

Antioxidant and antifungal activities *in vitro* of essential oils and extracts of twelve Algerian species of *Thymus* against some mycotoxigenic *Aspergillus* genera

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Abstract

The aim of the study was to determine the phenolic and flavonoid content of Essential Oils (EOs), chloroform and ethanolic extracts of 12 Algerian Thymus species and evaluate their antioxidant and antifungal activities. EOs (1.73±0.30- $15.00 \pm 1.24 \mu g/mg$), chloroform extracts $(33.8 \pm 2.42 -$ 160.93±3.88µg/mg) and ethanol extracts (27.01±3.56 -148.46±4.40µg/mg) showed considerable phenolic content. Flavonoids values of chloroform extracts ranged between 3.39±0.17 and 20.27±0.29µg/mL while ethanolic extracts values ranged between 2.81±0.11 and 26.64±0.18µg/mg. Results of DPPH showed that EOs, chloroform and ethanolic extracts exhibited strong radical scavenging activity (IC50=21.75±6.54-338.22±2.99µg/mL, 22.91±5.59-90.93±1.36µg/mL, and $33.51\pm5.72-103.80\pm4.54\mu$ g/m, respectively). Inhibition of β -

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This article is distributed under the terms of the Creative Commons Attribution Noncommercial License (by-nc 4.0) which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and source are credited. carotene bleaching was potentially performed by all EOs (66.48±2.41-94.06±2.68%), chloroform extracts (68.98±1.58-95.30±1.99%), and ethanolic extracts (62.15±2.51-92.36±1.15%). The antifungal activity of EOs and extracts was tested using the Minimum Inhibitory Concentration (MIC) and minimum fungicidal concentration (MFC). The EOs (0.1±0.00mg/mL-(0.1±0.00 mg/mL-1.06±0.46mg/mL), chloroform 1.06 ± 0.46 mg/mL) and ethanol $(0.1\pm0.00$ mg/mL -1.6 ± 0.00 mg/mL) showed remarkable antifungal activity against mycotoxigenic Aspergillus genera. The MFC of EOs (1.0±0.34mg/mL and >4.8mg/mL), chloroform (0.26 \pm 0.11mg/mL and >1.6 mg/mL) and ethanol (0.2±0.00mg/mL and >1.6 mg/mL) were fungicidal in nature higher than MICs. The findings of the study indicated that Thymus spp. EOs and extracts could be used as natural alternatives for food industry.

Introduction

Aspergillus spp. are widespread in nature and especially in the soil where they contribute to the biodegradation and recycling of organic matter. They are also used in several fields by performing beneficial roles such as the production of useful metabolites. Given its extreme economic importance linked to its useful and harmful effects, several works have been devoted to the genus *Aspergillus* in general.¹

Mycotoxins produced by these fungi cause food spoilage: Aflatoxins (AF), Ochratoxin A (OA), nidulotoxin, Sterigmatocystin (STC), emodin, ventilacton etc. Susceptibility to mycotoxins can vary greatly from one individual to another depending on the breed, physiological state or stress to which he is subjected. Likewise, the different mycotoxins induce different effects: some exerting a hepatotoxic or carcinogenic power, others proving to be estrogenic, immunotoxic, nephrotoxic or neurotoxic. Unlike bacterial toxins, the effects of which are immediate, mycotoxins have insidious effects, which manifest themselves in the more or less long term.

Several strategies have been used to control fungal growth and mycotoxin biosynthesis in foods. Compared to synthetic food additives, the changing consumer preference for more natural products and the reduction in the use of salt and sugar in foods for dietary reasons has stimulated the use of spices and/or aromatic plants, sodium and calories from these plants. The content is weak. Therefore, in recent years, much research has focused on using Essential Oils (EOs), extracts and oleoresins extracted from spices and aromatic herbs as alternative food preservatives.²

Thymus is considered to be one of the eight largest genera of the Lamiaceae and includes several species distributed throughout the coasts and even in internal regions and in arid zones. Many studies have shown that *Thymus* species exhibit antibacterial, antifungal, and antioxidant activities.³ These activities are mainly referred to the richness of EOs and to non-volatile compounds comprising polyphenols and flavonoids.

In Algeria, 12 species of *Thymus* colonize the territory of the country. Some of them are endemic to Algeria such as *Thymus pallescens* de Noah, *Thymus dreatensis* Batt., *Thymus guyonii* de Noah and *Thymus lanceolatus* Desf., others are endemic to North Africa such as *Thymus ciliatus* Desf., *Thymus fontanesii* Boiss. and Reut., *Thymus numidicus* Poiret., *Thymus munbyanus* Boiss. and Reut and *Thymus algeriensis* Boiss. and Reut. *Thymus gallescensis* is common and endemic to northern Algeria, while, *Thymus dreatensiss* rare and endemic to the Aures mountains (Batna region) and the Djurdjura mountains (region of the East) Kabylie.⁴

The aim of the study was to investigate the antioxidant activity and effect of *Thymus* spp. EOs and extract on the growth of *Aspergillus* species, isolated from stored wheat and corn.

Materials and Methods

Plant material

Thymus species were collected from different regions of Algeria in 2018 and then dried in the shade at an ambient temperature during seven days. The plants were identified in the laboratory of Ethnobotany and Natural Substances, Department of Natural Science, Ecole Normale Supérieure (ENS; Table 1).

Fungal strains and culture conditions

Aspergillus alliceus, Aspergillus carbonarus, Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus ochracus, Aspergillus parasiticus, Aspergillus tamari, Aspergillus terreus, Aspergillus candidus, Aspergillus glaucus, were isolated from stored wheat and corn. Fungal strains were transferred to fresh Potato Dextrose Agar (PDA) medium every two months in



order to avoid a decline in strain viability and stored at 4°C.

Extraction of EO

The EO from leaves of different species was extracted by hydrodistillation. The process consists of immersing the plant material (200g) in a bath of distilled water for three hours. The obtained organic EO solution was dried with anhydrous sodium sulfate (Na₂SO₄) weighed and stored in opaque brown colored vials, hermetically sealed and stored at 4° C to avoid any degradation.

Preparation of extracts

Leaves were prepared and left to dry in the shade for 2 weeks, then crushed in a blender to turn them into powder. The extraction was carried out by maceration using two solvents: chloroform and ethanol. The extracts were prepared by adding 10mL of the extraction solvent to 1g of each species powder. After stirring for 30 minutes, the mixture was kept standing for 24h at 4°C. The extracts were filtered using Whatman No.1 filter paper, then the filtrates were evaporated by a rotary evaporator.

Preparation of the inoculum

Spores from 7-day cultures of each fungus were recovered by washing the Petri dishes, with a volume of 10 ml of a sterile 0.1% (v/v) tween-80 solution. The number of spores was determined (1×10⁶ spores/mL) by counting using the Mallassez cell (depth 0.2mm, 1/400mm²) under a light microscope.

Determination of the content of total phenolic compounds

The determination of the total polyphenols was performed with the Folin-Ciocalteu colorimetric reagent according to the method of Dewanto *et al.*⁵ A volume of 125 μ L of each EO and extract were dissolved in 500 μ L of distilled water and 125 μ L of 10 times diluted Folin-Ciocalteu reagent. The solutions were mixed and incubated for 3 minutes. After the incubation 1.25mL of sodium carbonate solution Na₂CO₃ (7%) was added. The final mixture was shaken and incubated for 2 h in the dark at room temperature. The absorbance of each mixture was measured by a spectrophotometer at 760nm.

The concentration of total phenolics was calculated from the equation generated with the standard gallic acid and expressed in μg of acid equivalents gallic per mg of extract.

| Species | Collection site | Speciescode | Latitude/Longitude | Altitude [m] | |
|-----------------------------------|-----------------|-------------|------------------------|--------------|--|
| Thymus capitatus 1 | Tlemcen | TC1 | N34° 53 24/W1° 19 12 | 842 | |
| Thymus fontanesii | Medea | TF | N36° 16 03/E2° 45 00 | 981 | |
| Thymus capitatus 2 | Oran | TC2 | N35° 42 10/W0° 38 57 | 580 | |
| Thymus numidicus | Tizi Ouzou | TN | N36° 43 00/E 4° 03 00 | 184 m | |
| Thymus guyoni | Djelfa | TG | N34° 40 00/E3° 15 00 | 1 140 m | |
| Thymus lanceolatus | Medea | TL | / | / | |
| Thymus munbyanus subsp. ciliatus | Setif | TI | N36° 09 00 /E 5° 26 00 | 1 100 m | |
| Thymus vulgaris | Blida | TL | N36° 29 00/E2° 50 00 | 229 m | |
| Thymus algeriensis | Medea | TA | / | / | |
| Thymus munbyanus subsp. coloratus | Setif | ТО | / | / | |
| Thymus dreatensis | Tizi Ouzou | TD | / | / | |
| Thymus pallescens | Boumerdes | TP | N36° 46 00/E3° 28 00 | 2 m | |

Table 1. Collection sites and geographic coordinates of the 12 Algerian species of *Thymus* which served as source of EOs and extracts in the present study.



Determination of the content of flavonoids

The content of flavonoids of the EOs and extracts was determined following the method described by Pękal and Pyrzynska.⁶ One milliliter of each extract at a suitable dilution was added to the same volume of AlCl₃.6H₂O (2% dissolved in methanol). The mixture was vigorously shaken and incubated for 10 min at room temperature. The absorbance was measured at 440 nm. The contents were expressed as Quercetin Equivalents per Dry Weight (μ g QE/mg DW).

Evaluation of the free radical scavenging activity by the DPPH method

The protocol followed was that described by Nikhat *et al.*⁷ In dry test tubes, an amount of 2.9 mL of each EO and extract and Butyl-Hydroxytoluene (BHT) at different concentrations of EOs and extracts was mixed with 100μ L of the methanolic solution with 0.004% DPPH°. After shaking, the tubes are placed in the dark at room temperature for 30 minutes. The reading was taken by measuring the absorbance at 517 nm.

The results were expressed as anti-free radical activity where the inhibition of free radicals (DPPH°) in percentages (I%) was calculated by the following formula:

 $I\% = [(A_{blank} - A_{sample}) / A_{blank}] \times 100$

Where: A _{blank}: Absorbance of the control reaction containing all reagents except EO/extract; A _{sample}: Absorbance of the sample containing a tested dose of extract.

Evaluation of antioxidant activity by the β-carotene/linoleic acid method

The method used was that of Miraliakbari and Shahidi.⁸ A stock was prepared where 0.5 mg of β -carotene crystals was dissolved in 1mL of chloroform, then 1mL of the solution was moved to a flask containing 25µL of linoleic acid and 200µL of tween-40. After elimination of chloroform, 100mL of oxygen-enriched distilled water was added; 2.5mL of this mixture were moved to test tubes containing 350µL of extract diluted in Dimethyl Sulfoxide (DMSO). After shaking, the tubes were incubated at 50° C for 2h. A positive control (BHT) tube was prepared. The absorbance was estimated at 470 nm against a blank. Antioxidant activities (I%) were assessed as follows:

$$I\% = (A_t / A_0) \times 100$$

Where: A_0 : Absorbance of the sample and of the control at t=0 min; A_t : Absorbance of the sample and of the control at t=2h.

Determination of minimum inhibitory and fungicidal concentrations

The Minimum Inhibitory (MIC) and Fungicidal (MFC) concentrations of each crude extract were determined using the liquid dilution method reported by Prakash *et al.*⁹ Ten (10) μ L of the fungal suspension (1×10⁶ spores/mL) were inoculated into test tubes containing 10mL of the SMKY liquid medium at different concentrations of EOs (0.3mg/mL, 0.6mg/mL, 1.2mg/mL, 2.4mg/mL, 4.8mg/mL) and extracts (0.05; 0.1; 0.2; 0.4; 0.8; 1.6mg/mL). SMKY tubes containing DMSO were used as control. The tubes were homogenized and incubated at 28±2°C for 7 days. After incubation, observation of a range allows access to the MIC, which corresponds to the lowest concentration capable of inhibiting the growth of the microorganism. Tubes that showed complete inhibition were subcultured into Petri dishes containing 10mL of the PDA culture medium. When there is a resumption of mycelial growth, the concentration is called fungistatic (MFCs). However, if there is no resumption of growth of the mycelium because of permanent inhibition, it is called fungicide (MFCc).

Statistical analysis

All bioassays were performed in triplicate and data analysis was done on mean±SD subjected to one-way ANOVA using STA-TISTICA version 6 software. Means are separated by Tukey's post hoc test when ANOVA was significant (p<0.05).

Results and Discussion

Phenolic content and flavonoids

Phenolic compounds such as phenolic acids and flavonoids are considered to be the major contributors to the antioxidant capacity of plants. Therefore, assays for phenolics and flavonoids from the EOs and extracts of *Thymus* species were performed in this study. The results are presented in Table 2.

The level of flavonoids of chloroform and ethanolic extracts was relatively comparable. Furthermore, the content of the flavonoids was more or less different. The richness in flavonoids were observed in the chloroform extract of TF (20.27±0.29µg/g), TC1 (17.20±0.16 μg/g), TC2 (11.95±0.16μg/g), TG $(10.94\pm0.24\mu g/g)$, and TO $(9.53\pm0.16\mu g/g)$. Such appreciable contents were revealed in the ethanolic extracts of TC1 (26.64±0.18µg/g), TF (10.23±0.13µg/g), TN (9.54±0.22 µg/g), and TO $(8.41\pm0.21\mu g/g)$; on the other hand, the lowest flavonoid content recorded for the chloroform extracts was that of TP $(3.39\pm0.17\mu g/g)$ and TL $(3.68\pm0.13\mu g/g)$, and for the ethanolic extracts was that of TP (2.81 \pm 0.111µg/g), TI (3.19 \pm 0.08µg/g), and TD $(3.50\pm0.08\mu g/g)$. However, the amounts of flavonoids in ethanolic extracts were low in comparison with those obtained by the chloroform extracts. The exception was raised only with TC1, where the flavonoids were mainly found higher than the flavonoids present in the chloroform extract of TC1 (p<0.05). The content was very variable depending on the species and the fractions.

The group of phenolic compounds is one of the most distributed ubiquitous groups in plants. The antioxidants extracted from plants are mainly phenolic compounds. Phenolic compounds seem to be good candidates for their antioxidant activities due to the presence of numerous hydroxyls, which can react with free radicals. According to previous work, species of *Thymus* are rich in flavonoids;¹⁰ these data are comparable with our results since the tests revealed the presence of flavonoids with significant quantities.

Scavenger effect of the radical DPPH

The antioxidant activity was determined by the decrease in the absorbance of an alcoholic solution of DPPH at 517nm. Various studies have experimentally determined the abilities of natural extracts to scavenge free radicals.

The IC_{50} values for the EOs and extracts of *Thymus* spp., as well as for the reference, BHT are presented in Table 2. Based on this test, the EOs and extracts showed significant differences in their scavenging ability of the free radical.

The EOs, chloroform and ethanolic extracts of TA, TI and T4 were the most active with IC_{50} of $21.75\pm6.54\mu g/mL$, $22.91\pm5.59\mu g/mL$ and $33.51\pm5.72\mu g/mL$, respectively compared to the positive control BHT (p<0.05).



Studies conducted by some researchers have indicated that the antioxidant activity of EOs may be greater than that of the majority of compounds that are tested separately. It has been established that the activity of an EO is related to the possible synergistic effects between minor constituents. Ballester-Costa *et al.*,¹¹ showed that strong antioxidant activity of the EO could be

explained by the existence of hydroxylated constituents such as terpenes in their chemical profile. According to Amarti *et al.*,¹² the antioxidant activity of EO can be linked to the phenolic content. In a study on the EO of *Thymus vulgaris*, Jukic and Milos¹³ showed that the phenolic (thymol and carvacrol) and non-phenolic (linalool) chemotypes are able to reduce the 2,2-diphenyl radical 1-

| Fable 2. Content of total | phenolic compounds, | flavonoids, and Antioxidant | activity of EO and extracts. |
|----------------------------------|---------------------|-----------------------------|------------------------------|
|----------------------------------|---------------------|-----------------------------|------------------------------|

| Test samples | Total phenolic content | Flavonoids | DPPH (IC_{50}) | β-Carotene/linoleic |
|--------------------|-------------------------------|-------------------------|---|--------------------------------|
| EOs | (µ5/m5) | (µg/mg) | (µg/mi) | |
| TC1 | 15 00±1 9/f | nd | 282 22 ⊥ 7 35g | 88 16+1 52efg |
| TF | 19.46±1.41ef | nd | 252.22±1.55° | 90.83-9.87fg |
| тс9 | 6 33+1 90abcd | nd | 172 25+1 07° | 84 72+3 86def |
| TN | 2 93+1 47ab | nd | $338 22 \pm 2.00^{k}$ | 79.31 ± 3.90 de |
| ТС | 3.8+1.70 ^{abc} | nd | 276 58+2 718 | 70.39+5.45 ^{bc} |
| TL. | 1.86+0.70ª | nd | 23678 ± 2.84^{e} | 82 08+1 94def |
| TI | 10.86+2.24 ^{def} | nd | 255.77±2.64 | 94.06+2.685 |
| TL. | 3 93 + 2 00 abc | nd | 200.21 ± 1.01 221 48+5 35 ^d | $85.64 \pm 3.84^{\text{defg}}$ |
| ТΔ | 8 76+1 96 ^{cde} | nd | 21 75+6 54ª | 60.82+2.66ª |
| ТО | $9.63 \pm 3.30^{\text{de}}$ | nd | 21.13 ± 0.01 231.67 ± 4.96 de | 77 63+1 18 ^{cd} |
| <u>тр</u> | 7 13 ± 1 30bcd | nd | 201.01 ±4.20 | 67 60+1 12ab |
| тр | 1 73+0 30a | nd | 321 01+9 46 ^h | 66 48+2 41ab |
| RHT | nd | nd | 101.07 ± 1.10^{b} | 94 77+1 61g |
| Chloroform Extract | iid | iiu | 101.01 ± 1.10 | 01.11 ± 1.01 |
| TC1 | 146.53 ± 2.40^{g} | 17.20±0.16 ^h | $87.09 \pm 1.34^{\text{fg}}$ | 72.32±1.18 ^{ab} |
| TF | 160.93 ± 3.88^{h} | 20.27 ± 0.29^{k} | 56.16 ± 0.67^{bc} | 72.12 ± 8.28^{ab} |
| TC2 | 98.73±3.30 ^f | 11.95 ± 0.16^{g} | 47.03±3.16 ^b | 71.03±2.44 ^a |
| TN | $69.06 \pm 1.89^{\circ}$ | 8.11±0.21° | 31.80 ± 5.60^{a} | 81.27±2.79 ^{bcd} |
| TG | 89.46±1.90 ^d | 10.94 ± 0.24^{f} | 50.31±2.78 ^{bc} | 87.14±3.38 ^{cd} |
| TL | $70.53 \pm 1.80^{\circ}$ | 8.45±0.27° | 90.93 ± 1.36^{g} | $91.06 \pm 0.60^{\mathrm{de}}$ |
| TI | 57.13±1.70 ^b | 6.94±0.13 ^b | 22.91±5.59ª | 91.31±2.28 ^{de} |
| TL | 34.8±3.34ª | 3.68 ± 0.13^{a} | $90.31 \pm 1.49^{\text{fg}}$ | 74.27 ± 2.25^{ab} |
| TA | 47.04±2.36 ^e | 5.18±0.15 ^d | 55.87 ± 0.98^{bc} | 95.30±1.99 ^e |
| ТО | 85.06 ± 1.94^{d} | 9.53 ± 0.16^{e} | $66.65 \pm 0.21^{\text{de}}$ | $78.56\pm6.08^{\mathrm{abc}}$ |
| TD | 59.46 ± 1.70^{b} | 7.44±0.11 ^b | 69.63 ± 3.02^{e} | 87.41±1.42 ^{cde} |
| TP | 33.8 ± 2.42^{a} | 3.39 ± 0.17^{a} | 81.37 ± 2.08^{f} | 68.98 ± 1.58^{a} |
| BHT | nd | nd | 58.29 ± 3.51^{cd} | 94.77±1.61 ^e |
| Ethanolic Extract | | | | |
| TC1 | 148.46 ± 4.40^{g} | $26.64{\pm}0.18^{k}$ | 79.15 ± 1.64^{de} | 84.84 ± 1.12^{cdef} |
| TF | 87.93 ± 3.93^{f} | 10.23 ± 0.13^{h} | 49.43 ± 2.75^{b} | 83.70 ± 0.82^{cde} |
| TC2 | 61.26±1.81 ^{ce} | 7.24 ± 0.13^{d} | 50.34 ± 3.69^{b} | 90.69±1.38 ^{fgh} |
| TN | 82.66 ± 2.80^{f} | 9.54 ± 0.22^{g} | 33.51 ± 5.72^{a} | 62.15 ± 2.51^{a} |
| TG | 56.86 ± 1.52^{bc} | 5.86 ± 0.17^{e} | 58.93 ± 2.44^{bc} | 79.24±0.93 ^{bc} |
| TL | $50.8 \pm 4.88^{\mathrm{bd}}$ | $5.15 \pm 0.30^{\circ}$ | $86.44 \pm 1.29^{\text{ef}}$ | $85.45 \pm 3.05^{\text{def}}$ |
| TI | 29.06 ± 2.60^{a} | 3.19 ± 0.08^{ab} | 72.42 ± 3.02^{d} | $90.66 \pm 1.72^{\text{fgh}}$ |
| TL | 42.53 ± 1.89^{d} | $4.90 \pm 0.17^{\circ}$ | 88.06 ± 2.91^{ef} | 80.04±2.26 ^{bcd} |
| TA | 67.26 ± 1.70^{e} | 6.97 ± 0.15^{d} | 103.80 ± 4.54^{g} | 62.17±2.27ª |
| ТО | 54.6 ± 1.63^{bc} | 8.41 ± 0.21^{f} | $89.76 \pm 3.21^{\text{ef}}$ | 75.85±3.63 ^b |
| TD | 29.2±2.4ª | 3.50 ± 0.08^{b} | 97.22 ± 8.89^{fg} | $92.36 \pm 1.15^{\text{gh}}$ |
| TP | 27.01 ± 3.56^{a} | 2.81±0.11 ^a | $90.10 \pm 4.37^{\text{ef}}$ | 86.64 ± 1.46^{efg} |
| BHT | nd | nd | 69.41±3.81 ^{cd} | 94.77±1.61 ^h |





picrylhydrazyl, with a higher effect than that recorded for phenolic chemotypes.

On the other hand, the multiple researches that were conducted have established that the Thymus species are rich and promising sources of phenolics and flavonoids. The study by Kulšic et al.¹⁴ showed that the aqueous extract of the leaves of *Thymus vulgaris* exhibited significant antioxidant activity. In this extract, phenolics, rosmarinic acid, and caffeic acid may explain the exhibited activity.14 However, plant extracts containing a mixture of these compounds have given more or less satisfactory results, leading to the conclusion that the antioxidant activity of these compounds depends not only on the phenolic content but that the phenolic compounds can act synergistically, antagonistically or can independently affect the whole activity of the mixture. Amič et al.15 demonstrated the structure-function relationship of 29 flavonoids (flavones, flavonols and flavanones) and their capacities to trap DPPH, whereas, better activity essentially requires the 3', 4'ortho-dihydroxy structure on ring B and the 4-carbonyl group on ring C which confers stability to the flavonoxy radical (A).

Evaluation of antioxidant activity by the β-carotene/linoleic acid method

The β -carotene bleaching mechanism is a phenomenon mediated by a free radical. β -carotene undergoes rapid color change in the absence of antioxidants resulting in reduced absorption of the test solution with reaction time.

The highest antioxidant activity was again associated with the EO of the TF ($AA=90.83\pm2.87$ %) and chloroform and ethanolic

extracts of TA and TD (AA=95.30±1.99 %, 92.36±1.15%, respectively compared to the control BHT (p<0.05; Table 2).

The results obtained showed that the EOs and extracts were able to reduce lipid peroxidation in the B-carotene-linoleic acid system. These results suggest that they have a considerable capacity to react with free radicals to convert them into non-reactive species and to interrupt the chain of radical reactions. Compounds that possess this characteristic can be used in food systems. Maggi et al.¹⁶ found that the EOs possess lipid peroxidation inhibitory activity due to the presence of a high level of oxygenates. These same authors also report that the antioxidant activity of a compound is very often linked to the presence of easily oxidizable portions such as a hydroxvl group on a hydrocarbon. In addition, Deba et al.¹⁷ suggested that the antioxidant activity of phenolics lies in the fact that they have the capacity to give hydrogen atoms to free radicals (hydroperoxides of the reaction medium) resulting from oxidation linoleic acid and, consequently, stop the attack of these radicals on β -carotene. Sandhar et al.18 in their publication, indicated that flavonoids inhibit lipid peroxidation at an early stage through the scavenger activity of peroxide radicals as they can interrupt a chain of radical reactions through the property of hydrogen donation.

Antifungal activity of EOs and extracts

The results of MIC and MFC are summarized in Tables 3,4, and 5. There are no confirmed criteria for MIC endpoints for *in vitro* antimicrobial bioassays. Nevertheless, according to Aligiannis *et al.*,¹⁹ the antimicrobial activity is considered stronger when MIC values are between 0.05 mg/mL and 0.50 mg/mL, mod-

| Fungal species | s TC1 | TF | TC2 | TN | TG | TL | TI | TL | TA | TO | TD | ТР |
|----------------|----------------------------|-----------------------------|-------------------------|------------------------------|------------------------------|------------------------|------------------------------|-----------------------------|------------------------|------------------------|-----------------------------|----------------------------|
| MIC (mg/mL) | | | | | \sim | | | | | | | |
| A. alliceus | 0.8 ± 0.34^{b} | 2.4 ± 0.00^{abc} | 4.8±0.00 ^c | 2.0 ± 0.69^{ab} | $2.4 \pm 0.00^{\text{abc}}$ | 4.0±1.38 ^{ac} | 2.0 ± 0.69^{ab} | $2.4 \pm 0.00^{\text{abc}}$ | 4.0 ± 1.38^{ac} | 1.6±0.69 ^{ab} | $2.4 \pm 0.00^{\text{abc}}$ | 2.8±1.83 ^{abc} |
| A.carbonarus | 1.6 ± 0.69^{ab} | 2.4 ± 0.00^{b} | 0.6 ± 0.00^{a} | 0.8 ± 0.34^{ab} | 1.6 ± 0.69^{ab} | 1.2 ± 0.00^{ab} | 2.0 ± 0.69^{ab} | 1.6 ± 0.69^{ab} | 0.8 ± 0.34^{ab} | 1.6 ± 0.69^{ab} | 2.0 ± 0.69^{ab} | 1.6 ± 0.69^{ab} |
| A. flavus | 0.6 ± 0.00^{a} | 1.0 ± 0.34^{a} | 0.8 ± 0.34^{a} | 2.4 ± 2.7^{a} | 1.2 ± 0.00^{a} | 1.6 ± 0.69^{a} | 2.4 ± 0.00^{a} | 1.2 ± 0.00^{a} | 1.6 ± 0.69^{a} | 2.0 ± 0.69^{a} | 1.0 ± 0.34^{a} | 2.0 ± 0.69^{a} |
| A.fumigatus | 1.6 ± 0.69^{ab} | 1.2 ± 0.00^{a} | 1.0 ± 0.34^{a} | 0.8 ± 0.34^{a} | 2.0 ± 0.69^{ab} | 1.2 ± 0.00^{a} | 3.2 ± 1.38^{b} | 2.0 ± 0.69^{ab} | 1.2 ± 0.00^{a} | 1.2 ± 0.00^{a} | 1.2 ± 0.00^{a} | 2.0 ± 0.69^{ab} |
| A. niger | $0.8 \pm 0.34^{\circ}$ | 4.0 ± 1.38^{ab} | 1.8±1.03 ^{abc} | $0.8 \pm 0.34^{\circ}$ | 1.2 ± 0.00^{ac} | 4.0 ± 1.38^{ab} | 4.8 ± 0.00^{b} | 3.2 ± 1.38^{abc} | 2.4 ± 0.00^{abc} | 4.0 ± 1.38^{ab} | 3.6 ± 2.07^{abc} | 2.4 ± 0.00^{abc} |
| A.ochracus | 0.6 ± 0.00^{a} | $0.6 \pm 0.00^{\mathrm{a}}$ | 0.6 ± 0.00^{a} | 0.6 ± 0.00^{a} | 2.0 ± 0.69^{a} | 1.6 ± 0.69^{a} | 2.4 ± 0.00^{ab} | 2.0 ± 0.69^{a} | $2.0{\pm}0.69^{a}$ | 1.6 ± 0.69^{a} | 2.0 ± 0.69^{a} | 4.0 ± 1.38^{b} |
| A.parasiticus | 1.2 ± 0.00^{ab} | 0.6 ± 0.00^{b} | 0.8 ± 0.34^{ab} | $1.6 \pm 0.69^{\mathrm{ac}}$ | 4.8 ± 0.00^{d} | 1.2 ± 0.00^{ab} | $2.4 \pm 0.00^{\circ}$ | 1.2 ± 0.00^{ab} | 1.2 ± 0.00^{ab} | 1.2 ± 0.00^{ab} | 1.6±0.69 ^{ac} | 1.2 ± 0.00^{ab} |
| A. tamari | $2.4{\pm}0.00^{bd}$ | $4.8 \pm 0.00^{\circ}$ | 4.8 ± 0.00^{cd} | $4.0 \pm 1.38^{\circ}$ | 4.0 ± 1.38 ^{cd} | 1.2 ± 0.00^{ab} | $4.8 \pm 0.00^{\circ}$ | 2.4 ± 0.00^{bd} | 1.2 ± 0.00^{ab} | 1.2 ± 0.00^{ab} | 0.6 ± 0.00^{a} | 0.6 ± 0.00^{a} |
| A.terreus | 0.6 ± 0.00^{b} | 1.2 ± 0.00^{bc} | $0.8 \pm 0.34^{\circ}$ | $1.6 \pm 0.69^{\text{abc}}$ | $2.4{\pm}0.00^{a}$ | $2.4{\pm}0.00^{a}$ | $2.0 \pm 0.69^{\mathrm{ac}}$ | 1.6 ± 0.69^{abc} | 2.4 ± 0.00^{a} | $2.4{\pm}0.00^{a}$ | 1.0 ± 0.34^{bc} | 4.8 ± 0.00^{d} |
| A.candidus | 2.4 ± 0.00^{a} | 2.8 ± 1.83^{a} | 1.6 ± 0.69^{a} | 1.4 ± 0.91^{a} | 3.2 ± 1.38^{a} | 4.0 ± 1.38^{a} | $2.4{\pm}0.00^{a}$ | 2.4 ± 0.00^{a} | 4.0 ± 1.38^{a} | $2.4{\pm}2.07^{a}$ | 4.0 ± 1.38^{a} | 1.6 ± 0.69^{a} |
| A.glaucus | $0.6 \pm 0.00^{\circ}$ | $1.2\pm0.00^{\mathrm{abc}}$ | $0.6 \pm 0.00^{\circ}$ | $0.8 \pm 0.34^{\mathrm{ac}}$ | $2.4{\pm}0.00^{\mathrm{ab}}$ | 2.8 ± 1.83^{b} | 2.4 ± 0.00^{ab} | 1.6 ± 0.69^{abc} | 4.8 ± 0.00^{d} | 2.4 ± 0.00^{ab} | 2.4 ± 0.00^{ab} | $0.6 \pm 0.00^{\circ}$ |
| MFC (mg/mL) | | | | | | | | | | | | |
| A. alliceus | 1.0 ± 0.34^{d} | 3.2 ± 1.38^{bc} | >4.8 | 4.0±1.38 ^{abc} | 2.4 ± 0.00^{bd} | 4.8±0.00 ^{ac} | 4.8±0.00 ^{ac} | >4.8 | >4.8 | 2.4 ± 0.00^{bd} | >4.8ª | $2.4{\pm}0.00^{a}$ |
| A.carbonarus | 2.0 ± 0.69^{ab} | 4.0 ± 1.38^{b} | 2.0 ± 0.69^{ab} | 1.4 ± 0.91^{a} | 2.4 ± 0.00^{ab} | 2.0 ± 0.69^{ab} | 3.2 ± 1.38^{ab} | 2.4 ± 0.00^{ab} | 1.6 ± 0.69^{ab} | 2.4 ± 0.00^{ab} | 2.4 ± 0.00^{ab} | 4.8 ± 0.00^{ab} |
| A. flavus | 1.0 ± 0.34^{a} | 1.0 ± 0.34^{a} | 0.8 ± 0.34^{a} | >4.8 | 1.2 ± 0.00^{ab} | 2.0 ± 0.69^{bd} | >4.8 | 2.0 ± 0.69^{bd} | 2.4 ± 0.00^{d} | 4.8±0.00 ^c | 1.2 ± 0.00^{ab} | >4.8 |
| A.fumigatus | 3.2 ± 1.38^{ab} | $2.0{\pm}0.69^{a}$ | 1.2 ± 0.69^{a} | 1.4 ± 0.91^{a} | 2.0 ± 0.69^{a} | 2.4 ± 0.00^{a} | 4.8 ± 0.00^{b} | 4.8 ± 0.00^{ab} | 1.2 ± 0.00^{a} | 2.4 ± 0.00^{a} | >4.8 ^b | 4.8 ± 0.00^{b} |
| A. niger | 0.8 ± 0.34^{c} | >4.8 | 2.0 ± 0.69^{d} | 1.6 ± 0.69^{cd} | 4.8 ± 0.00^{b} | >4.8 | 4.8 ± 0.00 | 2.4 ± 0.00^{b} | >4.8 | >4.8 | 4.8 ± 0.00^{b} | 4.8 ± 0.00^{b} |
| A.ochracus | 1.0 ± 0.34^{ab} | 1.6 ± 0.69^{ab} | 0.6 ± 0.00^{a} | 0.6 ± 0.00^{a} | $4.0 \pm 1.38^{\circ}$ | 2.0 ± 0.69^{ab} | $4.8 \pm 0.00^{\circ}$ | 2.4 ± 0.00^{b} | >4.8 ^c | 2.4 ± 0.00^{b} | >4.8 | >4.8 |
| A.parasiticus | $2.0 \pm 0.69^{\text{bd}}$ | $0.8 \pm 0.34^{\circ}$ | 1.6 ± 0.69^{bcd} | 4.8 ± 0.00^{a} | >4.8ª | >4.8 | 2.4 ± 0.00^{b} | 2.4 ± 0.00^{b} | 4.8 ± 0.00^{a} | 1.2 ± 0.00^{a} | >4.8ª | $2.4 \pm 0.00a$ |
| A. tamari | >4.8 | >4.8 | >4.8 | >4.8 | >4.8 | >4.8 | >4.8 | 2.4 ± 0.00^{bc} | $3.2 \pm 1.38^{\circ}$ | 1.6 ± 0.69^{b} | 1.2 ± 0.00^{b} | $4.8 \pm 0.00^{\text{bc}}$ |
| A.terreus | 2.0 ± 0.69^{a} | 4.0±1.38 ^b | 2.4 ± 0.00^{a} | 2.4 ± 0.00^{a} | >4.8 | 4.8 ± 0.00^{b} | 2.4 ± 0.00^{a} | 2.4 ± 0.00^{a} | 2.4 ± 0.00^{a} | >4.8 | >4.8 | 2.4±0.00 b |
| A.candidus | >4.8 | >4.8 | 3.2 ± 1.38^{b} | 2.4 ± 0.00^{b} | >4.8 | >4.8 | 4.8 ± 0.00^{a} | >4.8 | 4.8 ± 0.00^{a} | >4.8 | 4.8 ± 0.00^{a} | 2.4 ± 0.00^{b} |
| A.glaucus | 1.6 ± 0.69^{ab} | 2.4±2.07 ^{abc} | 4.0±1.38 ^{bcd} | 1.0 ± 0.34^{a} | 2.0 ± 0.00^{abc} | >4.8 | 3.2±1.38 ^{abcd} | 4.8 ± 0.00^{cd} | >4.8 | >4.8 | 2.4 ± 0.00^{abc} | 2.4 ± 0.00^{abc} |

Table 3. MIC and MFC of Thymus EOs.

erate when they are between 0.6 mg/mL and 1.5 mg/mL and low when greater than 1.50 mg/mL.

The EOs and extracts of the 12 species were significantly effective towards target fungi (p<0.05). Generally, MIC values varied between 0.6 ± 0.00 mg/mL and 4.8 ± 0.00 mg/mL for EOs, between 0.1 ± 0.00 mg/mL and 1.06 ± 0.46 mg/mL for chloroform extracts and between 0.1 ± 0.00 mg/mL and 1.6 ± 0.00 mg/mL for ethanolic extract. According to this evaluation system, the EOs had moderate to low activity with respect to the tested fungi. The chloroform extracts exhibited good activity and the ethanolic extracts presented the same pattern of inhibitory activity.

EOs and extracts were fungicidal in nature higher than MICs as MFCs, with MFCs against all fungal strains being higher. The MFCs were found to be between 1.0 ± 0.34 mg/mL and >4.8mg/mL for EOs, 0.26 ± 0.11 mg/mL and >1.6mg/mL for chloroform extracts, 0.2 ± 0.00 mg/mL and >1.6mg/mL for ethanolic extracts.

Several chemical components recognized for their antifungal activities are present in *Thymus* spp. EOs as major or minor constituents. Generally, EOs manifest powerful antifungal potential against food fungi due to the abundance in phenolic compounds mainly thymol, carvacrol, linalool, γ -terpinene, and p-cymene indicating phenolic compounds as agents with high antifungal potential among all terpene components of EOs.²⁰

The effect of thymol seems to be similar to that of carvacrol because the different position of the hydroxyl group in their phenolic ring does not influence the degree of antimicrobial activity. Dikbas *et al.*,²¹ confirmed that the inhibition of fungi by thymol and carvacrol was similar or above that of the overall EOs.

Overall, the observed antifungal effect would then be attributable to one or more active molecules, present in high or low proportion in EOs. In this way, several active compounds may have high inhibition potential or they have synergistic/antagonistic effects, which could affect the inhibition capacity.

The results indicated also that all fungal strains were affected by both extracts. Similar to antifungal activity of EOs, antifungal activity of extracts can be related to some bioactive compounds such as phenolics and flavonoids. Lahmar *et al.*²² showed that the activity of phenolics against fungi can be attributed to the production of enzymatic inhibition of protein synthesis in the cell. In another studies, Zaïri *et al.*,²³ confirmed that the hydrophobicity of phenolics such as flavonoles is also a criterion of toxicity that allows them to intercalate in membrane phospholipids and exert their antifungal effects inside the cell.

Conclusions

The results of the present study showed that the EOs, chloroform and ethanolic extracts of Algerian *Thymus* spp. showed considerable antioxidant activity and antifungal potential. It would be useful to target one of the food products for which *Thymus* spp. EOs and organic extracts are used as an alternative to the synthetic preservative already used and see its behavior on several levels.

Table 4. MIC and MFC of Thymus chloroform extract.

| Fungal specie | es TC1 | TF | TC2 | TN | TG | TL | TI | TL | TA | TO | TD | TP |
|----------------|----------------------|--------------------------|-------------------------|--------------------------|------------------------|-------------------------------|------------------------|-----------------------------|-----------------------------|---------------------------|-------------------------|-------------------------------|
| MIC (mg/mL) | | | | | | | | | | | | |
| A. alliceus | 0.26 ± 0.11^{ab} | 0.33±0.11 ^{abc} | 0.1±0.00a | 0.13 ± 0.05^{a} | 0.33 ± 0.11^{abc} | $0.4\pm0.00^{\mathrm{abc}}$ | $0.16{\pm}0.05^a$ | $0.4 \pm 0.00^{\text{abc}}$ | 0.26 ± 0.11^{ab} | 0.33±0.11 ^{abc} | 0.66 ± 0.23^{bc} | $0.53 \pm 0.23^{\circ}$ |
| A. carbonarus | 0.16 ± 0.05^{a} | 0.13 ± 0.05^{a} | 0.16 ± 0.05^{a} | $0.26{\pm}0.11^{\rm ab}$ | 0.66 ± 0.23^{b} | $0.26{\pm}0.11^{ab}$ | 0.66 ± 0.23^{b} | 0.4 ± 0.00 ab | 0.53 ± 0.23^{ab} | 0.53 ± 0.23^{ab} | 0.4 ± 0.00^{ab} | 0.26 ± 0.11^{ab} |
| A. flavus | $0.33{\pm}0.11^{bc}$ | $0.16{\pm}0.05^{ab}$ | 0.26 ± 0.11^{abc} | $0.4 \pm 0.00^{\circ}$ | 0.16 ± 0.05 ab | 0.1 ± 0.00^{a} | $0.2{\pm}0.00^{ab}$ | $0.2{\pm}0.00^{ab}$ | $0.1 \pm 0.00^{\mathrm{a}}$ | $0.13{\pm}0.05^{a}$ | $0.13{\pm}0.05^{\rm a}$ | $0.33{\pm}0.11^{bc}$ |
| A. fumigatus | 0.53 ± 0.23^{ab} | 0.26 ± 0.11^{a} | 0.2 ± 0.00^{a} | 0.2 ± 0.17^{a} | 0.53 ± 0.23^{ab} | 0.33 ± 0.11^{a} | 0.33 ± 0.11^{a} | 0.26 ± 0.11^{a} | 0.4 ± 0.00^{a} | 0.33 ± 0.11^{a} | 0.66 ± 0.23^{ab} | 1.06 ± 0.46^{b} |
| A. niger | 0.2 ± 0.00^{a} | 0.2 ± 0.00^{a} | 0.33 ± 0.11^{ab} | 0.66 ± 0.23^{bc} | 0.66 ± 0.23^{bc} | 0.53 ± 0.23^{abc} | 0.53 ± 0.23^{abc} | 0.4±0.00 ^{abc} | $0.8 \pm 0.00^{\circ}$ | $0.4{\pm}0.00^{abc}$ | $0.16{\pm}0.05^a$ | $0.4{\pm}0.00^{abc}$ |
| A. ochracus | 0.13 ± 0.05^{a} | 0.33 ± 0.11^{ab} | 0.33 ± 0.11^{ab} | 0.26 ± 0.11^{a} | 0.13 ± 0.05^{a} | 0.33 ± 0.11^{ab} | 0.4 ± 0.00^{ab} | 0.66 ± 0.23^{b} | 0.66 ± 0.23^{b} | 0.26 ± 0.11^{a} | $0.13{\pm}0.05^{a}$ | 0.33 ± 0.11^{ab} |
| A. parasiticus | 0.8 ± 0.00^{b} | $0.66{\pm}0.23^{\rm b}$ | 0.1 ± 0.00^{a} | 0.26 ± 0.11^{a} | 0.13 ± 0.05^{a} | 0.26 ± 0.11^{a} | 0.2 ± 0.00^{a} | 0.2 ± 0.00^{a} | 0.13 ± 0.05^{a} | 0.1 ± 0.00^{a} | 0.2 ± 0.00^{a} | $0.66{\pm}0.23^{\rm b}$ |
| A. tamari | 0.1 ± 0.00^{a} | 0.46 ± 0.30^{a} | 0.66 ± 0.23^{a} | 0.13 ± 0.05^{a} | 0.33±0.11 ^a | 0.13 ± 0.05^{a} | 0.16 ± 0.05^{a} | 0.66 ± 0.23^{a} | 0.33±0.11 ^a | 0.2 ± 0.00^{a} | 1.33 ± 0.46^{b} | 0.53 ± 0.23^{a} |
| A. terreus | 0.33 ± 0.11^{ab} | 0.26 ± 0.11^{ab} | 0.1 ± 0.00^{a} | 0.26 ± 0.11^{ab} | 0.2 ± 0.00^{ab} | 0.3 ± 0.17^{ab} | 0.4 ± 0.00^{ab} | 0.33 ± 0.11^{ab} | $1.33 \pm 0.46^{\circ}$ | 0.53 ± 0.23^{ab} | 0.26 ± 0.11^{ab} | 0.66 ± 0.23^{b} |
| A. candidus | 0.26 ± 0.11^{ab} | 0.66 ± 0.23^{b} | 0.53 ± 0.23^{ab} | 0.26±0.11 ^{ab} | 0.26 ± 0.11^{ab} | 0.53 ± 0.23^{ab} | 0.26 ± 0.11^{ab} | 0.4 ± 0.00^{ab} | 0.4 ± 0.00^{ab} | 0.33±0.11 ^{ab} | 0.13 ± 0.05^{a} | 0.33±0.11 ^{ab} |
| A. glaucus | $0.13{\pm}0.05^{ab}$ | $0.4{\pm}0.00^{ab}$ | $0.66{\pm}0.23^{bc}$ | 0.1 ± 0.00^{a} | 0.13 ± 0.05^{ab} | 0.4 ± 0.00^{ab} | 0.33 ± 0.11^{ab} | 0.16 ± 0.05^{ab} | 0.16 ± 0.05^{ab} | 0.6 ± 0.34^{abc} | $1.06{\pm}0.46^{\rm b}$ | $0.33{\pm}0.11^{ab}$ |
| MFC (mg/mL) |) | | | | | | | | | | | |
| A. alliceus | 0.4 ± 0.00^{a} | 0.4 ± 0.00^{a} | 0.13 ± 0.05^{d} | 0.4 ± 0.00^{a} | 0.4 ± 0.00^{a} | 0.8 ± 0.00^{b} | 0.4 ± 0.00^{a} | 0.66 ± 0.23^{b} | 0.8 ± 0.00^{b} | $1.6 \pm 0.00^{\text{e}}$ | >1.6 | >1.6 |
| A. carbonarus | 0.26 ± 0.11^{a} | 0.4 ± 0.00^{a} | 0.33 ± 0.11^{a} | 0.4 ± 0.00^{a} | $0.8{\pm}0.00^{ab}$ | $0.53{\pm}0.23^{a}$ | $0.8{\pm}0.00^{ab}$ | $0.8{\pm}0.00^{ab}$ | $0.8{\pm}0.00^{ab}$ | $0.8{\pm}0.00^{ab}$ | >1.6 | $0.8{\pm}0.00^{\rm b}$ |
| A. flavus | 0.53 ± 0.23^{bc} | >1.6 | $0.8 \pm 0.00^{\circ}$ | 0.4 ± 0.00^{ab} | 0.4 ± 0.00^{ab} | 0.33 ± 0.11^{ab} | 0.26 ± 0.11^{ab} | 0.33±0.11 ^{ab} | 0.4 ± 0.00^{ab} | 0.16 ± 0.05^{a} | 0.33±0.11 ^{ab} | 0.8 ± 0.00^{d} |
| A. fumigatus | 0.8 ± 0.00^{a} | 0.8 ± 0.00^{a} | $0.26{\pm}0.11^{\rm b}$ | $0.33{\pm}0.11^{\rm b}$ | 0.8 ± 0.00^{a} | 0.8 ± 0.00^{a} | $0.4{\pm}0.00^{\rm b}$ | 0.46 ± 0.30^{b} | 0.8 ± 0.00^{a} | 1.6 ± 0.00^d | $1.6 {\pm} 0.00^{d}$ | 1.6 ± 0.00^{d} |
| A. niger | 0.8 ± 0.00^{ab} | 0.8 ± 0.00^{ab} | 0.8 ± 0.00^{ab} | 0.8 ± 0.00^{ab} | 0.8 ± 0.00^{ab} | $1.33 \pm 0.46^{\mathrm{ac}}$ | 1.6 ± 0.00^{cd} | >1.6 | >1.6 | 0.8 ± 0.00^{ab} | $0.66{\pm}0.23^{\rm b}$ | $1.33 \pm 0.46^{\mathrm{ac}}$ |
| A. ochracus | >1.6 | 0.4 ± 0.00^{a} | 0.4 ± 0.00^{a} | 0.4 ± 0.00^{a} | 0.33 ± 0.11^{a} | $0.53{\pm}0.23^{ab}$ | $1.6 \pm 0.00^{\circ}$ | $0.8{\pm}0.00^{\rm b}$ | $1.6 \pm 0.00^{\circ}$ | $0.8{\pm}0.00^{\rm b}$ | $0.8{\pm}0.00^{\rm b}$ | $0.53{\pm}0.23^{ab}$ |
| A. parasiticus | 0.8 ± 0.00^{b} | 0.53 ± 0.23^{b} | 0.66 ± 0.23^{ab} | 0.4 ± 0.00^{a} | 0.33 ± 0.11^{a} | $1.6 \pm 0.00^{\circ}$ | 0.33 ± 0.11^{a} | 0.66 ± 0.23^{ab} | 0.66 ± 0.23^{ab} | $1.6 \pm 0.00^{\circ}$ | 0.4 ± 0.00^{b} | >1.6 |
| A. tamari | 0.33 ± 0.11^{ab} | $0.4{\pm}0.00^d$ | $0.8 \pm 0.00^{\circ}$ | $0.26{\pm}0.11^{a}$ | 0.4 ± 0.00^{ab} | $0.4{\pm}0.00^{ab}$ | 0.53 ± 0.23^{abc} | 0.8±0.00 ^c | 0.66 ± 0.23^{bc} | 0.4 ± 0.00^{ab} | >1.6 | >1.6 |
| A. terreus | 0.4 ± 0.00^{a} | 0.4 ± 0.00^{a} | 0.16 ± 0.04 d | 0.4 ± 0.00^{a} | 0.4 ± 0.00^{a} | 0.4 ± 0.00^{a} | 0.8 ± 0.00^{b} | 0.8 ± 0.00^{b} | >1.6 | >1.6 | 0.4 ± 0.00^{a} | $1.6\pm0.00^{\mathrm{e}}$ |
| A. candidus | 0.33 ± 0.11^{ab} | 0.8 ± 0.00^{a} | $0.66{\pm}0.23^{ab}$ | 0.8 ± 0.00^{a} | 0.8 ± 0.00^{a} | 1.6 ± 0.00^{cd} | $0.4{\pm}0.00^{ab}$ | >1.6 | $1.33 \pm 0.46^{\circ}$ | $1.6{\pm}0.00^{cd}$ | $0.26{\pm}0.00^{c}$ | $0.66{\pm}0.23^{ab}$ |
| A. glaucus | 0.26 ± 0.11^{a} | 0.66 ± 0.23^{ab} | 0.8 ± 0.00^{ab} | 0.53 ± 0.23^{ab} | 0.26 ± 0.11^{a} | 1.6 ± 0.00^{bc} | 0.66 ± 0.23^{ab} | 0.33±0.11 ^a | >1.6 | 1.06 ± 0.46^{bd} | >1.6 | 0.8 ± 0.00^{ab} |



| Fungal specie | s TC1 | TF | TC2 | TN | TG | TL | TI | TL | TA | ТО | TD | TP |
|----------------|--------------------------|------------------------------|-----------------------------|-------------------------|----------------------------|-----------------------------|--------------------------|-------------------------|------------------------------|------------------------------|-----------------------------|-------------------------------|
| MIC (mg/mL) | | | | | | | | | | | | |
| A. alliceus | 0.4 ± 0.00^{ab} | 0.26±0.11 ^a | 0.16 ± 0.05^{a} | 0.4 ± 0.00^{ab} | $0.66 \pm 0.23^{\text{b}}$ | $1.6 \pm 0.00^{\mathrm{a}}$ | 0.13 ± 0.05^{a} | 0.26±0.11 ^a | $0.16 \pm 0.05^{\mathrm{a}}$ | 0.33±0.11a ^b | $0.66 \pm 0.23^{\text{b}}$ | 0.26±0.11 ^a |
| A. carbonarus | 0.8 ± 0.00^{ab} | 0.53 ± 0.23^{ab} | 0.53 ± 0.23^{ab} | 1.33 ± 0.46^{a} | 1.06 ± 0.46^{ab} | 0.66 ± 0.23^{ab} | $0.53{\pm}0.23^{ab}$ | $0.16{\pm}0.05^{\rm b}$ | 0.66 ± 0.23^{ab} | 1.33 ± 0.46^{a} | 0.53 ± 0.23^{ab} | 1.33 ± 0.46^{a} |
| A. flavus | 0.13 ± 0.05^{a} | 0.16 ± 0.05^{a} | 0.2 ± 0.00^{a} | 0.33 ± 0.11^{b} | 0.53 ± 0.23^{ab} | 0.1 ± 0.00^{a} | 0.33±0.11 ^{ab} | $0.66{\pm}0.23^{\rm b}$ | 0.66 ± 0.23^{b} | 0.26 ± 0.11^{ab} | $0.66{\pm}0.23^{\rm b}$ | 0.16 ± 0.05^{a} |
| A.fumigatus | 0.66 ± 0.23^{a} | 0.4 ± 0.00^{ab} | $1.06 \pm 0.46^{\text{bc}}$ | $1.6 \pm 0.00^{\circ}$ | $1.6 \pm 0.00^{\circ}$ | 0.26 ± 0.11^{a} | 1.06 ± 0.46^{bc} | 0.33±0.11 ^a | 0.16 ± 0.05^{a} | 0.66 ± 0.23^{ab} | 0.26 ± 0.11^{a} | 0.13 ± 0.05^{a} |
| A. niger | 0.53 ± 0.23^{a} | 1.33 ± 0.46^{b} | 0.53 ± 0.23^{a} | 0.26 ± 0.11^{a} | 0.26 ± 0.11^{a} | 0.66 ± 0.23^{a} | 0.16 ± 0.05^{a} | 0.26 ± 0.11^{a} | 0.53 ± 0.23^{a} | 0.33±0.11 ^a | 0.26 ± 0.11^{a} | 0.2 ± 0.00^{a} |
| A. ochracus | 0.13 ± 0.05^{a} | 0.26 ± 0.11^{ab} | 0.26 ± 0.11^{ab} | 0.2 ± 0.00^{ab} | $1.33 \pm 0.46^{\circ}$ | 0.33 ± 0.11 ab | 0.33 ± 0.11^{ab} | $1.33 \pm 0.46^{\circ}$ | 0.13 ± 0.05^{a} | 0.8 ± 0.00^{bc} | 0.66 ± 0.23^{ab} | 0.33 ± 0.11^{ab} |
| A. parasiticus | 0.26 ± 0.11^{a} | $0.8 \pm 0.00^{\mathrm{b}}$ | 0.13 ± 0.05^{a} | 0.33±0.11 ^{ab} | 0.26 ± 0.11^{a} | 0.13 ± 0.05^{a} | 0.3 ± 0.17^{a} | 0.2 ± 0.00^{a} | 0.33±0.11 ^{ab} | 0.26 ± 0.11^{a} | $1.33 \pm 0.46^{\circ}$ | 0.2 ± 0.00^{a} |
| A. tamari | 0.33±0.11 ^{abc} | 0.2 ± 0.00^{ab} | 0.33±0.11 ^{abc} | 0.2 ± 0.00^{ab} | 0.16 ± 0.05^{a} | 0.26 ± 0.11^{ab} | 0.2 ± 0.00^{ab} | $0.66 \pm 0.23^{\circ}$ | 0.13 ± 0.05^{a} | 0.16 ± 0.05^{a} | 0.53 ± 0.23^{bc} | $0.4 \pm 0.00^{\text{abc}}$ |
| A. terreus | 1.06 ± 0.46^{b} | 0.4 ± 0.00^{ab} | 0.16 ± 0.05^{a} | 0.4 ± 0.00^{ab} | 1.06 ± 0.46^{b} | 0.66 ± 0.23^{ab} | 0.16 ± 0.05^{a} | 0.53 ± 0.23^{ab} | 0.26±0.11 ^a | 0.66 ± 0.23^{ab} | 0.13 ± 0.05^{a} | 0.53 ± 0.23^{ab} |
| A. candidus | 0.16 ± 0.05^{a} | 0.1 ± 0.00^{a} | 0.1 ± 0.00^{a} | 0.33 ± 0.11^{ab} | $0.66 \pm 0.23^{\circ}$ | 0.2 ± 0.00^{a} | 0.33 ± 0.11^{ab} | 0.1 ± 0.00^{a} | 0.33 ± 0.11^{ab} | 0.1 ± 0.00^{a} | 1.6 ± 0.00^{bc} | 0.53 ± 0.23^{d} |
| A. glaucus | 0.53 ± 0.23^{a} | 0.33±0.11 ^a | 0.26 ± 0.11^{a} | 0.26 ± 0.11^{a} | 0.1 ± 0.00^{a} | 0.33±0.11 ^a | 1.6 ± 0.00^{b} | 0.26 ± 0.11^{a} | 0.13 ± 0.05^{a} | 0.4 ± 0.00^{a} | 0.33±0.11 ^a | 1.33 ± 0.46^{b} |
| MFC (mg/mL) | | | | | | | | | | | | |
| A. alliceus | >1.6 | $0.8 \pm 0.00^{\mathrm{ac}}$ | 0.66 ± 0.23^{a} | 1.33 ± 0.46^{cd} | >1.6 | >1.6 | 0.33±0.11 ^a | 0.53 ± 0.23^{a} | $0.8 \pm 0.00^{\mathrm{ac}}$ | 0.53 ± 0.23^{a} | 1.6 ± 0.00^{cd} | 0.53 ± 0.23^{a} |
| A. carbonarus | 1.06 ± 0.46^{ab} | 0.8 ± 0.00^{ab} | 1.6 ± 0.00^{a} | 1.6 ± 0.00^{a} | 1.33 ± 0.46^{ab} | 1.6 ± 0.00^{a} | 1.06 ± 0.46^{ab} | 0.66 ± 0.23^{a} | 1.06 ± 0.46^{ab} | 1.6 ± 0.00^{a} | 1.33 ± 0.46^{ab} | 1.6 ± 0.00^{a} |
| A. flavus | 0.53 ± 0.23^{ab} | 0.2 ± 0.00^{a} | >1.6 | 0.66 ± 0.23^{ab} | 0.8 ± 0.00^{abc} | 0.33±0.11 ^a | $0.8{\pm}0.00^{\rm abc}$ | 1.06 ± 0.46^{bc} | >1.6 | 0.66 ± 0.23^{ab} | 1.33±0.46 ^c | >1.6 |
| A. fumigatus | 0.8 ± 0.00^{abc} | $1.6 \pm 0.00^{\text{de}}$ | >1.6 | >1.6 | $1.6 \pm 0.00^{\text{de}}$ | 0.66 ± 0.23^{ab} | 1.33 ± 0.46^{cd} | 0.4 ± 0.00^{a} | 0.8 ± 0.00^{abc} | $1.06 \pm 0.46^{\text{bcd}}$ | $0.8 \pm 0.00 b^{cd}$ | 0.53 ± 0.23^{ab} |
| A. niger | $1.6{\pm}0.00^{bc}$ | $1.6{\pm}0.00^{bc}$ | $0.8 \pm 0.00^{\mathrm{a}}$ | 0.8 ± 0.00^{a} | $0.66{\pm}0.23^{a}$ | 0.8 ± 0.00^{a} | $0.66{\pm}0.23^{\rm a}$ | 1.33 ± 0.46^{abc} | 1.33 ± 0.46^{abc} | 0.8 ± 0.00^{a} | >1.6 | 1.06 ± 0.46^{ab} |
| A. ochracus | 0.4 ± 0.00^{b} | 1.6 ± 0.00^{ab} | 0.53 ± 0.23^{b} | >1.6 | 1.6 ± 0.00^{b} | 0.53 ± 0.23^{b} | >1.6 | >1.6 | 0.33 ± 0.11^{b} | >1.6 | $0.8 \pm 0.00^{\mathrm{b}}$ | $1.33 \pm 0.46^{\circ}$ |
| A. parasiticus | 1.6 ± 0.00^{cd} | $1.06 \pm 0.46^{\text{bc}}$ | $0.8{\pm}0.00^{ab}$ | $0.53{\pm}0.23^{ab}$ | $0.8{\pm}0.00^{ab}$ | 0.26 ± 0.11^{a} | 0.66 ± 0.23^{ab} | >1.6 | >1.6 | $0.66{\pm}0.23^{ab}$ | 1.6 ± 0.0^{cd} | 0.66 ± 0.00^{ab} |
| A. tamari | 0.53 ± 0.23^{ab} | $1.33 \pm 0.46^{\circ}$ | >1.6 | 0.66 ± 0.23^{ab} | 0.4 ± 0.00^{a} | 0.66 ± 0.23^{ab} | 0.33 ± 0.11^{a} | >1.6 | 0.26 ± 0.11^{a} | 0.8 ± 0.00^{abc} | $1.06 \pm 0.46^{\text{bc}}$ | >1.6 |
| A. terreus | >1.6 | $0.53{\pm}0.23^{a}$ | $0.53{\pm}0.23^{a}$ | >1.6 | $1.33{\pm}0.46^{\rm bc}$ | >1.6 | >1.6 | >1.6 | $0.8{\pm}0.00^{\rm ac}$ | $1.06{\pm}0.46^{\rm ac}$ | $0.8{\pm}0.00^{\rm ac}$ | $1.06 \pm 0.46^{\mathrm{ac}}$ |
| A. candidus | $0.66{\pm}0.23^{ab}$ | 0.33 ± 0.11^{a} | $0.8 \pm 0.00^{\text{abc}}$ | 0.4 ± 0.00^{a} | >1.6 | 0.66 ± 0.23^{ab} | 1.06 ± 0.46^{bc} | 0.4 ± 0.00^{a} | $1.33 \pm 0.46^{\circ}$ | 0.53 ± 0.23^{ab} | >1.6 | >1.6 |
| A. glaucus | >1.6 | 0.66 ± 0.23^{a} | 0.8 ± 0.00^{a} | 0.53 ± 0.23^{a} | 0.53 ± 0.23^{a} | 0.4 ± 0.00^{a} | >1.6 | 0.66 ± 0.23^{a} | >1.6 | 1.06 ± 0.46^{a} | 1.06 ± 0.46^{a} | >1.6 |

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