ 391.2714 $[M+H]^+$ 279.1283 167.0419 149.0049	Dioctyl phthalate (isomer II)	Phthalic acid derivative	(Net et al., 2015; Khaled et al., 2019)
20	27.55	739.7314 **$[2M+Na]^+$**	381.2806 $[M+Na]^+$ 359.3035 $[M+H]^+$ 341.2940 267.2514	Dihydroxy hexadecane	Alkanes	(Hussein et al., 2018)
21	29.69	395.3731	337.2488 309.2655	Octacosane	Alkanes	(Hussein et al., 2018)

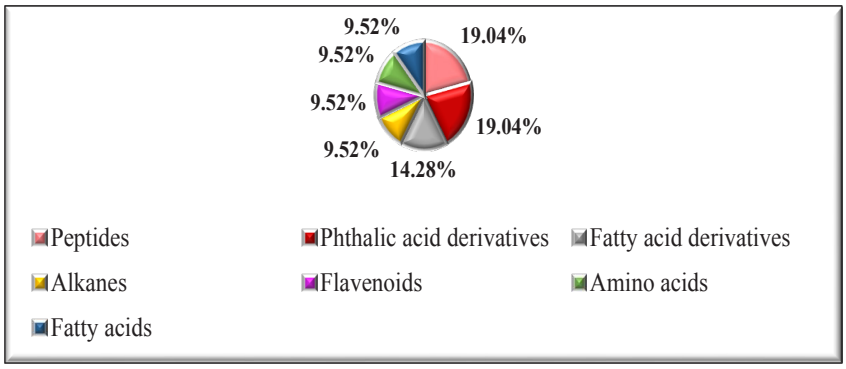

Fig. 7. Percentage of metabolites chemical groups identified by LC-ESI-MS in *D. tharense* PS 11 methanol extract

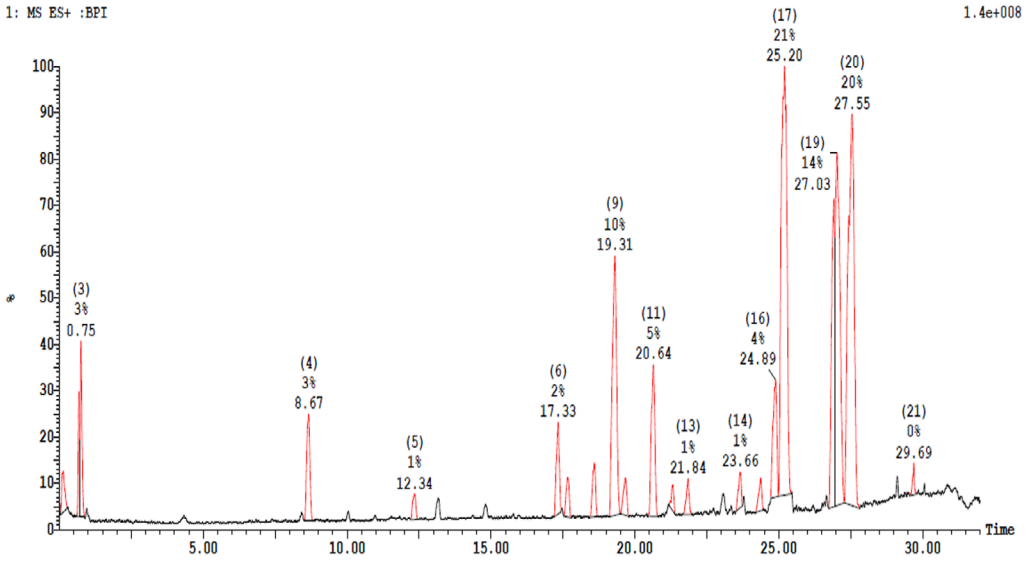

Fig. 8. LC-ES-MS chromatogram of MeOH fraction of *D. tharense* PS 11

Fig. 9. Tomato plants after 87 days of seed sowing; (A) negative control, in which seeds are sown in soil free from the pathogen, (B) *D. tharense* treatment, in which seeds are coated with the extract and sown in soil that contains the pathogen, (C) positive control, in which seeds are sown in soil that contains the pathogen and (D) control of *D. tharense* treatment, in which seeds are coated with *D. tharense* extract and sown in soil free from the pathogen

TABLE 5. Percentage of vascular wilt disease incidence

Treatments	Percentage of vascular wilt disease incidence			
	After 16 days	**After 32 days**	**After 48 days**	**After 64 days**
Negative control	0±0 [a]	0±0 [c]	3.33±3.33 [g]	3.33±3.33[i]
Positive control	16.66±3.33 [b]	16.66±3.33 [d]	23.33±3.33 [f]	26.66±3.33 [k]
D. tharense extract	0±0 [a]	3.33±3.33 [e]	10±0 [h]	10±0 [j]
D. tharense extract + *F. oxysporum* f. sp. *lycopersici*	0±0 [a]	6.66±3.33 [e]	13.33±6.66 [h,f]	13.33±6.66 [j]
LSD at p ≤ 0.005	**5.43**	**13.31**	**1331**	**13.31**

All values are mean (n=3) ± standard error.

Values with the same letters in the same column are not significantly different at P≤ 0.05.

Percentage of Tomato seed germination

The tomato seeds germinated 8 days after been sowed in soil. The maximum percentage of seed germination was observed after 22 days (Table 6), whereas the highest percentage of seed germination reached up to 63.33% in case of positive control, followed by the control of *D. tharense* extract (30%) and in *D. tharense* treatment, in which the seeds were sown in soil containing the pathogen (26.66%).

Effects of algal extracts on tomato growth

Effect of algal extracts on root and shoot length

There was no significant difference in plant height between negative control, positive control, or *D. tharense* treatments (Table 7). The shoot length of *D. tharense* control was higher than the plants shoot length of *D. tharense* treatment, where the soil contains the pathogen (Fig. 10, B & D).

Effect of algal extracts on fresh weight and yield

The highest total fresh weight was observed in the following treatments: positive control (260gm), *D. tharense* control (170gm), and *D. tharense* treatment, in which the soil contains the pathogen (100gm). *D. tharense* extract promotes plant fruiting and has a great effect on plant yield. The highest plant yield was, nevertheless, observed in *D. tharense* control (30.33g) and *D. tharense* treatment, in which the soil contains the pathogen (18.66g) (Table 7).

Plant pigments

The highest ratio of chlorophylls was observed in control of *D. tharense* treatment (0.699 mg/ml) (Table 7). *D. tharense* extract increases the amount of plant carotenoids. The lowest concentration of carotenoids was observed in negative control, through. There was a significant difference in the concentration of chlorophylls and carotenoids between the control of *D. tharense* treatment and the negative control.

TABLE 6. Percentage of tomato seeds germination

Treatments	Percentage of seeds germination		
	After 8 days	**After 16 days**	**After 22 days**
Negative control	6.66±3.33 [a]	23±12.01 [c]	26.66±14.52 [e]
Positive control	30±10 [b]	60±5.77 [d]	63.33±6.66 [f]
D. tharense extract	6.66±6.66 [a]	30±10 [c]	30±10 [e]
D. tharense extract + *F. oxysporum* **f. sp**. *lycopersici*	23.33±14.5 [b]	26.66±14.5 [c]	26.66±14.5 [e]
LSD at P≤ 0.005	**31.22**	**36.05**	**39.56**

All values are mean (n=3) ± standard error.

Values with the same letters in the same column are not significantly different at P≤ 0.05.

TABLE 7. Plant growth characteristics after 113 days as affected by *D. tharense* extract or not under either soil infected with *F. oxysporum* f. sp. lycopersici or soil free from pathogen

Treatments	Negative control	Positive control	*D. tharense* extract	*D. tharense* extract + *F. oxysporum*	LSD P≤ 0.05
Total fresh weight (g)	95	260	170	100	--
Total yield (g)	0	0	30.33	18.66	--
Root length (cm)	172.37± ^{j}	222.3± ^{j}	232.58± ^{j}	23.25± 1.03 ^{j}	**7.52**
Shoot length (cm)	53.50.28± ^{k}	60.53.46± ^{k}	57.44.9± ^{k}	51.250.75± ^{k}	**9.7**
Plant height (cm)	70.52.63± ^{l}	82.588..4± ^{l}	80.47.35± ^{l}	74.51.75± ^{l}	**15.55**
Chl a (mg/mL)	0.152±0.056 ^{a}	0.101±0.05 ^{a}	0.417±0.053 ^{b}	0.125±0.032 ^{a}	**0.138**
Chl b (mg/mL)	0.244±0.09 ^{c}	0.169±0.007 ^{c}	0.281±0.02 ^{c}	0.121±0.019 ^{c}	**0.155**
Chl a+b (mg/mL)	0.397±0.147 ^{d}	0.27±0.012 ^{f}	0.699±0.007 ^{e}	0.247±0.051 ^{f}	**0.279**
Carotenoids (mg/mL)	0.048±0.002 ^{g}	0.051±0.008 ^{g}	0.268±0.018 ^{h}	0.132±0.006 ^{i}	**0.035**

All values are mean (n=3) ± standard error except total fresh weight and total yield.
Values with the same letters in the same row are not significantly different at P≤ 0.05.

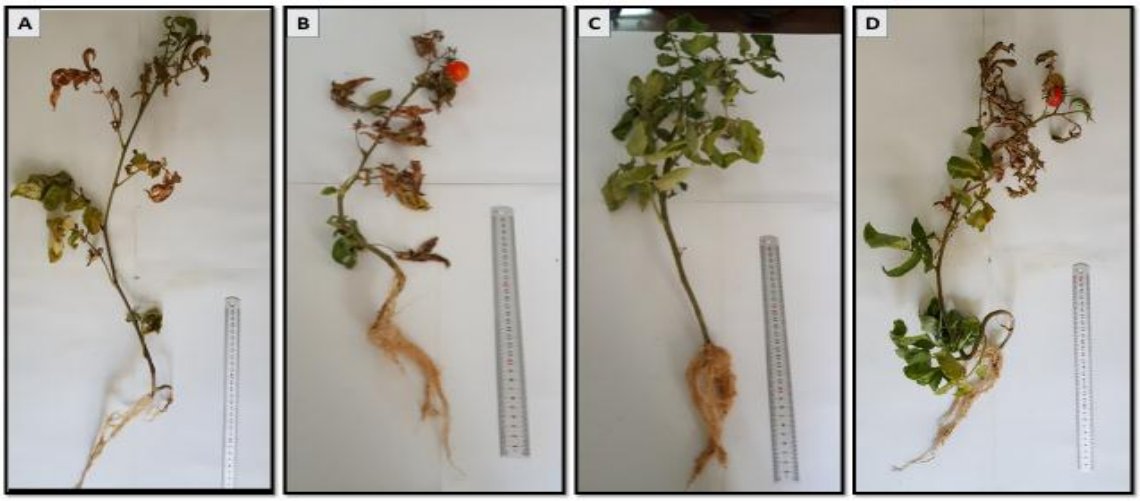

Fig. 10. Tomato plant length of different treatments after 113 days. (A) negative control, in which seeds are sown in soil free from the pathogen, (B) *D. tharense* treatment, in which seeds are coated with the extract and sown in soil that contains the pathogen, (C) positive control, in which seeds are sown in soil that contains the pathogen and (D) control of *D. tharense* treatment, in which seeds are coated with the extract and sown in soil free from the pathogen

Discussion

The isolated algal strain was morphologically and genetically identified as *Desertifilum tharense* (Dadheech et al., 2012). Although *Desertifilum tharense* and *Desertifilum salkalinema* are different in morphology and habitat; these two species are genetically homogeneous. The cells of *D. salkalinema* are thinner and longer than *D. tharense* cells. Cells of *D. salkalinema* are 2.08 ± 0.80µm wide and 5.14 ± 0.51µm long (Cai et al., 2017), while cells of *D. tharense* are 2.61 ± 0.140µm wide and 4.016 ± 0.108µm long. With regard to habitat, *D. salkalinema* is usually isolated from alkaline water, whereas the habitat of *D. tharense* is of low salinity and alkalinity, thus these two species show

different ecological characteristics (Dadheech et al., 2012; Cai et al., 2017).

Cyanobacteria are recognized as a potent source of bioactive compounds with antibacterial and antifungal activities (Wijesekara & Manage, 2017). In the present study, the antifungal activity of *D. tharense* intracellular and extracellular metabolites was evaluated against *F. oxysporum*. In addition, extract concentration was affected by the type of organic solvent. The highest extract concentration was obtained by acetone and this observation was not matched with the results of Abdel-Aal et al. (2015) who reported that the highest extraction yield from *Spirogyra longata* was obtained by methanol. Furthermore, our results disagree with Lotfi et al. (2021) who reported that the methanol maintained the highest percentage of extract in *Ulva lactuca*, *Ulva fasciata* and *Cladophora sericea*.

The highest antifungal activity against *F. oxysporum* was observed by *D. tharense* methanol extract and this agrees with Wijesekara & Manage (2017) who found that the methanol extract of *Oscillatoria* sp. possesses the highest antifungal and antibacterial activity against *Staphylococcus aureus* and *Candida albicans*. In addition, this outcome aligns with Alrefaey et al. (2019) who reported that the methanol extract of *Anabaena azolla* exhibits the highest antifungal activity against *Fusarium oxysporum, Penicillium expansum, Aspergillus niger* and *Candida albicans*.

Desertifilum tharense methanol extract, which exhibited the highest antifungal activity against *F. oxysporum* f. sp. *Lycopersici*, was analyzed by GC-MS and LC-ESI-MS for identification of bioactive compounds. Moreover, the four compounds were detected in *D. tharense* methanol fraction by GC/MS. Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-, and di-n-octyl phthalate compounds, which are potent antifungal compounds (Celis et al., 2011; Akhtar et al., 2020). On the other hand, the LC-ESI-MS was used to identify the polar compounds in *D. tharense* methanol fraction. Twenty-one chromatographic peaks were detected in the LC-ESI-MS of *D. tharense* methanol fraction.

Two amino acids were observed; the peak 4 with a molecular anion at 178.0935 *m/z* $[M+Na]^+$ showed a fragment ion at 156.0939 *m/z* which indicates the presence of histidine (Thiele et al., 2008), while the peak 5 with a molecular anion at 203.0933 *m/z* $[M+Na]^+$ showed a fragment ion at 181.1309 *m/z* which indicates the presence of tyrosine (Thiele et al., 2008). Campanella et al. (2002) reported that the amino acids e.g histidine, tyrosine, and phenylalanine, were identified by the analysis of *Spirulina* sp. extract.

Four peptides were detected, two dipeptides were represented as peaks 1 and 2, while the remaining were tetrapeptides (peaks 3 and 7). The peak 1 showed a molecular ion peak at 264.8113 *m/z* $[M+H]^+$, and the appearance of 221.0129 *m/z* fragment indicated a loss of CO^- and NH_3 which indicates the presence of peptides. Correspondingly, the appearance of a fragment ion at *m/z* 118.1547 indicates the presence of valine, and the other fragments indicate a successive loss of H_2O molecule which indicates the presence of phenylalanyl structure. Thus, the peak 1 could be assigned as phenylalanyl valine (Steigenberger et al., 2019). As for the peak 2, it showed the molecular ion peak at 266.9915 *m/z* $[M+H]^+$. The appearance of *m/z* 118.9663 fragment indicates the presence of threonine, so compound 2 is tentatively identified as phenylalanyl threonine (Steigenberger et al., 2019). In turn, peak 3 showed a molecular ion peak at 439.0763 *m/z* $[M+H]^+$ and illustrated a product ion at *m/z* 118.9526 as well, which indicated the presence of threonine. Peak 3 also displayed fragments at 365.0549 *m/z* after loss of 74 da due to loss of H_2O, CO^- and NH_3 and 242.8575 *m/z* after loss of 120 da, which indicates the presence of phenylalanyl. Hence, compound 3 was tentatively identified to be threoninyl phenylalanyl alanyl threonine (Huang et al., 2019). Similarly, the appearance of molecular ion peak for compound 7 at *m/z* 378.3013 $[M+H]^+$ and other fragments, after the loss of CO^- and NH_3, also indicates the presence of tetrapeptide, which is alanyl alanyl serinyl methionine (Huang et al., 2019).

Three fatty acids were detected at 6,8 and 11, and the derivatives represented as peaks 12,13 and 14. Peak 6 with a molecular anion at 437.2108 *m/z* $[M+H]^+$ showed a fragment 275.3391 *m/z* after the loss of 162 Da indicating a hexoside moiety. Subsequently, compound 6 was tentatively assigned as octadecapentaenoic acid-O-hexoside (Napolitano *et al.*, 2018). Nevertheless, the molecular anion of peaks 8 and 11 was 331.1773 *m/z* $[M+H]^+$ and 333.3584 *m/z* $[M+H]^+$; thus, compound 8 was tentatively identified as trihydroxyoctadecenoic acid, and compound 11 was assigned as trihydroxy octadecanoic acid (Napolitano *et al.*, 2018). Molecular ion peak of compound 12 was 425.1948

m/z $[M+Na]^+$. Fragments of peak 12 after loss of 22 Da were 403.1767 *m/z*, but changed to 361.1830 *m/z* after loss of 42 Da, which indicates the presence of acetate. Therefore, compound 12 could be identified as trihydroxy propanoyl hexadecanoic acid acetate(Napolitano et al., 2018). Compound 13 with mass spectrum 543.1926 *m/z* $[M+H]^+$ was assigned as linoleic anhydride, and peak 14 was linoleic cholestanyl ester due to the presence of molecular ion at 678.5082 *m/z* $[M+H]^+$. Jassbi et al. (2013) reported that octadecenoic acid was the major fatty acid in the extract of *Hypnea flagelliformis*, *Cystoseira myrica* and *Sargassum boveanum*, *and* it possesses *antibacterial action against Staph. aureus* and *Bacillus subtilis.*

Peaks 9 and 10 showed the same quasi-molecular ion peak at *m/z* 579 $[2M+Na]^+$. They also showed the same fragments at 279 and 149 *m/z*, which indicate the presence of phthalic acid derivatives. Thus, compounds 9 and 10 are dioctyl phthalate (Net et al., 2015; Khaled et al., 2019). *Pertaining to* peaks 18 and 19, they showed the same molecular ion peak at *m/z* 803 $[2M+Na]^+$ and the same fragment at *m/z* 391 $[M+H]^+$ and 149. *Hence,* compounds 18 and 19 were tentatively assigned as dioctyl phthalate (Net et al., 2015; Khaled et al., 2019). Phthalic acid esters are known as plasticizers in polymer materials, whereas Husein et al. (2014) reported that plants or algae such as *Lythrum* sp. and *Sargassum* sp. that usually grow in water flow can synthesize phthalates compounds that are known for their antimicrobial activity.

Two compounds were detected as flavonoids (peaks 16 and 17) showing the same base peak at *m/z* 353 $[M+Na]^+$, and the same fragment at *m/z* 331 $[M+H]^+$ resulted from the loss of Na (22 amu). On the bases of the mass fragments, peaks 16 and 17 were identified as trihydroxy dimethoxy flavone (Marzouk et al., 2018).

Peak 20 showed a molecular ion peak at 739.7314 *m/z* $[2M+Na]^+$ and a fragment ion at 359.3035 *m/z* $[M+H]^+$. Hence, compound 20 was identified as dihydroxyhexadecane (Hussein *et al.*, 2018). Compound 21 was identified as octacosane due to the appearance of 395.3731 *m/z* $[M+H]^+$ fragment ion peak (Hussein et al., 2018). Patel et al. (2020) reported that the red alga *Gracillaria corticata* is able to produce octacosane molecule.

Desertifilum tharense methanol extract was applied as a biocontrol agent against tomato wilt pathogen; *F. oxysporum*. Notably, the highest percentage of disease incidence was observed in positive control, while the lowest percentage of disease incidence was observed in negative control and *D. tharense* treatments. These results agree with Alwathnani & Perveen (2012) who found that the percentage of disease incidence was higher in tomato plants infected with *F. oxysporum* f. sp. *lycopersici* than in tomato plants treated with *Nostoc linckia*. The highest percentage of seed germination was observed in the positive control, followed by *D. tharense* treatments. These results disagree with Alwathnani & Perveen (2012) who found that the highest *percentage* of tomato seed germination was observed in seeds treated with the cyanobacterium *Nostoc linckia*. Positive control of tomato plants presented high total fresh weight and plant height. Interestingly, this observation is because of the positive effect of *F. oxysporum* volatiles that enhance plant growth in addition to the fact that it was found to be in compliance with our observation Bitas et al. (2015), who found that *F. oxysporum* produces several volatiles that cause growth enhancement and augment of *Arabidopsis thaliana* and tobacco height. *D. tharense* methanol extract has a positive effect on tomato plant yield. *Desertifilum tharense* methanol extract contains phenolics, flavonoids, amino acids, and fatty acids that enhance plant growth, yield, and make the plant more resistant to biotic and abiotic stress. In corroboration of our results, Kim & Kim (2008) reported that extracts of cyanobacterium *Nostoc commune* FA-103 have a suppression effect to *Fusarium oxysporum* f. sp. *lycopersici* growth, sporulation and wilt infection of tomato pathogen either in *vitro* or in *vivo*. Abdel-Hafez et al. (2015) found that metabolites of *Nostoc muscorum* and *Oscillatoria* sp. have potential fungicide effect to control purple blotch disease of onion that is caused by *Alternaria porri* under greenhouse conditions, and therefore, cyanobacteria metabolites can be used as biocontrol agents of plant diseases, and this was in accordance with our observations.

Conclusion

In-*vitro* and in-*vivo* assay results clarify that *D. tharense* P11 methanol extract was the most effective extract in growth reduction of *F. oxysporum* f. sp. *lycopersici* and control of vascular wilt disease on tomato plants. Bioactive compounds also identified in *D. tharense* P11 methanol extract can play a double role as biocontrol of vascular wilt disease in tomato and bio-simulant properties.

Finally, in field application of *D. tharense* P11 methanol extract, it is considered a natural, cheap and safe agrochemical

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Authors' contributions: M. kottb and A. Alallaf: Proposed the idea of this study; A. Alallaf: designed the experimental work, made the measurements, analyzed and interpreted the data, performed the calculations and statistical analysis, wrote the manuscript, and acted as the corresponding author. A. El-Sayed: analysis of molecular data. All authors: manuscript drafting and revision.

Ethics approval: Not applicable.

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مستخلص الميثانول لطحلب *Desertifilum tharense* تأثير فعال في تثبيط مرض الذبول الوعائى الناتج من فطر *Fusarium oxysporum* f. sp. *lycopersici* وتعزيز نمو نبات الطماطم

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يعتبر تطبيق السيانوبكتريا في الزراعة بديلاً واعدًا لمبيدات الفطريات الكيميائية لأنها غنية بالعديد من المواد النشطة بيولوجيًا. فى البحث الحالى تم معملياً اختبار قدرة المستخلصات المختلفة الناتجة من طحلب *Desertifilum tharense* PS 11 فى تثبيط فطر *Fusarium oxysporum*. أوضحت النتائج أن أعلى تأثير فى تثبيط نمو الفطر يعزى إلى مستخلص الميثانول من طحلب *D. tharense* (19.11%).

ثم تم تحليل مستخلص الميثانول بواسطة جهاز كروماتوجرافيا الغاز (GC/MS) وجهاز الاستشراب السائل المزود بمقياس طيف الكتلة (LC-ESI-MS). أظهرت النتائج أن مستخلص الميثانول غنى بالعديد من المركبات النشطة بيولوجياً مثل الفينولات، والالكانات، والأحماض الأمينية، والبيبتيدات، والأحماض الدهنية، ومشتقات حمض الفثاليك. بالاضافة إلى ذلك، تم حقلياً دراسة تأثير مستخلص الميثانول فى المكافحة البيولوجية لمرض الذبول الفيوزارى لنبات الطماطم الذى يسببه فطر الفيوزاريم وتعزيز نمو النبات. أظهرت النتائج أن مستخلص الميثانول كان له تأثير فعال فى الحد من الاصابة بمرض الذبول الوعائى للطماطم الذى يسببه فطر الفيوزاريم وتعزيز نمو النبات وزيادة الانتاجية. أكدت نتائج الدراسة الحالية أن مستخلص الميثانول لطحلب *D. tharense* مجال مستقبلى واعد فى المكافحة البيولوجية لفطر الفيوزاريم وزيادة نمو وانتاجية نبات الطماطم.