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Survey of marine-derived fungal biota and efficacy of its metabolites as plant growth promoter

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ABSTRACT

Marine fungi inhabiting algae represent an ecologically and taxonomically interesting group of microorganisms. Because algal host is the best known for fungi, the information of fungi associated with algae is necessary for discovering secondary metabolites. Marine macroalgae associated with fungal community like *Ulva Lactuca linnaeus*, *Enteromorpha intestinalis Linnaeus*, and *Dictyota sp* were carried out for surveying fungal biota. Those algae were collected from Suez Canal and seashore in the Mediterranean Sea at Port Said governorate. The study aims to investigate the role of these fungi and their secondary metabolites on plant growth. The most abundant of fungal community are phylum Ascomycota and genera *Penicillium*, *Cladosporium*, *Fusarium* and *Aspergillus*.26, marine fungi were isolated from algal samples and culturing on plates with Czapek's yeast extract agar amended with chloramphenicol and Rose Bengal. After identification of organisms by macroscopic and microscopic observation, isolated fungi are let to produce natural secondary metabolites into the media after a week, 50% ethyl acetate in water was added to broth media, then incubated at room temperature. The crude extract was collected after using separating funnel for solvent separation and using rotavapor for extraction. Secondary metabolites identified using GC-MS analysis as Linalool from *Trichoderma harzainum* while Methyl jasmonate and Nylidrin from *Penicillium crustosum*. Raw secondary metabolites of the taxa *Penicillium crustosum* and *Trichoderma harzainum* give increasing in length of shoot and root system of (eggplant) *Solanum melongena* and also affects the whole plant by increasing its dry weight.

Keywords:
Marine algae, Marine derived-fungi, *Penicillium crustosum*, Secondary metabolites, Trichoderma harzainum..

1. INTRODUCTION

Marine habitats are complex and harbor a broad spectrum of fungal taxa that have been identified and described [1]. Most origin of marine fungi revealed to be from soil environment, such as *Fusarium* spp. *Penicillium* spp., *Aspergillus* spp. Taxa of Marine-derived fungi seem to have diversity in habitats, like mangrove wood, algae, fish, sponge's deep sea and marine sediments.

Marine derived fungi are classified according to habitat to facultative marine fungi that are soil origin or fresh water origin and have the ability to flourish in marine environment while obligate marine fungi flourish only in marine environment [2], [3], and [4]. Fungi from marine environment are good source of natural secondary metabolites unlike fungi from soil environment because of their adaption to harsh conditions such as fluctuation pressure and high salinity [4]. Facultative and obligate classification of marine fungi could be expressed as marine-derived fungi and marine isolates of samples were not verified [5]. Marine environments are extremely complex and host a wide diversity of fungal species [1]. Nowadays, an online database (www.marinefungi.org) was available to obtain more details on the taxonomy of marine-derived fungi, with all information of marine fungal species [6].

Algae are considered an excellent host for many microorganisms [7; 8] especially its thallus surface. Microorganisms like bacteria and fungi affect positively on algal growth and ecosystem [9; 10]. Physical and biochemical processes in the surface of algal thallus, play a role in community association with algae [11; 12]. Thirty percent of marine derived-fungi are isolated from algae [13]. Marine fungi play an important role in finding structurally unique secondary metabolites, some of which show promising pharmacological activities, providing useful clues for drug discovery [14].

Close to 40% of all natural products in China was covered [14] displayed various bioactivities. The main reported biological activities were cytotoxicity against some cancer cell lines, antimicrobia activity, and antiviral activity, which accounted for 13%, 9%, and 3% of all natural products reported.

Exophilone new secondary metabolite with other nine known compounds, were isolated from *Exophiala oligosperma* which was deep-sea-derived fungus [15].

Marine derived-fungi have a new and many promising sources of natural bioactive secondary metabolites, also marine microalgae, cyanobacteria and bacteria produce secondary metabolites [16]. The first isolated marine secondary metabolites from marine derived-fungi *Acremonium chrysogenum* was cephalosporine C [17]. Other secondary metabolite was isolated from sponge host *Penicillium chrysogenum* can produce sorbicillactone A, which has cytotoxicity against leukemia [17, 18, 19].

Global studies and researches of marine fungi showed more than 270 new identified bioactive secondary metabolites from early 1990 to middle of 2002. More than 70% of metabolic compounds were isolated from sponge host and plant derived-fungi. From 2002 to 2006, 330 new natural compounds were recorded including large number of new carbon skeletons [17, 18, 19]. This finding give huge interest in marine derived fungi as a source of new biological active metabolism.

A variety of bioactive fungal secondary metabolites were obtained by the genus *Trichoderma* spp. and *Penicillium* spp. as well as many other fungal taxa is well known [19, 20]. Natural secondary metabolites are different chemical compounds such as enzymes, antibiotics and growth regulators which produced by microorganisms for protection and existence when symbiotic relationships, mineral transport and competition as can kill competitor microorganisms [21, 22].

Some *Trichoderma* spp. can affect negatively on phytopathogens through production of natural secondary metabolites that affect metabolism of the plant and cause increase of plant growth furthermore plant productivity is increased [23].

Plant biomass are promoted by several *Trichoderma* strains as indole-3- acetic acid (IAA) are produced causing increase of root growth [24, 25]. Many researchers are able to identify and isolate natural secondary metabolites from *T. harzianum* (6-pentyl-α -pyrone) and *T. koningii* (koninginins A-C, E, G) that act as plant regulators 26, 27, 28, 29].

Trichoderma virens prevent outgrowth of wheat coleoptiles at 10^{-4} and 10^{-3} M through its natural secondary metabolites trichocaranes A – D and carotane skeleton [30].

Viridiol is a natural secondary metabolite produced by *T. virens* and has the ability to inhibit plant growth [31] and also prevent biosynthesis of aflatoxin through inhibition of 5'-hydroxyaverantin dehydrogenase enzyme [32, 33].

Targets of the research were (i) isolation and identification of marine fungi associated with *Ulva lactuca* Linnaeus, *Enteromorpha intestinalis* Linnaeus, and *Dictyota* sp. Also, (ii) analysis and study natural secondary metabolic compounds released by marine derived-fungi like *Trichoderma sp*., *Penicillium sp*, and many other genera which isolated from selected algal taxa; as well as studying effect of metabolic compounds on growth of the plant.

2. MATERIAL AND METHODS

2.1. Site and location characters

Suez Canal is considered an arm of the Red Sea as it connects Gulf of Suez with Port Said on the Mediterranean Sea through artificial waterway from north to south across the Isthmus of Suez in north-eastern Egypt.

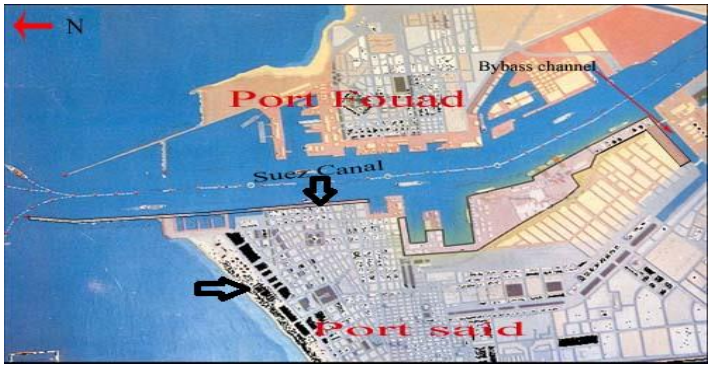


Figure 1: Side of samples collection from Suez Canal and Port Said sea shore. (Arrows)

2.2. Sample collection

Marine algal thalli (*Ulva lactuca, Encephalitozoon intestinalis,* and *Dictyota* sp.) were collected from Suez Canal and sea shore at Port Said District, Egypt (31° 15' 36" N - 032° 17' 24" E) through November and December 2021 (autumn season) during low tide (Fig. 1). Contamination is prevented through transporting samples to presterilized polythene bags and kept at 7°C in lab refrigerator (as many as 40 algal thallus for each species) and then used for fungal isolation. The surfaces were washed with sterile seawater, and then sliced into small pieces (3mm x 2mm).

Figure 2: Showing algal thalli growing in Port Said sea shore

2.3. Fungal isolation and identification

Forty thalli of candidate's algal taxa were collected to test the existence of marine fungi associated with these algae. An overall of 48 Petri plates were incubated and placed at 27^{o}C for 4 days to let the formation of colonies, it necessary to register the colony forming unit (CFU) of each fungal colony per gram of wet weight of each algal thallus.

Discs of 3 x 2 mm were cut from algal thallus then washed by sterile seawater for 3 min. then incubation of algal thallus discs in petri dishes with potato dextrose agar media (pre-prepared in lab., filtrate of 250 gm boiled potato tuber – 20gm agar then completed to one liter by water) and / or Czapek - yeast extract agar (CYA, 30gm sucrose, 3gm sodium nitrate, 1gm potassium dihydrogen phosphate, 0.5gm potassium chloride, 0.5gm magnesium sulphate, 5gm yeast extract, and 2gm agar, then completed to one liter by water) , all media were containing half strength sea water SW for yeast and mold isolation; to prevent contamination with bacteria Rose Bengal (1/15000) and chloramphenicol (250 mg/L) were added to media then incubation plates for 4 days at 27°C. After incubation period resulted cultures were sub-cultured to fresh petri dishes containing Czapek's media and incubated for 6 days at 27°C to allow sporulation of fungi then cultures were stored at 4°C in lab refrigerator.

Morphological characters of marine fungi

To identify fungal biota morphologically, colony color and diameter, reverse and exudates were determined and recorded as represent macro-morphological characters. Mycelium, conidia and phialides were observed as represent micro-morphological characters [34].

2.4. Examination of fungal crude metabolites as eggplant plant growth promoting

Seed germinated in small pots of ten centimeter depth contain humus and sand with 1:1 ratio for seven days. In March 2021, sterilized eggplant (*Solanum melongena*) seedling was introduced to sterilized soil for two hours. Pots contain humus and sand were presented in greenhouse characterized by 14-10 h light dark cycle, 60% relative humidity and 17-20 ^{0}C. *Trichoderma* and *Penicillium* secondary metabolite were collected and concentrated using rotary evaporator for 3 hrs. 500 µL to 1500 uL of *Trichoderma* and *Penicillium* secondary metabolite filtrate (in ethyl acetate) were inoculated to soil near crown zone After 5, 14 and 21 days of sowing while control test inoculated with fresh media containing fertilizers. The experiment was done using 7 pots with 3 seedling in every pot replicate three times.

2.5. Extraction of fungal metabolites

Trichodema and Penicillium were allowed to produce their natural secondary metabolic compounds in number of flasks which contain 200 ml of Czapek broth media and two or three discs CZ agar media that contain tested fungi then incubation at 25 ^{0}C statically for 28 days. After incubation period, mycelium biomass was observed and filtrated, ethyl acetate (3 × 500 mL for each 1 L of broth) was

added to filtrate, evaporation process used to get secondary metabolites crude extract. The crude extract was collected after using separating funnel for solvent separation and using rotavapor for extraction.

2.6. GC-MS

The chemical composition of samples analysis were performed using GC-TSQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m x 0.25 mm x 0.25 µm film thickness). The column oven temperature was initially held at 60°C and then increased by 6°C /min to 250°C withhold 1 min then increased to 300 wiht 30 C/min. The injector temperature was kept at 270°C. Helium was used as a carrier gas at a constant flow rate of 1 ml/min. The solvent delay was 4 min and diluted samples of 1 µl were injected automatically using Autosampler AS3000 coupled with GC in the split mode. EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 50–650 in full scan mode. The ion source and transfer line were set at 200 °C and 280 °C respectively. The components were identified by comparison of their mass spectra with those of WILEY 09 and NIST14 mass spectral database [34].

2.7. Molecular identification of marine fungi

2.7.1. Extraction of DNA

Potato dextrose agar media (PDA) was centrifuged at 10000 g for three min, supernatant was discarded and mycelium was homogenized in 400 µL of TBS buffer (0.4 M NaCl, 10 mM Tris-HCl pH 8 and 2 mM EDTA pH 8), after that sample was vortexed for one minute. 20 mg/ml of Proteinase K and 40 µL of 20% SDS were added. Incubation the mixture at 60 ^{0}C for three hrs., then 300 µL of 6 M NaCl were added , tubes were vortexed for half one min before centrifuged at 10000 g for thirty minutes at 4 ^{0}C. Supernatants were added to isopropanol contain tubes of equal volume then incubation at 20 ^{0}C for one hour. centrifugation of tubes at 10000 g for twenty minutes at 4 ^{0}C , centrifugation of pellet after washing with 70% at maximum speed for ten minutes , after that subsequently recovered the pellet and dried at 37 ^{0}C for five minutes , DNA was suspend in 300 µL of Tris-EDTA buffer (TE buffer), finally stored at -20 ^{0}C. Agarose gel technique was applied for estimation of DNA under UV rays.

2.7.2. PCR amplification and sequencing

PCR amplification depend on change in temperatures as denaturation step occur at 94 ^{0}C for 2 min followed by hybridization step at 72 ^{0}C for 1min , finally elongation step at 72 ^{0}C for 10 min. after amplification products of PCR were analyzed by gel electrophoresis that have 1% Agarose gel in 1X TBE buffer stained with SYBR SAFE (1%). For fungal identification PCR products have been sequencing only perfect query sequences as ITS (Internal Transcribed Spacer) regions of rDNA were identified as ITS1 (50-GGA GTA AAA GTC GT A ACA GG-3 0) and ITS4 (50-TCC TCC GCT TATTGA TATGC-3 0). 4 µL Solis Biodyne FIREPOL, 5 µL of fungal DNA, 0.75 µL of fungal primer at 10 µM, were combined in 25 µL volume. Then identified by Megablast Algorithm of NCBI's Nucleotide BLAST Tool, The Fungi Ref Seq ITS databases (Fungi FTP:ftp://ftp.ncbi.nlm.nih.gov /refseq/Targ etedLoci/Fungi/) was targeted as ITS region was recorded

3. RESULTS and DISCUSSION

Through this treatise, a total number of 31 species belong to 16 genera, have been isolated from different algal species (*Ulva lactuca, Encephalitozoon intestinalis,* and *Dictyota* sp.). Isolated fungi belong to four classes (Table 1) of which Ascomycota (anamorphic) as 23 species represent 74.19 % of

isolated taxa. Then Mitosporic fungi that represented by 5 species constituting 16. 3% of the total fungi. While class *Zygomycota* exemplified by 2 species accounting for 6. 45 % of the total fungi. While class Ascomycota (teleomorphic) came next represented by only one species (3.23%).

Table (1): Number of isolated species from different algal taxa

Algal / **Classes**	***Ulva. Lactuca***	***E. intestinalis***	***Dictyota* sp.**	**Total**	**%**
	No. of spp. Isolated	**No. of spp. Isolated**	**No. of spp. Isolated**		
Mitosporic fungi	**4**	**5**	**0**	**5**	**16.13**
Ascomycota (teleomorphic)	**1**	**1**	**0**	**1**	**3.23**
Ascomycota (anamorphic)	**14**	**13**	**17**	**23**	**74.19**
Zygomycota	**2**	**1**	**0**	**2**	**6.45**
Total No. of species	**21**	**16**	**17**	**31**	**100.00**

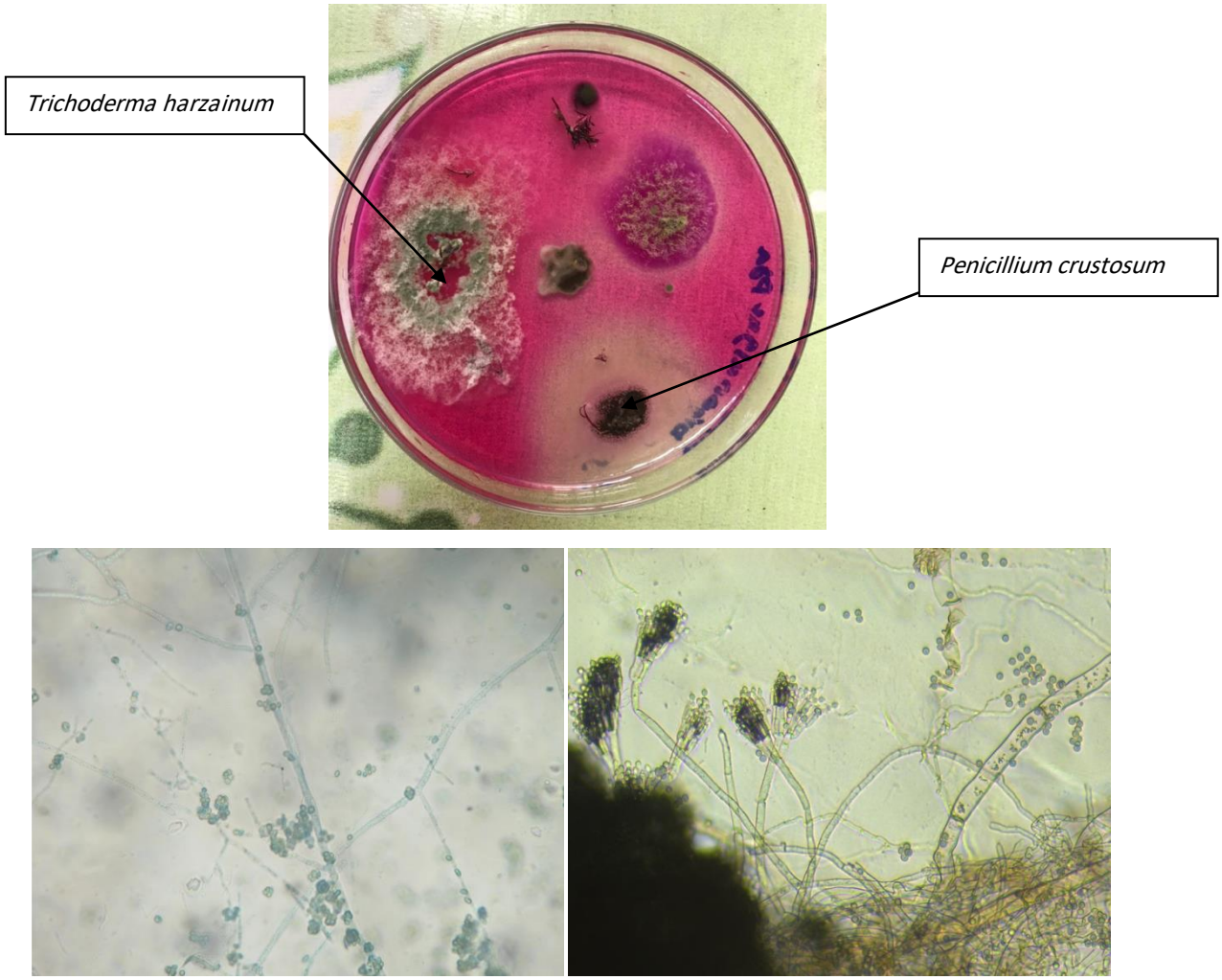


Figure 3: Morphological identification of *Trichoderma harzainum* and *Penicillium crustosum.*

Species richness:

Richness of species means the number of species belonging to each genus isolated throughout the present study. The genera recorded are given in Table 2. It is clearly evident, from the Table, that *Aspergillus* is the richest by showing a spectrum 6 species (including anamorph stage of one *Eurotium* sp.). *Cladosporium* and *Penicillium* comes next by being represented by 3 species each. After them *Trichoderma, Fusarium, Alternaria, Acremonium* and *Curvullaria, by* showing two species each. Only one species were represented. *A. niger* is the most dominant among all Aspergilli, *F. oxysporum* among all Fusaria, while *P. chrysogenum* among all Penicillia.

Table (2): GC-MS compound names, structure, retention time, Molecular formula and molecular weight.

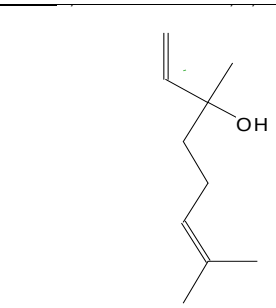
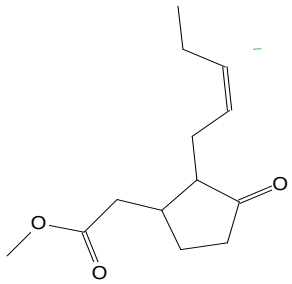
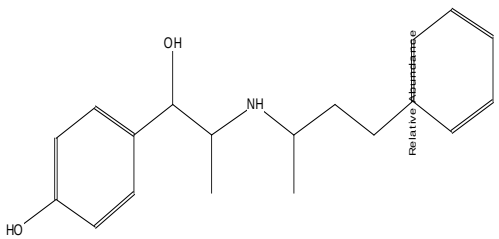
Name	Structure	Retention time	Molecular formula	Molecular weight
Linalool		7.66	C10H18O	154
Methyl jasmonate		24.55	C13H20O3	224
Nylidrin		18.40	C19H25NO2	299

Table (2): Genera and species richness of isolated fungi from algal taxa

No.	Algal taxa / Fungal genera	*Ulva lactuca*	*E. intestinalis*	*Dictyota* sp.	total No. Of Species
		No. of species	No. of species	No. of species	
1	***Aspergillus*** *	5	3	3	6
2	***Penicillium***	3	2	4	4

3	***Cladosporium***	1	2	3	3
4	***Trichoderma***	2	2	1	2
5	***Alternaria***	1	1	1	2
6	***Acremonium***	1	1	1	2
7	***Fusarium***	2	1	0	2
8	***Curvullaria***	0	1	2	2
9	***Paecilomyces***	1	0	0	1
10	***Botryotrichum***	1	0	0	1
11	***Chrysosporium***	0	1	0	1
12	***Stemphylium***	1	0	1	1
13	***Ulocladium***	1	0	0	1
14	***Mucor***	0	1	1	1
15	***Syncephalastrum***	1	0	0	1
16	***Rhodotorula***	1	1	0	1
////	**Total**	**21**	**16**	**17**	**31**

*including ascosporic taxa

Total fungal count:

Fungal counts were expressed as total number of colony forming units per millimeter wet algal thallus (cfu/wmm). The data of Table 3 show there is apparent difference in fungal counts among tested algal species. *Ulva lactuca* showed a mean colony count of 833.6 cfu/wmm, *Encephalitozoon intestinalis* revealed a mean colony count of 500.1, while *Dictyota* sp. Accommodates only 483 cfu/wmm.

Frequency of species:

It is based on the percentage number of cases of isolation (regardless of colony count). The data of Table 3 revealed that, in view of frequency values, recorded species could be temporarily divided into four ecological classes as follows: High occurrence group (H), including species showing frequency values of 80 % or more out of 5 cases; Moderate occurrence (M), from 60 – 80 %; Low occurrence (L), species showing frequency values between 40 % and 60 %; Rare occurrence (R), less than 40 %.

Table 3. Main of total count (MTC, colonies/ mm wet algal thallus), number of cases of isolation (NCI, out of five algal samples), percentage frequency (F) and frequency classes (FC) of isolated fungal taxa.

Fungal taxa	*U. lactuca*				*E. intestinalis*				*Dictyota* sp.			
	MTC	**NC1**	**%F**	**FC**	**MTC**	**NC1**	**%F**	**FC**	**MTC**	**NC1**	**%F**	**FC**
Aspergillus flavus	16.7*	4	80	H	0	0	0	0	33.3	2	40	L
A. niger	50	3	60	M	0	0	0	0	16.7	4	80	H
A. terreus	33.3	4	80	H	33.3	1	20	R	0	0	0	0

A. . Sydowii	16.7	2	40	L	16.7	1	20	R	0	0	0	0
A. ochraceous	0	0	0	0	16.7	1	20	R	16.7	1	20	R
A. galacus	16.7	2	40	L	0	0	0	0	0	2	40	L
Penicillium chrysogenum	83.3	3	60	M	50	1	20	R	0	0	0	0
P. citrinum	33.3	3	60	M	16.7	1	20	R	0	0	0	0
P. crustosum	16.7	2	40	L	0	0	0	0	16.7	1	20	R
P. sp.	0	0	0	0	0	0	0	0	33.3	2	40	L
Cladosporium cladosporioides (Fresen) V.	116.7	5	100	H	83.3	2	40	L	0	0	0	0
C.herbarum (Pers.) Link	0	0	0	0	33.3	1	20	R	33.3	2	40	L
C. sp.	0	0	0	0	0	0	0	0	16.7	3	60	M
Acremonium terricola	16,7	1	20	R	0	0	0	0	33.3	2	40	L
Acr. sp.	0	0	0	0	33.3	1	20	R	0	0	0	0
Alternaria alternata (Fr.) Keissl.	16.7	1	20	R	0	0	0	0	33.3	3	60	M
Alt. tenuissima Samuel Paul Wiltshire	0	0	0	0	16.7	1	20	R	0	0	0	0
Curvularia sp.	0	0	0	0	16.7	1	20	R	16.7	1	20	R
Curv. lunata (Wakker) Boedijn	0	0	0	0	0	0	0	0	16.7	1	20	R
Fusarium oxysporum	50	3	60	M	0	0	0	0	83.2	3	60	M
F. solania	16.7	2	40	L	16.7	3	60	M	0	0	0	0
Trichoderma harzianum	100	3	60	L	50	3	60	M	0	0	0	0
T. koningii	50	2	40	L	0	0	0	0	50	1	20	R
Botryotrichum piluliferum	16.7	1	20	R	0	0	0	0	0	0	0	0
Chrysosporium sp.	0	0	0	0	16.7	2	40	L	0	0	0	0
Mucor racemosus	0	0	0	0	16.7	1	20	R	33.3	2	40	L
Paecilomyces sp.	16.7	1	20	R	0	0	0	0	33.3	2	40	L
Rhodotorula sp.	133.3	3	60	M	83.3	4	80	H	0	0	0	0

Stemphylium sp.	16.7	1	20	L	0	0	0	0	0	0	0	0
Syncephalastrum racemosum Cohn ex J. Schröt.	16.7	1	20	L	0	0	0	0	0	0	0	0
Ulocladium atrum	16.7	1	20	L	0	0	0	0	16.7	2	40	L
Total	833.6	//////////			500.1	//////////			483	//////////		

Abbreviations: TC: Total count, CI: Cases of isolation, F: Frequency and FC: Frequency classes.

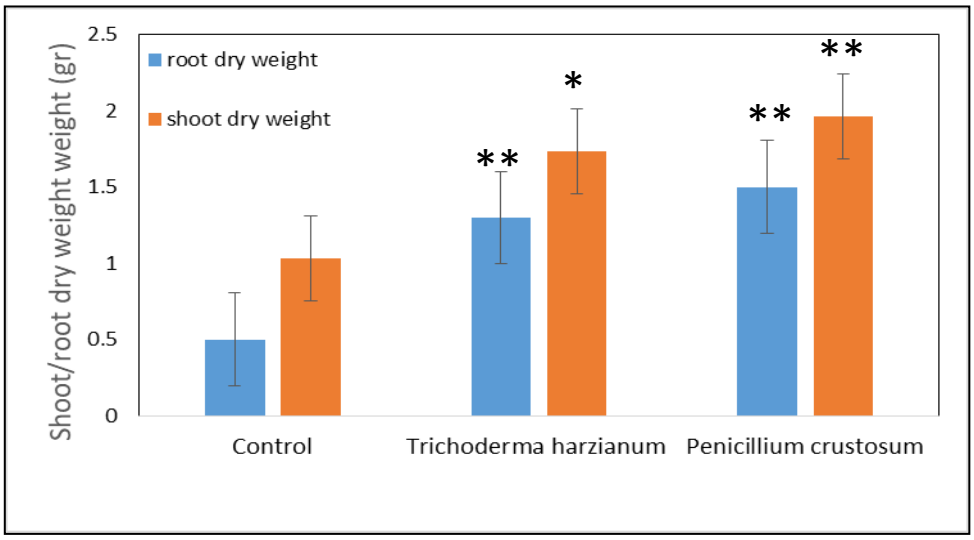


Figure 3: Effect of *Trichoderma harzianum* and *Penicillium crustosum* secondary metabolites on the dry weight of *Solanum melongena*. Data are the means of three replicates (*n*= 3) asterisks indicate significant differences from the control at $p < 0.01$.

Impact of raw fungal secondary metabolites (RSM) on plant growth

Data of Table 4 show clearly that both *Penicillium crustosum* and *Trichoderma harzianum* enhanced eggplant growth although apparently, there is no difference in growth promotion between candidate taxa but obviously, the differences were between tested species and control.

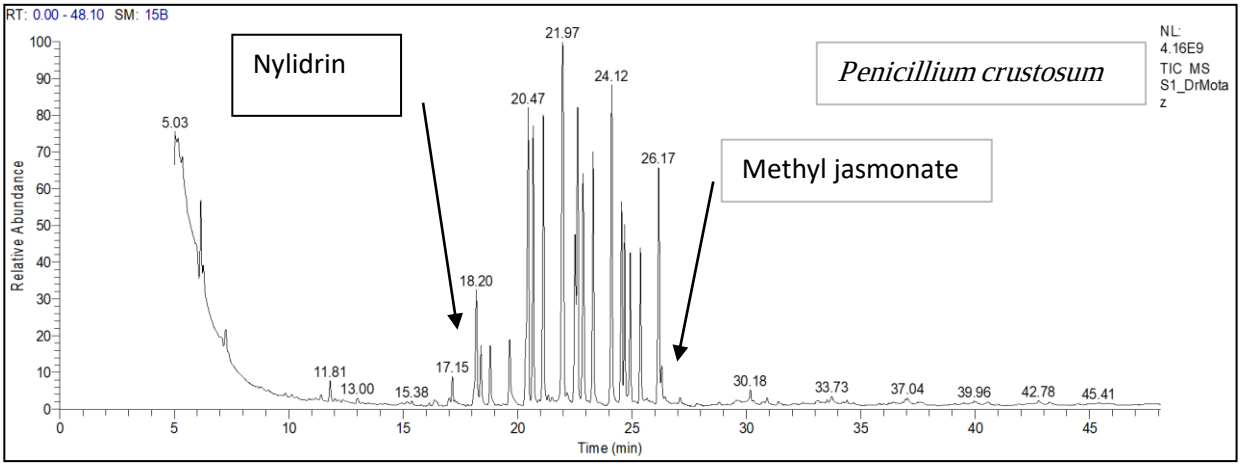
Table 4. Metabolites measurement effect of *Trichoderma harzianum* and *Penicillium crustosum* on the growth of (eggplant) *Solanum melongena* seedling. *In vitro*

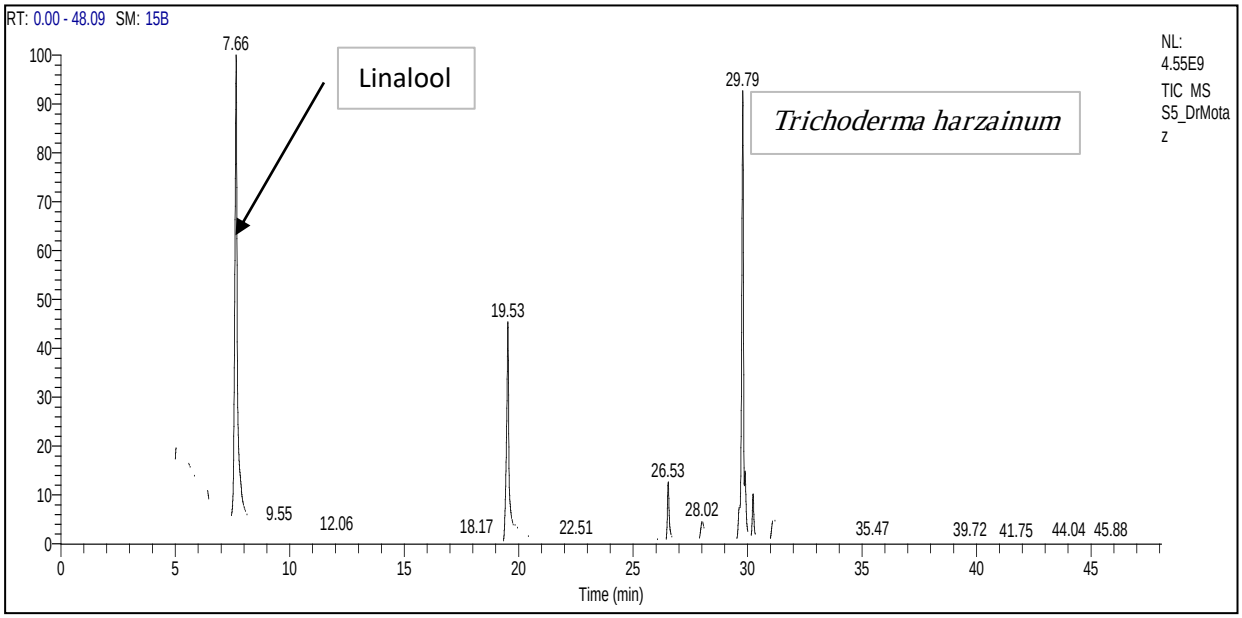
Plant part / Treatment	**Control**	***T. harzianum***	***P. crustosum***
Seed germination N/L*	**6 / 3**	**7 / 4**	**8 / 4**
Length (cm) [whole plant]	**46.7**	**50**	**47.5**
Total Leaves (big & small)	**8**	**13**	**10**
Root length (cm)	**27.5**	**26**	**27.5**

Shoot length (cm)	**18**	**24**	**22**
Wet weight (gm)	**19.2**	**21.9**	**20**
Dry Weight (gm) [whole plant]	**2.1**	**2.5**	**2.3**
Root dry wt	**1.2**	**1.1**	**1.2**

N/L* number of seeds / longevity

Identification of marine fungal biota associated with macroalgae was the main intention of search. Meanwhile, investigating crude metabolites of isolated fungi in plant growth. The two fungus are molecular identified as *Trichoderma harzianum* and *Penicillium crustosum.* The two fungi show rich growth in petri dish and good amount of secondary metabolites compared with other taxa.





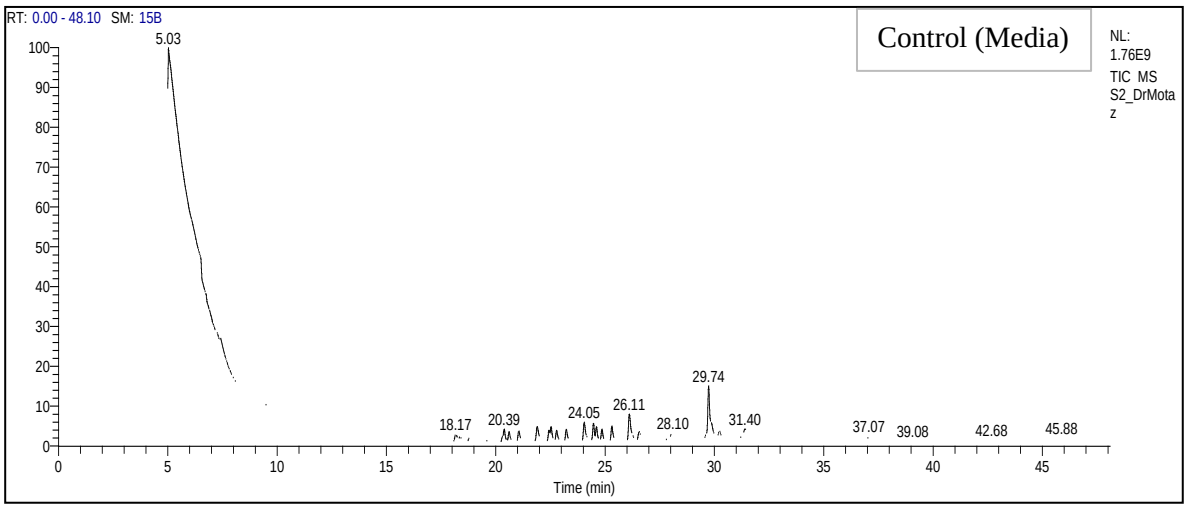


Figure 4: GC-MS spectrum for identified compounds.

To assure reasonable and fair characterization of the mycobiota of candidate algal taxa, two parameters have been adopted in order to avoid over or under estimation of fungal populations. These parameters are species density, based on total number of colonies forming units (cfu) per millimeter wet algal thallus; and species frequency, based on the number of cases of isolation of each species (regardless of its number of colonies on the isolation plates).

In view of species richness i.e. number of species revealed by each genus, the genera *Cladosporium* ,*Penicillium* and *Aspergillus* were the most exhibit a broad spectrum of 6, 4, 3 species respectively. They were followed by *Acremonium, Alternaria, Curvularia, Fusarium* and *Trichoderma* by representing two species; other taxa represented only by one species. The same finding was reported by [12- 15].

As for total count i.e. colony forming units (cfu) per millimeter wet algal thallus, data shows that counts apparently differ from one algal to other. While *Ulva lactuca* showed a main total count of 883.6 cfu/ colonies/ mm wet algal thallus, *E. intestinalis* revealed a main of 500.1 cfu/mmw thallus; still *Dictyota* sp. exhibit only 483 cfu/ colonies/ mm wet algal thallus.

According to the frequency value, isolated fungi were diversified into four ecological groups: High, Moderate, Low and rare frequency classes. High frequency group, involved species showing frequency of 80 % or more out of 5 cases, specified to this group: *Aspergillus flavus*, *A. niger*, *A. terreus*, *Cladosporium cladosporioides*, *Rhizopus stolonifer*, and *Rhodotorula* sp. Moderate frequency group, encompass species rendering frequency from 60 – 80 %. Assigned to this group species of common isolated penicillin, such as, *Penicillium chrysogenum, P. citrinum*; this group includes *Trichoderma harzianum* ,*Cladosporium* sp., , *F. solani, Alternaria alternate, Fusarium oxysporum*, Low frequency group, consisting of species having frequency values between 40 % and 60 %. Among these species: *Acremonium terricola, Aspergillus sydowii, A. galacus, Chrysosporium* sp., *Cladosporium herbarum, Mucor racemosus*, *Penicillium crustosum*, *Trichoderma koningii*, and others many taxa. Rare frequency group, comprised taxa showing frequency less than less than 40 %. This group comprises the following species: *Aspergillus ochraceous*, *Alternaria tenuissima*, *Curvularia lunata*, *Curvularia* sp., and *Botryotrichum piluliferum*

The results obtained in this research; as for species richness, total count and species frequency; agree to some extent with the results obtained in many studies all over the world: [12, 6, 35, 15, and 36]. It is worthy to mention that, also our data of fungi associated with marine macro algae are largely agreement with many results of marine sediment fungal biota; especially species of the genera belonging to aspergilli, penicilli, fusaria, *Alternaria*, *Cladosporium* and *Rhodotorua*; which recorded by many researchers: [37, 38 – 39].

eggplant (*Solanum melongena*) experiment study effect of plant growth regulators extracted from fungal culture filtrate as secondary metabolic compounds that cause elongation of shoot of eggplant seedlings[40,41]. It's necessary to screen and identify natural secondary metabolites in established methods [42].

(Pang et al, 2021) studied the impact of fungal secondary metabolites on supply nutrients, suppress diseases protect from biotic and abiotic environmental stresses and plant growth promotion [43]. Theses metabolites effect positively on growth of plant and preventing plant diseases so enhancing plant growth.

The *in vitro* effect of raw secondary metabolites (RSM) of *Penicillium crustosum* and *Trichoderma harzianum* on eggplant lead to increasing length of root and stem, enhance seed germination, wet and dry weight increased also and numbers of leaves per plant (Table 4). Germination of seed enhanced by both secondary metabolites, number of germinated seeds, length of root and shoot length (Table 4) and plant growth. While *P. crustosum* revealed 8/4, 47.5, 10, 27.5, 22, 20, 2.3, and 1.2 of plant growth morphology respectively; *T. harzianum* showed 7/4, 50, 13, 26, 24, 21.9, 2.5, and 1.1 respectively. Control appeared only 6/3, 46.7, 8, 27.5, 18, 19.2, 2.1, and 1.2.

The metabolism of plant host was affect by secondary metabolites of several *Trichoderma* strains [43,44, 45], these metabolites enhance defense mechanisms of the plant and regulate growth of plant which has been inspected In this treatise we indicate that Raw fungal secondary metabolites (RSM) proved to be a promising as plant growth promoter molecules .Generally, *Trichoderma* produces natural secondary metabolic compounds and hydrolytic enzymes which released extracellularly which display diverse tasks, such as biocontrol agents, promoters for plant growth and other important function [43, 46].

RSM were produced in a huge amount from isolates of *Trichoderma* indicating that IAA (indole acetic acid) would be exist in the SM , IAA induced the growth of tested plant so it considered to be a remarkable and important compound. Our results of *Trichoderma harzianum* are agreement with several data obtained by many researcher all over the world [43- 47]. As for *Penicillium crustosum*, several researcher reported that a diversity of *Penicillium* species prevent pathogens through production of effective compounds. *Penicillium* species solubilize insoluble inorganic phosphate solubilize insoluble inorganic phosphate when respiration process as emission protons or NH_4^+ assimilation [48]. *Penicillium* species can affect positively on growth of the plant by producing hydrocyanic acid, indole acetic acid and siderophores [49]. Indole acetic acid induce length of root, germination of seed and formation of root. [50]. Linalool monotepene has phytotoxic effect against *Lactuca sativa*, *Lepidium sativum*, and *Portulaca oleracea* [51]. According [52] Seed germination, hypocotyl growth regulation, root elongation, petal expansion, and apical hook growth are all covered by Jasmonic acid broad spectrum of action. It was reported that Roots grown on Methyl Jasmonate showed that apical meristem integrity was altered by Methyl Jasmonate induction. [53].

Growth and yield of maize and wheat plant induced by natural secondary metabolic compounds of *P. bilaii* [51, 52]. Length of shoot induced after inoculation with *P. viridicatum*, *P. aurantiogriseum*, *P. herquei* and *P. variabile* [53, 54]. Dry weight of tobacco, sesame and maize increased because *P.*

aurantiogriseum excrete siderophores and indole acetic acid also can dissolve inorganic phosphate. Plant growth of eggplant was enhanced after treatment seedling with RSMs.

4. CONCLUSION

Biological application, taxonomy and ecology of marine derived-fungi are poorly known and little available information although studding over the hundred years [55]. From our research finding suggestions, marine derived-fungi resorted to macroalgae as temperate substrate for protection [56]. Results also proved that the importance of natural secondary metabolites. The ethyl acetate extract from raw secondary metabolites of *Trichoderma harzianum* and *Penicillium crustosum* gives a very promising results in length and dry weight of eggplant parts as ethyl acetate extract have high biological control on soil fungal pathogen effect on the eggplant *Solanum melongena*. More finding on the biological control fungi from marine environment and secondary metabolites need to be investigated.

5. REFERENCES

[1] Amend A, Burgaud G, Cunliffe M, Edgcomb VP, Ettinger CL, Gutiérrez MH, Heitman J, Hom EFY, Laniri G, Jones AC, Kagami M, Picard KT, Quandt CA, Raghukumar S, Riquelme M, Stajich J, Vargas-Muñiz J, Walker A, Yarden O, Gladfelter AS. Fungi in the marine environment: Open questions and unsolved problems.2019, doi: https://doi.org/10.1128/mBio.01189-18.

[2] Kohlmeyer J, Volkmann-Kohlmeyer B. Fungi from coral reefs: a commentary. Mycol Res. 2003, doi: 10.1017/s0953756203227775.

[3] Li Q, Wang G. Diversity of fungal isolates from three Hawaiian marine sponges. Microbiol Res. 164:233–41. 2009, doi: 10.1016/j.micres.2007.07.002.

[4] Bonugli-Santos RC, Dos Santos Vasconcelos MR, Passarini MRZ, Vieira GAL, Lopes VCP, Mainardi PH, Dos Santos JA, de Azevedo DL, Otero IVR, da Silva Yoshida AM, Feitosa VA, Pessoa A Jr, Sette LD. Marine-derived fungi: diversity of enzymes and biotechnological applications. Front Microbiol. 6:269–84. 2015, doi: 10.3389/fmicb.2015.00269.

[5] C. Osterhage. Isolation, structure determination and biological activity assessment of secondary metabolites from marine-derived fungi. PhD Thesis university of Carolo-Wilhelmina at Braunschweig; 2001. https://doi.org/10.24355/dbbs.084-200511080100-40.

[6] Jones, E.B.G., Pang, KL., Abdel-Wahab, M.A. et al. An online resource for marine fungi. Fungal Diversity 96, 347–433. 2019. https://doi.org/10.1007/s13225-019-00426-5.

[6] Godinho, V. M., Furbino, L. E., Santiago, I. F., Pellizzari, F. M., Yokoya, N. S., Pupo, D. … Rosa, L. H. Diversity and bioprospecting of fungal communities associated with endemic and cold-adapted macroalgae in Antarctica. The ISME Journal, 7(7), 1434 –1451. 2013. https://doi.org/10.1038/ismej.2013.77.

[7] Wichard, T., Charrier, B., Mineur, F., Bothwell, J. H., Clerck, O. De, & Coates, J. C. The green seaweed Ulva/: a model system to study morphogenesis. Frontiers in Plant Science, 6, 72. 2015. https://doi.org/ 10.3389/fpls.2015.00072.

[8] Y. Matsuo, H. Imagawa, M. Nishizawa, Y. Shizuri. Isolation of an algal morphogenesis inducer from a marine bacterium. Science, 307(5715). 2014. doi: 10.1126/science.1105486.

[9] Richards, T. A., Jones, M. D. M., Leonard, G., & Bass, D. Marine fungi: their ecology and molecular diversity. Annual Review of Marine Science, 4(1), 495 – 522. 2012. https://doi.org/10.1146/annurev-marine-120710 - 100802.

[10] Egan, S., Harder, T., Burke, C., Steinberg, P. D., Kjelleberg, S., & Thomas, T. The seaweed holobiont: understanding seaweed-bacteria interactions. FEMS Microbiology Reviews, 37(3), 462 – 476. 2013. https://doi.org/ 10.1111/1574 -6976.12011.

[11] Tujula, N. a, Crocetti, G. R., Burke, C., Thomas, T., Holmstrom, C., & Kjelleberg, S. Variability and abundance of the epiphytic bacterial community associated with a green marine Ulvacean alga. The ISME Journal, 4(2), 301– 311. 2010. https://doi.org/10.1038/ ismej.2009.107.

[12] Gnavi, G., Garzoli, L., Poli, A., Prigione, V., Burgaud, G., & Varese, G. C. The culturable mycobiota of *Flabellia petiolata*: first survey of marine fungi associated to a Mediterranean green alga. PLoS ONE, 12(4), 1– 20. 2017. https://doi.org/10.1371/ journal.pone.0175941.

[13] Liu Z, Frank M, Yu X, Yu H, Tran-Cong NM, Gao Y, Proksch P. Secondary Metabolites from Marine-Derived Fungi from China. Prog Chem Org Nat Prod. 111:81-153. 2020. Doi: 10.1007/978-3-030-37865-3_2. PMID: 32114663.

[14] Hong, Ming-Jun, Meng-Jiao Hao, Guang-Yu Zhang, Hou-Jin Li, Zong-Ze Shao, Xiu-Pian Liu, Wen-Zhe Ma, Jun Xu, Taifo Mahmud, and Wen-Jian Lan. "Exophilone, a Tetrahydrocarbazol-1-one Analogue with Anti-Pulmonary Fibrosis Activity from the Deep-Sea Fungus Exophiala oligosperma MCCC 3A01264" Marine Drugs 20, no. 7: 448. 2022. https://doi.org/10.3390/md20070448.

[16] Butler, M.S. The role of natural product chemistry in drug discovery. J. Nat. Prod. 67, 2141–2153. 2004. https://doi.org/10.1021/np040106y.

[14] König GM, Kehraus S, Seibert SF, Abdel-Lateff A, Müller D. Natural products from marine organisms and their associated microbes. Chembiochem 7, 229–238. 2006. doi: 10.1002/cbic.200500087.

[15] John W. Blunt, Brent R. Copp, Wan-Ping Hu, Murray H. G. Munro, Peter T. Northcotec and Michèle R. Prinsep. Marine natural products. Nat. Prod. Rep. 25, 35–94. 2008. https://doi.org/10.1039/B701534H.

[16] Dalla Bona A, Formaggio F, Peggion C, Kaptein B, Broxterman QB, Galdiero S, Galdiero M, Vitiello M, Benedetti E, Toniolo C. Synthesis, conformation, and bioactivity of novel analogues of the antiviral lipopeptide halovir A. J Pept Sci. Dec;12(12):748-57. 2006, doi: 10.1002/psc.808. PMID: 17131285.

[17] Bringmann, G.; Gulder, T.A.M.; Lang, G.; Schmitt, S.; Stöhr, R.; Wiese, J.; Nagel, K.; Imhoff, J.F. Large-Scale Biotechnological Production of the Antileukemic Marine Natural Product Sorbicillactone A. Mar. Drugs. 5, 23-30. 2007, https://doi.org/10.3390/md502023.

[18] Blunt JW, Copp BR, Hu WP, Munro MH, Northcote PT, Prinsep MR. Marine natural products. Natural product reports. 2007 Feb; 24(1):31-86. doi: 10.1039/b603047p.

[19] Ghisalberti EL, Sivasithamparam K. Antifungal antibiotics produced by *Trichoderma* spp. Soil Biology and Biochemistry, 23, 1011-1020. 1991, https://doi.org/10.1016/0038-0717(91)90036-J.

[20] Reino JL, Guerrero RF, Hernández-Galán R, Collado IG. Secondary metabolites from species of the biocontrol agent *Trichoderma*. Phytochemistry Review, 7, 89-123. 2008. DOI: 10.1007/s11101-006-9032-2.

[21] Demain AL, Fang A. The natural functions of secondary metabolites. Advances in Biochemical Engineering-Biotechnology, 69, 1-39. 2000, doi: 10.1007/3-540-44964-7_1.

[22] Chiang Y, Lee K, Sanchez JF, Keller NP, Wang CCC. Unlocking fungal cryptic natural products. Natural Product Communications, 4, 1505–1510. 2009. https://doi.org/10.1177/1934578X0900401113.

[23] Vinale F, Ghisalberti EL, Sivasithamparam K, Marra R, Ritieni A, Ferracane R, Woo SL, Lorito M. Factors affecting the production of *Trichoderma harzianum* secondary metabolites during the interaction with different plant pathogens. Letters in Applied Microbiology, 48, 705–711. 2009, doi: 10.1111/j.1472-765X.2009.02599.x

[24] Le Doan T, El-Hajii M, Rebuffat S, Rajeswari MR, Bodo B. Fluorescein studies on the interaction of trichorzianine A IIIc with model membranes. Biochimica et Biophysica Acta, 858, 1–5. 1986.

[25] Rebuffat S, El Hajji M, Hennig P, Davoust D, Bodo B. Isolation, sequence and conformation of seven trichorzianines B from *Trichoderma harzianum*. International Journal of Peptide and Protein Research, 34, 200-210. 1989. https://doi.org/10.1111/j.1399-3011.1989.tb00231.x.

[26] Benítez T, Rincón AM, Limón MC, Codón AC. Biocontrol mechanisms of Trichoderma strains. International Microbiology, 7, 249-260. 2004.

[27] Contreras-Cornejo HA, Macìas-Rodrìguez L, Cortés-Penagos C, López-Bucio J. *Trichoderma virens*, a plant beneficial fungus, enhances biomass production and promotes lateral root growth through an auxin-dependent mechanism in Arabidopsis. Plant Physiology. 149, 1579–1592. 2009, https://doi.org/10.1104/pp.108.130369.

[28] Hoyos-Carvajal L, Orduz S, Bissett J. Growth stimulation in bean (*Phaseolus vulgaris* L.) by *Trichoderma*. Biological Control, 51, 409–416. 2009, https://doi.org/10.1016/j.biocontrol.2009.07.018.

[30] Cutler HG, Himmetsbach DS, Arrendale RF, Cole PD, Cox RH. Koninginin A: a novel plant regulator from *Trichoderma koningii*. Agricultural and Biological Chemistry, 53, 2605-2611. 1989, https://doi.org/10.1080/00021369.1989.10869746.

[31] Parker SR, Cutler HG, Schreiner PR .Koninginin C: a biologically active natural product from *Trichoderma koningii*. Bioscience Biotechnology and Biochemistry, 59, 1126-1127. 1995, https://doi.org/10.1271/bbb.59.1747.

[32] Parker SR, Cutler HG, Schreiner PR. Koninginin E: isolation of a biologically active natural product from *Trichoderma koningii*. Bioscience Biotechnology and Biochemistry, 59, 1747-1749. 1995, https://doi.org/10.1271/bbb.59.1747.

[33] Macías FA, Varela RM, Simonet AM, Cutler HG, Cutler SJ, Eden MA, Hill RA. Bioactive carotanes from *Trichoderma virens*. Journal of Natural Products, 63, 1197-1200. 2000, doi: 10.1021/np000121c.

[34] Dugan M. F. The American Phytopathological Society (APS). 2017. https://doi.org/10.1094/9780890545041.

[34] Mamoun S. M. Abd El-Kareem, Mohamed Abd El Fattah Rabbih, Ezzat Taha Mohamed Selim, Elsherbiny Abd El-monem Elsherbiny, Ayman Yasen El-Khateeb. Application of GC/EIMS in

Combination with Semi-Empirical Calculations for Identification and Investigation of Some Volatile Components in Basil Essential Oil. International Journal of Analytical Mass Spectrometry and Chromatography, 4, 14-25. 2016, doi: 10.4236/ijamsc.2016.41002.

[35] Zuccaro A, Roth A, Kneip S, Dersch P. Characterization of a new inducible promoter for protein expression in Aspergillus niger, using a green fluorescent protein reporter system. Journal of Biotechnology 131: S256-S257. 2007, doi:10.1016/j.jbiotec.2007.07.464.

[36] Zuccaro, A., and J. I. Mitchell. Fungal communities of seaweeds, p. 533–579. In J. Dighton, J. F. White, Jr., and P. Oudemans (ed.), The fungal community, 3rd ed. CRC Press, New York, NY. 2005,

[37] Zhang, T.; Wang, N. F.; Zhang, Y. Q; Liu, H. Y. and Yu, L. Y. Diversity and distribution of fungal communities in the marine sediments of Kongsfjorden, Svalbard (High Arctic). Sci. Rep. 5, 14524. 2015, doi: 10.1038/srep14524.

[38] Chen, Z. et al. Purification and characterization of a novel antifungal protein secreted by Penicillium chrysogenum from an Arctic sediment. Appl. Microbiol. Biot. 97, 10381–10390. 2013, doi: 10.1007/s00253-013-4800-6

[39] Xu, W., Pang, K. L. & Luo, Z. H. High fungal diversity and abundance recovered in the deep-sea sediments of the Pacific Ocean. Microb. Ecol. 68, 688–698. 2014, doi: 10.1007/s00248-014-0448-8

[40] Hamayun M, Khan SA, Kim HY, Chaudhary MF, Hwang YH, Shin DH, Kim IK, Lee BH, Lee IJ: Gibberellin Production and Plant Growth Enhancement by Newly Isolated Strain of *Scolecobasidium tshawytschae*. J Microbiol Biotechnol 2008, https://doi.org/10.4014/jmb.0809.520.

[41] Choi WY, Rim SO, Lee JH, Lee JM, Lee IJ, Cho KJ, Rhee IK, Kwon JB, Kim JG: Isolation of gibberellins producing fungi from the root of several Sesamum indicum plants. Journal of Microbiology and Biotechnology, 15:22-28. 2005.

[42] Higgs RE, James AZ, Jeffrey DG, Matthew DH: Rapid method to estimate the presence of secondary metabolites in microbial extracts. Appl Environ Microbiol, 67:371-376. 2001, doi: 10.1128/AEM.67.1.371-376.2001.

[43] Pang Z, Chen J, Wang T, Gao C, Li Z, Guo L, Xu J and Cheng Y. Linking Plant Secondary Metabolites and Plant Microbiomes: A Review. Front. Plant Sci. 12:621276. 2021. doi: 10.3389/fpls.2021.621276

[43] Vinale, F.; Sivasithamparam, K.; Ghisalberti, E.L.; Woo, S.L.; Nigro, M.; Marra, R.; Lombardi, N.; Pascale, A.; Ruocco, M.; Lanzuise, S.; et al. Trichoderma secondary metabolites active on plants and fungal pathogens. The Open Mycology Journal. 2014, doi**:** 10.2174/1874437001408010127.

[44] Vinale, F.; Nigro, N.; Sivasithamparam, K.; Flematti, G.; Ghisalberti, E.L.; Ruocco, M.; Varlese, R.; Marra, R.; Lanzuise, S.; Eid, A.; et al. Harzianic acid: A novel siderophore from *Trichoderma harzianum*. FEMS Microbial Letters. 347, 123–129. 2013, doi: 10.1111/1574-6968.12231.

[45] Vinale, F.; Sivasithamparam, K.; Ghisalberti, E.L.; Ruocco, M.; Woo, S.; Lorito, M. *Trichoderma* secondary metabolites that affect plant metabolism. Natural Product Communication. 7, 1545–1550. 2012, doi: 10.1177/1934578X1200701133.

[46] López-Bucio, J., Pelagio-Flores, R., and Herrera-Estrella, A. *Trichoderma* as biostimulant: Exploiting the multilevel properties of a plant beneficial fungus. Scientia Horticulturae 196:109–123. 2015. https://doi.org/10.1016/j.scienta.2015.08.043.

[47] Hermosa, R., Viterbo, A., Chet, I., and Monte, E. Plant beneficial effects of *Trichoderma* and of its genes. Microbiology 158:17–25. 2012, doi: 10.1099/mic.0.052274-0.

[48] Illmer, P., & Schinner, F. Solubilization of inorganic calcium phosphates- solubilization mechanisms. Soil Biology & Biochemistry, 27(3), 257–263. 1995, https://doi.org/10.1016/0038-0717 (94)00190-C.

[49] Sreevidya, M., & Gopalakrishnan, S. *Penicillium citrinum* VFI-51 as biocontrol agent to control charcoal rot of sorghum (*Sorghum bicolor* (L.) Moench). African Journal of Microbiology Research, 10(19), 669–674. 2016. https://doi.org/10.5897/AJMR2015.7831.

[50] Ahemad, M., & Kibret, M. Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective. Journal of King Abdulaziz University: Science, 26(1), 1–20. 2014. https://doi.org/10.1016/j.jksus.2013.05.001.

[51] Abd-ElGawad AM, El Gendy AE-NG, Assaeed AM, Al-Rowaily SL, Alharthi AS, Mohamed TA, Nassar MI, Dewir YH, Elshamy AI. Phytotoxic Effects of Plant Essential Oils: A Systematic Review and Structure-Activity Relationship Based on Chemometric Analyses. Plants. 2021; 10(1):36. https://doi.org/10.3390/plants10010036.

[51] Kim, Y.-S., Yeung, E. C., Hahn, E.-J., and Paek, K.-Y. (2007). Combined effects of phytohormone, indole-3-butyric acid, and methyl jasmonate on root growth and ginsenoside production in adventitious root cultures of Panax ginseng CA Meyer. Biotechnol. Lett. 29, 1789–1792. doi: 10.1007/s10529-007-9442-2

[51] Leggett, M., M.K. Newlands, D. Greenshields, L. West, S. Inman, and M.E. Koivunen. 2015. Maize yield response to a phosphorus- solubi-lizing microbial inoculant in field trials. J. Agric. Sci. 153(8):1464 – 1478. http://dx.doi.org/10.1017/S0021859614001166

[52] Gleddie, S. C., Hnatowich, G. L., & Polonenko, D. R. (1991, February). A summary of wheat response to Provide@ (Penicillium bilaji) in western Canada. In Proceedings of the Alberta Soil Science Workshop (pp. 306–313). Lethbridge, Alberta.

[53] I. Khokhar, M., S., Haider, I., Mukhtar, A., Ali, S. Mushtaq, , & Ashfaq, M. (2013). "Effect of *Penicillium* species culture filtrate on seedling growth of wheat." International Research Journal of Agricultural Science and Soil Science, 3(1), 24–29. Jan. 2013

[54] X., Zhao,Y., Ni, H., Zhao, X., Liu, B., Bipo, B., Shi, Q., Ma, and H., Liu. "Plant growth-promoting ability and control efficacy of *Penicillium aurantiogriseum* 44M-3 against sesame *Fusarium* wilt disease." Biocontrol science and technology. 31:12, 1314-1329, Jul. 2021. DOI: 10.1080/09583157.2021.1946011.

[55] R. I. Jones and J. Grey, "Biogenic methane in freshwater food webs." Freshwater Biology, vol. 56,n no. 2, pp.213-229, Oct. 2010, doi:10.1111/j.1365-2427.2010.02494.x.

[56] D. Bridge and B. M. Spooner, "Non-lichenized Antarctic fungi: transient visitors or members of a cryptic ecosystem?," Fungal Ecology, vol. 5, no. 4, pp. 381–394, Aug. 2012, https://doi.org/10.1016/j.funeco.2012.01.007.