Mycological Progress (2023) 22:58
https://doi.org/10.1007/s11557-023-01902-8

ORIGINAL ARTICLE

Selection of electron-deficient substances as antifungal candidates

Wafa Mihoubi[1] · Haitham Elleuch[2] · Manel Boudabbous[1] · Emna Ketata[1] · Ines Borgi[1] · Farhat Rezgui[2] · Ali Gargouri[1]

Received: 2 February 2023 / Revised: 28 May 2023 / Accepted: 4 June 2023 / Published online: 26 July 2023

Abstract
A new class of bioactive compounds synthesized from Morita-Baylis–Hillman adducts (MBH) showed antioxidant and anti-melanogenesis activities.Therefore, the present researchwork explores the relationship between antifungal activity and the responsible chemical function of MBH adducts and their derivatives (alcohols, acetates, phosphonates and hydrazono phosphonates). It was against the phyto-pathogenic fungi*Aspergillus niger, Fusarium oxysporum, Penicillium occitanis, Trichoderma reesei, Stachybotrys microspora, Fusarium solani,Trichoderma parceramosum, Fusarium aethiopicum*, *Alternaria alternata* and *Aspergillus flavus* using the agar diffusion method. Our results showed that acetates exhibited varying degrees of antifungal activity against several fungi tested, while single alcohol revealed a weaker activity. The five-membered ring derivative was the most potent with an inhibition zone diameter of 4.75 ± 0.21, 6.1 ± 0.14, 4.35 ± 0.21, 3.9 ± 0.14, 4.54 ± 0.11, 3.55 ± 0.07, 3 ± 0, 3.2 ± 0.2, 5.36 ± 0.26 and 5.06 ± 0.5 cm against *F. oxysporum, T. parceramosum, S. microspora, T. reesei, F. solani, P. occitanis, A. niger, F. aethiopicum, A. alternata*and *A. flavus,* respectively. Compared to the positive control, i.e., the nystatin, the most tested compounds exhibited moderate to strong growth inhibitory effects, depending on the radical group. The originality of this work is that several adducts were evaluated, for the first time. Acetates or alcohols with five and six-membered rings exhibited good antifungal activity. Linear or cyclic molecules coupled to five carbons generally carried an antifungal activity. The five-membered carbon acetates have thus been proven to be the most effective derivatives.

Keywords Antifungal · Morita-Baylis–Hillman adducts · Alcohols · Acetates · Phosphonates · Hydrazono phosphonates · Plate assay

Introduction

Despite the significant importance of natural products such as fungal metabolites or toxins, which can pose risks to animals and humans, their full exploration has yet to be achieved. Mycotoxins are organic compounds that are typically non-volatiles and essentially produced by fungi in the form of secondary metabolites. For centuries, people have been aware of their toxic effects. However, due to the decreased use of antifungal medicines advised for use in food, the negative effects of toxins only recently have come to be given significant relevance, which inevitably threats our health (Weidenborner 2001).

Our study focuses on fungi known to produce toxins, namely *Aspergillus, Trichoderma, Fusarium, Penicillium and Stachybotrys* (Braise et al. 2009). These fungi and other fungus pathogens are known to harm plants severely, resulting in major losses. Furthermore, they seriously jeopardize food security, as emphasized by Xiao et al. (2014). In addition to these dangers, the evolution of drug resistance has emerged as a critical problem, demanding the creation of new and stronger compounds. Despite the efforts to discover new classes of antimicrobial agents, the control of pathogenic microorganisms remains a challenge due to the emergence of drug-resistant strains (Pinto et al. 2006). Microbial resistance to commonly used antibiotics and antifungals is the main component of the global health problem, given the large number of cases where conventional treatment of

Section Editor: Ji-Kai Liu

✉ Haitham Elleuch
haitham.elleuch@cst.rnu.tn

[1] Laboratoire de Biotechnologie Moléculaire Des Eucaryotes, Centre de Biotechnologie de Sfax B.P, Université de Sfax, 1177, 3018 Sfax, Tunisia

[2] Laboratoire de Chimie Organique Structurale LR99ES14, Faculté Des Sciences de Tunis, Université El Manar, Tunis, Tunisia

infections has failed. A review of the literature reveals that many plant extracts provide a variety of known and unknown compounds, such as essential oils, with antifungal activity (Pinto et al. 2006; Tabanca et al. 2007; Tullio et al. 2007; Dutta et al. 2007). It is trusty to mention that natural products have certain advantages over synthetic molecules, and are biodegradable, non-toxic, with high antifungal activity, and inexpensive and non-specific qualities. However, the development of resistance by pathogenic fungi against natural products poses a significant challenge. It would therefore be interesting to find a new family of synthetic compounds to search for molecules with interesting functionalities.

Morita-Baylis–Hillman (MBH) adducts have been shown to be an important class of bioactive products that can be easily synthesized and inexpensive (Lima–Junior CG, Vasconcellos MLAA 2012). Moreover, they exhibit potent biological activities such as antimicrobial (Sa et al. 2014; Kumar et al. 2013), antifungal (Das et al. 2007), antimalarial (Kundu et al. 1999; Narender et al. 2005), Leishmanicidal (S.C.O., Sousa et al. 2017) and anti-proliferative activity on human tumor cell lines (Kohn et al. 2006).

In order to search for new antimicrobial compounds, we have looked at the MBH adducts and their derivatives which have not shown any cytotoxic effects (Ketata et al. 2019) and antioxidant (Elleuch et al. 2018). Here, we plan to evaluate the antifungal activity of MBH adducts and their derivatives against *Aspergillus, Trichoderma, Fusarium, Penicillium and Stachybotrys* fungal strains.

To this end, four sets of cyclic MBH adducts (alcohols, acetates, phosphonates and hydrazono phosphonates) were newly synthesized. They were evaluated for their antifungal activity, using the "agar well diffusion" method.

Materials and methods

MBH adducts preparation

The MBH products 1, 2, 3 and 4 corresponding to alcohols, acetates, phosphonates and hydrazono-phosphonates, respectively, were prepared according to previous reports (Gatri and Gaied 2002; Kwong et al. 2007; Elleuch et al. 2016; Elleuch et al. 2017; Luo et al. 2002 ; Moghadam et al. 2007) and tested at 10^{-3} dilution in DMSO.

Fungal cultures

Fungal strains were grown on potato dextrose agar (PDA) medium at 30 °C for 7 days until sporulation. Spores were collected by scraping the plates and filtering through sterile cotton. The spores were stored at 10^9 spores/mL in 20% Glycerol and at -80 °C. Liquid cultures were performed in Mandels' medium. The composition of the slightly modified Mandels' medium (Mandels et al. 1962) per liter was as follows: 2 g KH_2PO_4, 1.4 g $(NH_4)_2SO_4$, 1 g yeast extract, 0.3 g $CaCl_2{\cdot}2H_2O$, 0.3 g $MgSO_4{\cdot}7H_2O$, 1 mL Tween 80 and 1 mL trace element solution composed of 1.6 g/L $MnSO_4$, 2 g/L $ZnSO_4$, 0.5 g/L $CuSO_4$, 0.5 g/L $CoSO_4$, was supplemented by adding 2% glucose. The medium was sterilized at 121 °C for 20 min. Before use, 100 µg/mL Ampicillin and 12.5 µg/mL Tetracycline were added to avoid bacterial contamination.

Biological screening

Biological Screening was assessed by the agar well diffusion method which is widely used to evaluate antimicrobial activities. This method is simple, inexpensive, easy to reproduce and easy to read andinterpret (Magaldi et al. 2004). Indeed, 100 µL of fungal inoculum of 10^9 spores/mL was spread on a solid PDA medium in square Petri dishes (120 mm × 120 mm). Subsequently, wells of 7 mm in diameter are made in these plates. Besides, 100 µL of each MBH product was loaded in wells dug in agar plates (7 mm diameter holes). DMSO was also added as a control as the MBH adducts were suspended in such a solvent, as well as *Nystatin*, the positive control. To serve as a control, DMSO was added as the solvent for the MBH adducts, and *Nystatin* was included as the positive control. The plates were incubated for 72 h at 30 °C, under aerobic conditions. After incubation, confluent fungal growth was observed. The diameter of the growth inhibition zone was measured in cm. The antifungal activities, in terms of the diameters of inhibition zones, are reported on the graphs. Tested fungi were *Fusarium oxysporum* (Skouri-Gargouri & Gargouri 2008) *Trichoderma reesei* (named RutC30) (Montenecourt & Eveleigh 1979), *Stachybotrys microspora* (N1) (Amouri & Gargouri 2006), *Fusarium solani* (S3) (Boudabbous et al. 2017), *Penicillium occitanis* (CT1) (Ayadi et al. 2011),

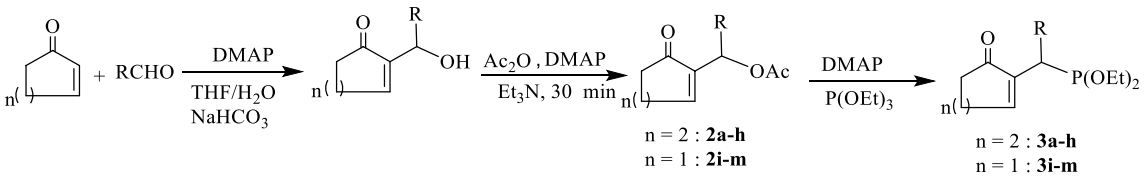
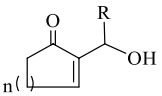
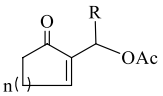
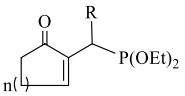
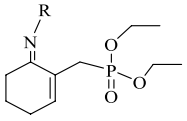


Fig. 1 MBH alcohols and acetates synthesis

Table 1 Chemical structure of cyclic MBH adducts

Alcohol (1)	Acetate (2)	Phosphonate (3)	Hydrazonophosphonate (4)
 n = 2 : **1a-f** n = 1 : **1g-l**	 n = 2 : **2a-f** n = 1 : **2g-l**	 n = 2 : **3a-f** n = 1 : **3g-l**	 **4a-d**
n = 2 (six-membered ring) **a**: R = H, **b**: R = CH_3, **c**: R = $(CH_2)_2CH_3$, **d**: R = Ph, **e**: R = CH_2CH_3, **f**: R = $CH(CH_3)_2$			**a:** R = NH-Ph$(NO_2)_2$, **b:** R = NH-Ph(NO_2), **c:** R = NH-SO_2Ph-Me
n = 1 (five-membered ring) **g**: R = H, **h**: R = Ph, **i**: R = CH_3, j: R = CH_2CH_3 , **k**: R = $(CH_2)_2CH_3$, **l** : R = $CH(CH_3)_2$			

Aspergillus niger (A1) (Hadj-Taieb et al. 1996), *Fusarium aethiopicum, Alternaria alternata*and *Aspergillus flavus.*

Fungicidal activities

The serial dilutions of MBH compounds were performed to produce volumes of 200 µL per Eppendorf tube. The final concentrations ranging from 10^{-1} to 10^{-4} M. 100 µL of the fungal suspension with a concentration of 10^8 spores/mL were added to each tube. Negative control wells contained the fungi in Mandels' medium. After incubation at 30 °C for 24 h, an amount of 100 µL was spread on PDA media and incubated at 30 °C for 5 days. The fungicidal activity was determined by assessing the number of colonies-forming units.

Chemical analysis

Melting points were identified on a Kofler melting apparatus and were uncorrected. ^{1}H and ^{13}C-NMR spectra were performed on a Varian 500 MHz spectrometer. The chemical shifts (δ) and coupling constants (*J*) are expressed in ppm and Hertz, respectively. Microanalyses and mass spectrometry analyses were carried out on a Carlo Erba EA 1102 and on a 3200 QTRAP (Applied Biosystem SCIEX), respectively. All solvents and reagents were obtained from commercial sources and purified before use if necessary. Merck Kieselgel 60F254 plates were used for TLC, and Merck Silica gel 60 (0.063–0.100 mm) for column chromatography.

Statistical analysis

The results were expressed as the mean ± standard deviation (SD). The statistical significance of the differences was evaluated by Student's t-test. $P < 0.05$ was considered to indicate a statistically significant difference.

Results and discussions

Cyclic acetates (2a-1) of MBH 2 were synthesized by the reaction of acetic anhydride on the corresponding alcohols (1a-1), in the presence of the triethylamine and DMAP as catalysts (Fig. 1, Table 1).

Cyclic acetates of MBH (2a-1) were reacted with triethylphosphite in the presence of DMAP or imidazole under solvent-free conditions to derive the synthesis of allylic phosphonates (3a-1) (Fig. 1, Table 1). The synthesis of these MBH adducts is outlined in Fig. 1 and summarized in Table 1, providing a concise description of each step involved. It is described in detail in the previous works reported by Elleuch et al. (2016–2018) and Ketata et al. (2019).

The phosphonate 3a, in turn, reacts with hydrazine in refluxing methanol to form the hydrazono-phosphonates 4a-c (Fig. 2, Table 1) (Elleuch et al. 2016, 2018).

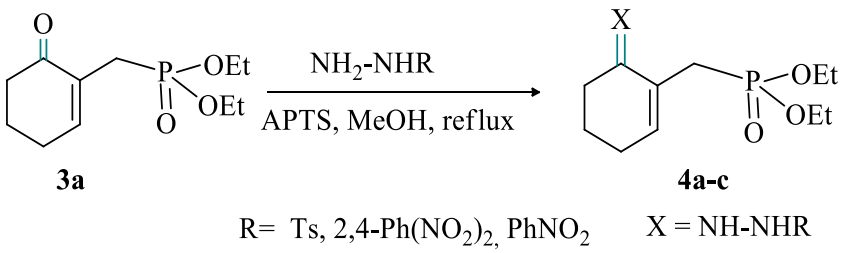
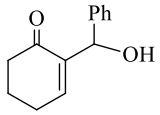
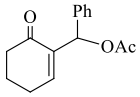
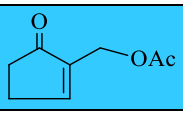
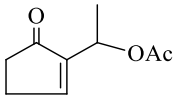
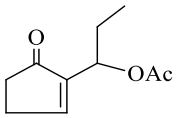
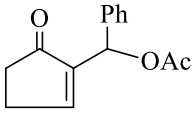
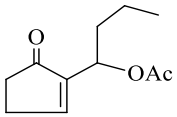
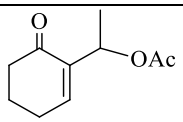
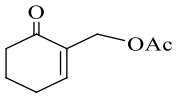
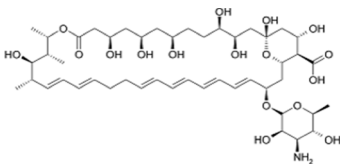


Fig. 2 Hydrazonophosphonates **4a-c** synthesis

Table 2 Zone of inhibition diameter values of MHB antifungal products against *Fusarium oxysporum, Trichoderma parceramosum, Stachybotrys microspora* and *Trichoderma reesei*

		Diameter (cm)			
Product	**Formule**	***Fusarium oxysporum***	***Trichoderma parceramosum***	***Stachybotrys microspora***	***Trichoderma reesei***
1d		**1.5 ± 0.14**	**4.05 ± 0.07**	**2.6 ± 0.14**	**2.95 ± 0.07**
2d		**1.7 ± 0.14**	**3.35 ± 0.07**	**3.6 ± 0.28**	**ND**
2g		**4.75 ± 0.21**	**4.35 ± 0.21**	**6.1 ± 0.14**	**4.85 ± 0.07**
2i		**2.6 ± 0.0**	**3.55 ± 0.07**	**4.1 ± 0.14**	**3.9 ± 0.14**
2j		**2.3 ± 0.0**	**4.1 ± 0.14**	**3.95 ± 0.07**	**3.05 ± 0.07**
2h		**2.15 ± 0.21**	**3.7 ± 0.14**	**3.35 ± 0.21**	**3,6 ± 0.0**
2k		**2 ± 0.0**	**3.85 ± 0.07**	**3.55 ± 0.21**	**3.05 ± 0.07**
2b		**1.4 ± 0.0**	**3.95 ± 0.07**	**2,5 ± 0.0**	**3.55 ± 0.07**
2a		**ND**	**ND**	**ND**	**2.5 ± 0.0**
Nystatine		**2.3 ± 0.1**	**3.0 ± 0.1**	**2.5 ± 0.1**	**3.5 ± 0.01**

Study of structure–activity relationship

The fungistatic/fungicidal potential of all the synthetic MBH adducts and their derivatives were examined against the mentioned fungi. However, in the results section, only the compounds exhibiting significant antifungal activity are presented and discussed.

Antifungal activity of the alcohol family

The screening for antifungal activity of the alcohol family shows that only the six-membered adduct 1d, bearing the phenyl group (R = Ph), exhibits antifungal activity. It was against the following fungi: *F. oxysporun, T. parceramosum, S. microspora* and *T. reesei* with growth inhibition

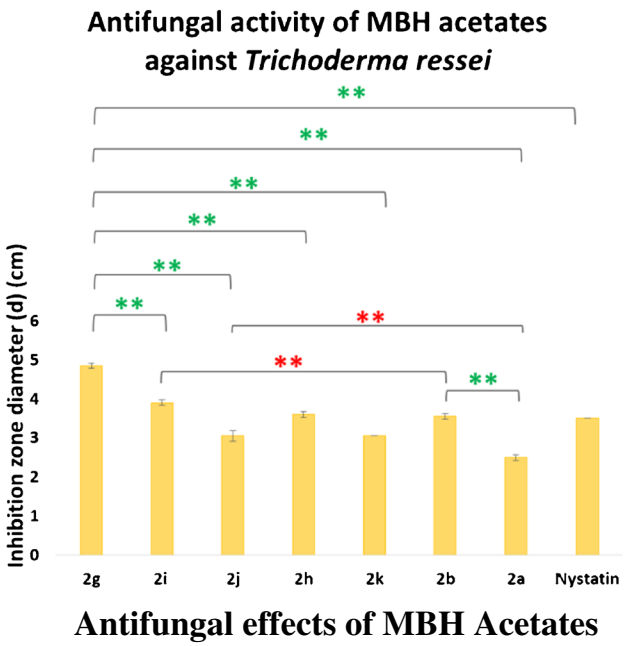


Fig. 3 Growth-inhibition zone diameters of the acetates family against *Trichoderma reesei* strain. The green color is used when the antifungal effect is related to the functional group and the red when it is related to the radical group. All tests were carried out in triplicate (mean ± SD; $n = 3$; $^{*}p < 0.05$, $^{**}p < 0.005$; p-values were calculated with Student's test). All the MHB products were tested at (100 µL/well), while *Nystatin* was tested at (30 µL/well) from the same concentration (10^{-1} M)

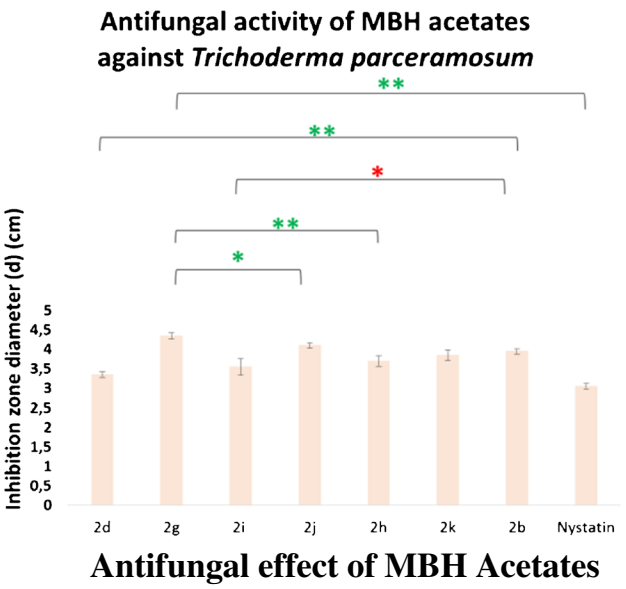


Fig. 4 Growth-inhibition zone diameters of the acetate family against *Trichoderma parceramosum* strain. The green color is used when the antifungal effect is related to the functional group and the red one when it is associated with the radical group. All tests were carried out in triplicate (mean ± SD; $n = 3$; $^{*}p < 0.05$, $^{**}p < 0.005$). p-values were calculated with Student's test. All the MHB products were tested at (100 µL/well), while *Nystatin* was tested at (30 µL/well) from the same concentration (10^{-1} M)

diameters, respectively: 1.5 ± 0.14, 4.05 ± 0.07, 2.6 ± 0.14, and 2.95 ± 0.07 cm (Table 2). Indeed, many synthetic or natural drugs contain this structural core of six-membered rings (de Carvalho et al. 2013; Khan et al. 2012). It should be noted that *T. parceramosum* is the most sensitive to alcohol 1d.

Antifungal activities of the acetate family

MBH 2 acetates were synthesized in a single step by there action between MBH alcohols and acetic anhydride in the presence of trimethylamine using DMAP as a catalyst. This synthetic approach is rapid (30 min) and inexpensive.

Most of the synthesized MBH2 acetates exhibit an antifungal activity that depends on theradical group R.

Antifungal activity against *Trichoderma reesei* RutC30 strain

The antifungal activity of the newly synthesized acetates having different radical groups was screened against the *Trichoderma reesei* (RutC30) strain. This adducts exhibited varying degrees of antifungal activities, depending on the radical group. Indeed, the MBH adduct 2a, a simple six-membered carbons acetate (R = H), presented an inhibition zone diameter of only d = 2.5 ± 0. This activity is lower than that of compound 2 g which is simple five-membered carbons acetate. It has a significant inhibitory effect (at $p \leq 0.05$) (d = 4.85 ± 0.07) on the growth of *T. reesei* (Fig. 3). In addition, five-membered carbons acetate (R = Me) 2i (d = 3.9 ± 0.14) showed a significant antifungal effect compared to the effect of the six-membered carbons acetate (R = Me) 2b with d = 3.55 ± 0.07 (Table 2). Furthermore, by comparing the different diameters of the zone of growth inhibition (d) of the six-membered carbon acetates MBH with different radical groups, it is found that the antifungal effect depends on the radical group. Indeed, when R = Me, d(2b) = 3.55 ± 0.07 ≥ d(2a) = 2.5 ± 0 when R = H (Table 2).

On the other hand, for MBH five-membered carbons acetates with different groups, the obtained results have revealed that when R = H (2 g), d = 4.85 ± 0.07, while the activity is significantly reduced when R = Me (d = 3.9 ± 0.14), as when R = Alkyl, Aryl (Table 2). Thus, the simple five-membered carbons acetate (R = H) 2 g has the highest inhibition potency against *Trichoderma reesei* (Fig. 3).

Antifungal activity against *Trichoderma parceramosum* strain

The present study on antifungal activity against *T. parceramosum* has shown that there is no significant difference in antifungal effect between the five membered-carbons acetates

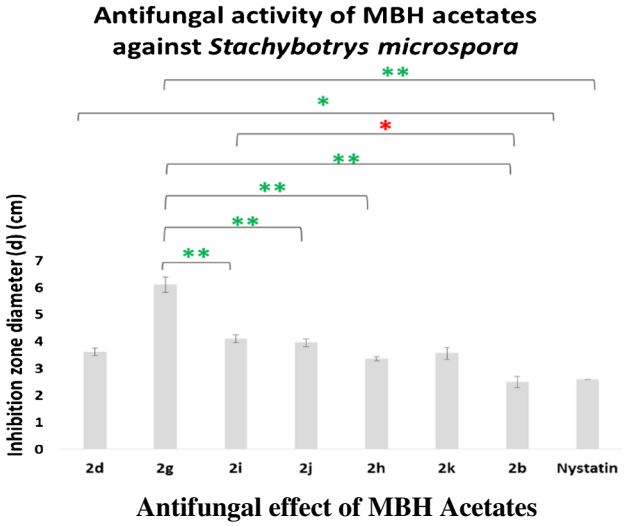


Fig. 5 Growth inhibition zone diameters of the compounds of the acetate family against *Stachybotrys microspora* strain. The green color is used when the antifungal effect is related to the functional group and the red one when it is linked to the radical group. All tests were carried out in triplicate (mean ± SD; $n=3$; $^{*}p<0.05$, $^{**}p<0.005$). *p*-values were calculated with Student's test. All the MHB products are tested at (100µL/well), while *Nystatin* was tested at (30 µL/well) from the same concentration (10^{-1} M)

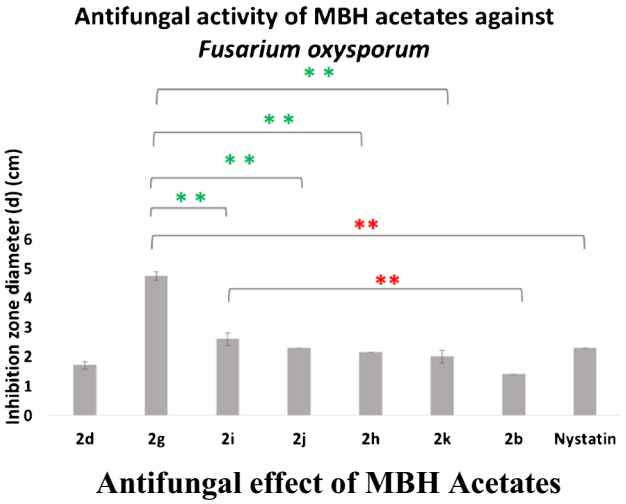


Fig. 6 Growth inhibition zone diameters of the compounds of the acetate family against *Fusarium oxysporum* strain. The green color is used when the antifungal effect is related to the functional group and the red one when it is linked to the radical group. All tests were carried out in triplicate (mean ± SD; $n=3$; $^{*}p<0.05$, $^{**}p<0.005$). *p*-values were calculated with student's t-test. All the MHB products were tested at (100 µL/well), while *Nystatin* was tested at (30 µL/well) from the same concentration (10^{-1} M)

and their counterparts (six-membered carbons) as can be seen in Table 2. Indeed, the diameters of growth inhibition were d(2d(R = Ph)) = 3.35 ± 0.07 ≈ d(2 h(R = Ph)) = 3.7 ± 0.14, as well as for 2i(R = Me), d = 3.55 ± 0.07, and its counterpart 2b(R = Me), d = 3.95 ± 0.07 (Fig. 4; Table 2). Moreover, the simple five-membered acetate 2 g (R = H) has the highest antifungal potency (d = 4.35 ± 0.21), which is reduced in the case of R = Me or R = Ph.

Antifungal activity against *Stachybotrys microspora* strain

Likewise, the five-membered carbon acetate has the highest antifungal activity against *Stachybotrys microspora*. Radical group replacement significantly reduces the antifungal activity against *S. microspora*. As shown in the Table 2, the acetate 2 g (R = H) has the highest antifungal activity (d = 6.1 ± 0.14). However, for R = alkyl, longer carbon chains have the least effective antifungal activity: d (2 g(R = H)) = 6.1 ± 0.14 ≥ d (2i(R = CH_3)) = 4.1 ± 0.14 ≥ d (2j(R = CH_2CH_3)) = 3.95 ± 0.07 ≥ d (2 k(R = $CH_2CH_2CH_3$)) = 3.55 ± 0.21 (Fig. 5; Table 2). In addition, the five-membered acetate 2 h (R = Ph), with a moderate steric hindrance, shows less antifungal activity (d (2 h) = 3.35 ± 0.21) than acetate 2 k (R = n-Pr) with d(2 k) = 3.55 ± 0.21. However, the antifungal activity of the six-membered ring acetate 2d with phenyl group (R = Ph), d(2d) = 3.60 ± 0.28 is greater than that of the acetate with methyl group 2b(R = Me), d(2b) = 2.5 ± 0.0.

Antifungal effect of MBH against *Fusarium oxysporum*

Among the tested compounds, the MBH 2 g adduct displayed the most pronounced antifungal activity (d = 4.75 ± 0.21) against *Fusarium oxysporum*. Similarly, the radical substitution modifies the potential for antifungal activity: d (2 g(R = H)) = 4.75 ± 0.2 1 ≥ d(2i(R = CH_3)) = 2.6 ± 0 ≥ d (2j(R = CH_2CH_3)) = 2.3 ± 0 ≥ d (2 k(R = $CH_2CH_2CH_3$)) = 1.4 ± 0 (Fig. 6; Table 2).

The MBH six-membered ring acetates 2b (R = Me, d = 1.4 ± 0) and 2d (R = Ph, d = 1.7 ± 0.14) show the weakest antifungal activity against *F. oxysporum*.

Antifungal activity against *Fusarium solani*, *Penicillium occitanis* and *Aspergillus niger* strains

The inhibitory effect of MBH five-membered carbon acetates on *Fusariumsolani* and *Penicilliumoccitanis* was observed, with the degree of inhibition varying depending on the specific radical involved. Acetate 2 g (R = H) exhibits the highest antifungal activity. In contrast, when R = Me, Alkyl or Ph, the respective compounds (2i, 2j and 2 h) share a rather moderate antifungal potential (Table 3).

It should be noted that the five-membered MBH acetate 2 g (R = H) was the only compound capable of inhibiting the growth of *Aspergillus niger* (Table 3).

Compared to the antifungal activity of *Nystatin* as a potent commercial antifungal product, most of the tested MBH

adducts exhibited moderate to strong inhibitory effects. Simple acetate 2 g (R = H) appears to have the highest antifungal activity against *Fusarium solani* and *Aspergillus niger*.

All the MHB products were tested at (100 µL/well) while *Nystatin* was tested at (30 µL/well) from the same concentration (10^{-1} M).

Compared to control nystatin, compounds 2i, 2j, 2 h, 2 k and 2a showed variable antifungal activity, while 2 g exhibited a broad spectrum of biological activity on all tested fungi.

Antifungal activity against *Aspergillus flavus,Alternaria alternata* and *Fusarium aethiopicum* strains

As shown in Table 4, the growth of *Fusarium aethiopicum*, *Alternaria alternata* and *Aspergillus flavus*, newly isolated strains in our laboratory, was strongly inhibited by the five-membered MBH acetate (R = H) (2 g). It is followed by a simple six-membered carbons acetate (R = H) and the antifungal activity weakens when the R group is longer (R = $(CH_2)_2CH_3$) or even none against *Alternaria alternata*. Of the three fungi, *Alternaria alternata* and *Aspergillus flavus* strains exhibit the highest sensitivity to compound 2 g. When comparing the antifungal activity of 2 g to that of the standard fungicide *Nystatin*, 2 g is proven to exhibitgreat potential as a fungicide and may have a more promising future in this regard.

It is worthy to recall that the MBH products were tested at (100 µL/well) and *Nystatin* was tested at (30 µL/well) from the same concentration (10^{-1} M). Despite this difference of the tested volume, the simple six-membered acetate 2a (R = H) has a good antifungal activity against *F. aethiopicum*, *A. alternata* and *A. flavus* strains. Acetate 2 k (R = n-Pr) demonstrated a weak antifungal activity. However, the antifungal activity of the five-membered ring acetate 2 g was found to be better than that of 2 k and 2a, except against *F. aethiopicum* which appears more sensitive to 2a. The results suggest that the introduction of an alkyl group would reduce the antifungal activity.

Regarding the strains under study, it should be noted that the majority of them are well known as phytopathogens (*F.*

Table 3 Values of the diameter of the zone of inhibition of MHB antifungal products against *Fusarium solani, Penicillium occitanis* and *Aspergillus niger*

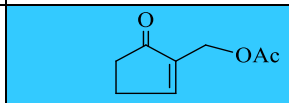
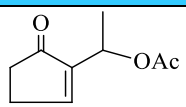
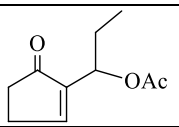
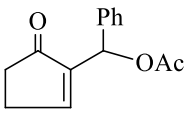
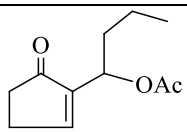
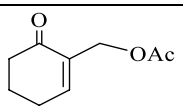
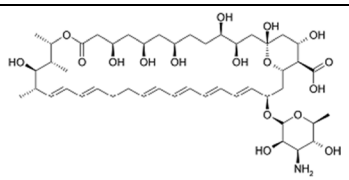
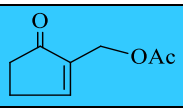
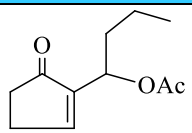
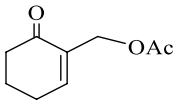
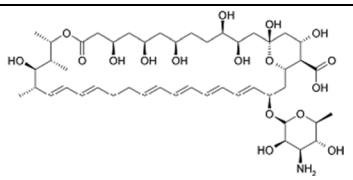
		Diameter (cm)		
Product	**Formule**	***Fusarium solani***	***Penicillium occitanis***	***Aspergillus niger***
2g		**4.54 ± 0.11**	**3.55 ± 0.07**	**3 ± 0.0**
2i		**2.05 ± 0.07**	**2 .3 ± 0.14**	**0**
2j		**2.05 ± 0.07**	**2.1 ± 0.14**	**0**
2h		**2.15 ± 0.21**	**2.2 ± 0.28**	**0**
2k		**1.85 ± 0.07**	**1.9 ± 0.14**	**0.66 ± 0.16**
2a		**3.8 ± 0.14**	**ND**	**1.70 ± 0.16**
Nystatin		**1.06 ± 0.11**	**1.50 ± 0.11**	**1.40 ± 0.14**

Table 4 Values of the diameter of the zone of inhibition of MHB antifungal products against *Fusarium aethiopicum*, *Alternaria alternata* and *Aspergillus flavus* strains

		Diameter (cm)		
Product	**Formule**	***Fusarium aethiopicum***	***Alternaria alternata***	***Aspergillus flavus***
2g		**3.2 ± 0.2**	**5.36 ± 0.26**	**5.06 ± 0.50**
2k		**2.80 ± 0.28**	**0**	**3.06 ± 0.11**
2a		**4.40 ± 0.34**	**4.10 ± 0.66**	**4.00 ± 0.11**
Nystatin		**3.66 ± 0.15**	**3.30 ± 0.14**	**3.66 ± 0.15**

oxysporum, F. solani, A. alternata, A. niger and A. flavus) than others (*T. reesei, S. microspora, T. parceramosum, P. occitanis, F. aethiopicum*).

Fusarium oxysporum is a devastating fungal pathogen, already known to secrete degrading enzymes called "plant cell wall degrading enzymes" (CWDE) such as pectinases (Bravo-Ruiz et al. 2016; Bravo-Ruiz et al. 2017). Similarly, *Fusarium solani* is capable of causing disease in many agriculturally important crops (Arie 2019). Typically, the *F. solani* species complex (FSSC) causes stem and/or root decay of the infected host plant, and the degree of necrosis correlates with the severity of the disease (Coleman 2016). *Aspergillus flavus* is a plant pathogen that attacks economically important crops, such as corn and peanuts, spices, flax seeds, cereals, and sometimes dried fruits (e.g. figs) (Hedayati et al. 2007). *A. flavus* is often studied as a contaminant producing mycotoxins such as aflatoxins harmful to humans (Gravesen et al. 1994). *Alternaria* is also known worldwide as both a common plant pathogen and an airborne allergen. More specifically, *A. alternata* is recognized as the aero-allergen type species. In the majority of cases, health problems among humans and animals have been associated with this species (Gravesen et al. 1994; de Luca, 2007). Finally, some of these phytopathogenic fungi, such as *A. niger or T. reesei* and *Penicillium* sp., can be used as biological control agents. They would protect the plant against the attack of other fungal strains and provide it with useful biomolecules (Bravo-Ruiz et al. 2017; Peng et al. 2021; Manzar et al. 2022). In this context, *Aspergillus niger* is known to solubilize potassium and phosphate, as well as to produce phytohormones (Mundim et al. 2022).

Assessment of fungicidal activity of 2 g compound

To further deepen our investigation, we determined the fungicidal activity of the synthesized compound 2 g, which stood out as having the best antifungal power against most of the tested fungi. It was tested against two fungi *Aspergillus niger* and *Penicillium occitanis*. Hence, not only does it offer good fungicidal

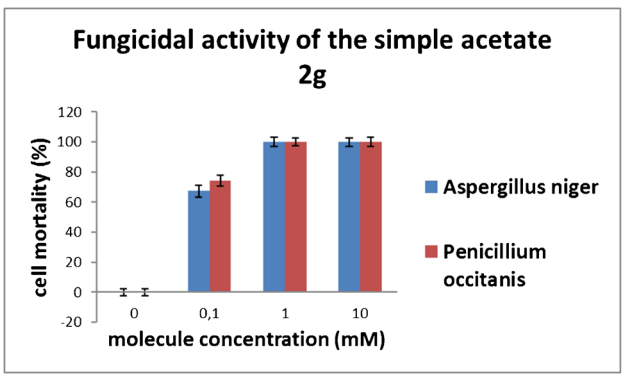


Fig. 7 Fungicidal activity of 2 g compound against *Aspergillus niger* and *Penicillium occitanis*. It was determined by observing the lowest concentration of the drug at which no visible growth exists, and it is expressed by the percentage of cell mortality

power (70%) at a concentration of 0.1 mM, but also100% inhibition of fungal growth at 1 mM, as shown in Fig. 7.

The screening of antifungal activity demonstrated the ability of MBH adducts possess the ability to inhibit fungal growth. Indeed, among the tested adducts the alcohol 1d(R = Ph) and MBH acetates were the only antifungal products. Thus, the antifungal activity depends on the functional group, and the five-membered ring acetate (2 g) having R = H is the most active against all tested fungi.

The introduction of radical substituents at the ortho position of the n-membered carbon acetate increased or decreased antifungal activity depending on the core of the compound. Notably, compound 2 g is lipophilic (absence of OH group), suggesting that this property could facilitate its penetration into the fungal cell, and thus promoting its activity (Han al. 2019; (Elleuchet al. 2018). The acetate moiety would be responsible for the antifungal effect and the radical H would ensure the effectiveness of its antifungal power.

MBH Phosphonates and hydrazonophosphonates families

MBH phosphonates were synthesized by reacting MBH acetates with triethylphosphite in the presence of DMAP. The hydrazonophosphonates were synthesized by reacting MBH phosphonates with hydrazines. Although all of these adducts were tested, they failed to inhibit fungal growth.

Conclusion

MBH adducts are of great interest in different applications, as shown previously, thanks to their antioxidant activity (Sun et al. 2019). They can be used as additives to prevent lipid oxidation or as anti-melanogenesis(Ketata et al. 2019) as well as for their antifungal activities. We adopted the agar diffusion method to evaluate the antifungal activity of several MBH adducts (Magaldi et al. 2004). The present research work has shown that MBH adducts (alcohols (1), acetates (2)) are endowed with interesting antifungal activity against the most known fungal phytopathogens and/or toxin producers, namely the genera *Aspergillus, Alternaria, Fusarium, Penicillium, Trichoderma*. This allows us to conclude that these compounds can be potential candidates as effective antifungals in the fields of agriculture, agro-foods and pharmaceutical industry (Saremi et al. 2018; Han et al. 2019; Srivastava et al. 2019). The MHB products were then classified according to the effectiveness of their antifungal activity evaluated by the diameter of the fungal growth inhibition zone.

This study has also discovered that the 2 g five-membered carbon acetate has the most effective growth inhibitor activity against all tested fungi, compared to six-carbon acetate. Besides, it has demonstrated that the antifungal activity is often inversely proportional not only to the length of the radical but also to its complexity.

Acknowledgements The authors thank the DGRST and the Ministry of Higher Education Tunisia for financial support of this work.

Authors Contributions W.M, H.E and AG: idea presentation, W.M, H.E, M.B and A.G: investigation, W.M, H.E, M.B, E.K and I.B carried out the experiments, W.M and H.E drafted the manuscript, A.G and F.R checked the manuscript, A.G and F.R supervising the finding of this work, A.G: funding acquisition, all authors discussed the results and contributed to the final manuscript.

Funding The authors received no specific funding for this work. This work was supported by grants from the"Ministère de l'Enseignement Supérieur et la Recherche Scientifique" (Tunisia).

Data availability All raw data are available from the Centre de Biotechnologie de Sfax, Sfax, Tunisia.

Declarations

Ethical approval Not applicable.

Consent to participate Not applicable.

Consent to publish Not applicable.

Conflict of interest The authors declare no competing financial interest.

We certify that there is no conflict of interests to declare and all authors Wafa Mihoubi, Haitham Elleuch, Manel Boudabbous, Emna Ketata, Ines Borgi, Farhat Rezgui and Ali Gargouri mutually agree to submit this original work.

References

Amouri B, Gargouri A (2006) Characterization of a novel β-glucosidase from a *Stachybotrys* strain. Biochem Eng J 32(3):191–197. https://doi.org/10.1016/j.bej.2006.09.022

Arie T (2019) *Fusarium* diseases of cultivated plants, control, diagnosis, and molecular and genetic studies. J Pestic Sci 44(4):275–281. https://doi.org/10.1584/jpestics.J19-03

AyadiM TS, Trigui-Lahiani H, Hadj-Taïeb N, Jaoua M, Gargouri A (2011) Constitutive over-expression of pectinases in *Penicillium occitanis* CT1 mutant is transcriptionally regulated. Biotech Lett 33(6):1139–1144. https://doi.org/10.1007/s10529-011-0546-3

Boudabbous M, Ben Hmad I, Saibi W, Belghith H, Gargouri A (2017) Trans-glycosylation capacity of a highly glycosylated multi-specific B-glucosidase from *Fusarium solani*. Bioprocess Biosyst Eng 40(4):559–571. https://doi.org/10.1007/s00449-016-1721-7

Braise S, Encinas A, Keck J, Nising CF (2009) Chemistry and biology of mycotoxins and related fungal metabolites. Chem Rev 109(09):3903–3990. https://doi.org/10.1021/cr050001f

Bravo-Ruiz G, Di Pietro A, Roncero G, MI, (2016) Combined action of the major secreted exo- and endo polygalacturonases is required for full virulence of *Fusarium oxysporum*. Mol Plant Pathol 17(3):339–353. https://doi.org/10.1111/mpp.12283

Bravo-Ruiz G, HadjSassi A, Marcet-Houben M, Di Pietro A, Gargouri A, Gabaldon T, G. Roncero MI, (2017) Regulatory mechanisms of a highly pectinolytic mutant of *Penicillium occitanis* and functional analysis of a candidate gene in the plant pathogen *Fusarium oxysporum*. Front Microbiol 8:1627. https://doi.org/10.3389/fmicb.2017.01627

Coleman JJ (2016) The *Fusarium solani* species complex: ubiquitous pathogens of agricultural importance. Mol Plant Pathol 17(2):146–158. https://doi.org/10.1111/mpp.12289

Das B, Chowdhury N, Damodar K, Banejee J (2007) A mild and efficient stereo selective synthesis of (*Z*)- and (*E*)-allyl sulfides and potent antifungal agent, (*Z*)-3-(4-Methoxybenzylidene)thiochroman-4-one from Morita–Baylis–Hillman Acetates. Chem Pharm Bull 55(8):1274–1276. https://doi.org/10.1248/cpb.55.1274

De Carvalho GSG, Dias RMP, Pavan FR, Leite CQF, Silva VL, Diniz CG, De Paula DTS, Coimbra ES, Retailleau P, Da Silva AD (2013) Synthesis, cytotoxicity, antibacterial and anti-leishmanial activities of Imidazolidine and Hexahydro pyrimidine derivativ. Med Chem 9(3):351–359. https://doi.org/10.2174/1573406411309030005

De Lucca AJ (2007) Harmful fungi in both agriculture and medicine. Revista Iberoamericana de Micología 24(1):3–13. http://www.reviberoammicol.com/2007-24/003013.pdf

Dutta BK, Karmakar S, Naglot A, Aich JC, Begam M (2007) Anticandidial activity of some essential oils of a mega biodiversity hotspot in India. Mycoses 50(2):121–124. https://doi.org/10.1111/j.1439-0507.2006.01332.x

Elleuch H, Ayadi M, Bouajila J, Rezgui F (2016) Synthesis of a series of γ-Keto Allyl Phosphonates. J Org Chem 81(5):1757–1761. https://doi.org/10.1021/acs.joc.5b02106

Elleuch H, Mihoubi W, Mihoubi M, Ketata E, Gargouri A, Rezgui F (2018) Potential antioxidant activity of Morita-Baylis-Hillman adducts. Bioorg Chem 78:24–28. https://doi.org/10.1016/j.bioorg.2018.03.004

Elleuch H, Baioui N, Bouajila J, Rezgui F (2017) Chemoselective reaction of ethane-1,2-dithiol, hydrazines, and hydroxylamine onto γ-ketoallyl phosphonates and phosphine oxides. Arkivoc, part iv:265–272. https://doi.org/10.24820/ark.5550190.p009.957

Gatri R, El Gaied MM (2002) Imidazole-catalysed Baylis-Hillman reactions: a new route to allylic alcohols from aldehydes and cyclic enones. Tetrahedron Lett 43(43):7835–7836. https://doi.org/10.1016/S0040-4039(02)01515-0

Gravesen S, Frisvad JC, and Samson RA (1994). Microfungi. Copenhagen, Munksgaard.168. https://www.cabdirect.org/cabdirect/abstract/19961201406

Hadj-Taieb N, Ayadi M, Gargouri A (1996) Selection of a constitutive hyper-pectinolytic mutant from a *Penicillium* strain. Biotechnol Prog 14:921–928. https://doi.org/10.1016/S0921-0423(96)80335-2

Han Y, Zhao J, Zhang B, Shen Q, Shang Q, Li P (2019) Effect of a novel antifungal peptide P852 on cell morphology and membrane permeability of *Fusarium oxysporum*. Biophysica Acta - Biomembranes 2:532–539. https://doi.org/10.1016/j.bbamem.2018.10.018

Hedayati MT, Pasqualotto AC, Warn PA, Bowyer P, Denning DW (2007) *Aspergillus flavus*: human pathogen, allergen and mycotoxin producer. Microbiology 153(6):1677–1692. https://doi.org/10.1099/mic.0.2007/007641-0

Ketata E, Elleuch H, Neifar A, Mihoubi W, Ayadi W, Marrakchi N, Rezgui F, Gargouri A (2019) Anti-melanogenesis potential of a new series of Morita–Baylis–Hillman adducts in B16F10 melanoma cell line. Bioorg Chem 84:17–23. https://doi.org/10.1016/j.bioorg.2018.11.028

Khan N, Shreaz S, Bhatia R, Ahmad SI, Muralidhar S, Manzoor N, Khan LA (2012) Anticandidal activity of curcumin and methyl cinnamaldehyde. Fitoterapia 83(3):434–440. https://doi.org/10.1016/j.fitote.2011.12.003

Kohn LK, Pavam CH, Veronese D, Coelho F, De Carvalho JE, Wanda AP (2006) Anti proliferative effect of Baylis-Hillman adducts and a new phthalide derivative on human tumor cell lines. Eur J Med Chem 41(6):738–744. https://doi.org/10.1016/j.ejmech.2006.03.006

Kumar AS, Kanakaraju S, Prasanna B, Chandramouli GVP (2013) Synthesis, molecular docking studies and antibacterial evaluation of Baylis-Hillman adducts of coumarin and pyran derivatives using ionic liquid under microwave irradiation. Chem Sci Trans 2(2):561–569. https://doi.org/10.7598/cst2013.415

Kundu MK, Sundar N, Kumar SK, Bhat SV, Biswas S, Valecha N (1999) Anti malarial activity of 3-hydroxyalkyl-2-methylenepropionic acid derivatives. Bioorg Med Chem Lett 9(5):731–736. https://doi.org/10.1016/S0960-894X(99)00057-8

Kwong CK-W, Huang R, Zhang M, Shi M, Toy PT (2007) Bifunctional polymeric organo catalysts and their application in the cooperative catalysis of Morita–Baylis–Hillman reactions. Chem Eur J 13(8):2369–2376. https://doi.org/10.1002/chem.200601197

Lima–Junior CG, Vasconcellos MLAA, (2012) Morita–Baylis–Hillman adducts: Biological activities and potentialities to the discovery of new cheaper drugs. Bioorg Med Chem 20(13):3954–3971. https://doi.org/10.1016/j.bmc.2012.04.061

Luo S, Zhang B, He J, Janczuk A, Wang PG, Cheng J-P (2002) Aqueous Baylis-Hillman reactions of cyclopent-2-enone using imidazole as catalyst. Tetrahedron Lett 43(41):7369–7371. https://doi.org/10.1016/S0040-4039(02)01716-1

Mandels M, Parrish FW, Reese ET (1962) Sophorose as an inducer of cellulase in *Trichoderma viride*. J Bacteriol 83(2):400–408. https://doi.org/10.1128/jb.83.2.400-408.1962

Manzar N, Kashyap AS, Goutam RS, Rajawat MVS, Sharma PK, Sharma SK, Singh HV (2022) *Trichoderma*: Advent of versatile biocontrol agent, its secrets and insights into mechanism of biocontrol potential. Sustainability 14(19):12786. https://doi.org/10.3390/su141912786

Magaldi S, Mata-Essayag S, Hartung de Capriles C, Perez C, Colella MT, Olaizola C, Ontiveros Y (2004) Well diffusion for antifungal susceptibility testing. Int J Infect Dis 8(1):39–45. https://doi.org/10.1016/j.ijid.2003.03.002

Moghadam M, Tangestaninejad S, Mirkhani V, Mohammadpoor-Baltork I, Taghavi SA (2007) Highly efficient and selective acetylation of alcohols and phenols with acetic anhydride catalyzed by a high-valent tin(IV) porphyrin, $Sn(TPP)(BF_4)_2$. J Mol Catal A Chem 274(1–2):217–223. https://doi.org/10.1016/j.molcata.2007.05.012

Montenecourt BS, Eveleigh DE (1979) Selective screening methods for the isolation of high yielding cellulase mutants of *Trichoderma reesei*. Adv Chem Ser 181:289–301. https://doi.org/10.1021/ba-1979-0181.ch014

Mundim GSM, Aciel GM, Mendes GO (2022) *Aspergillus niger* as a biological input for improving vegetable seedling production. Microorganisms 10(4):674. https://doi.org/10.3390/microorganisms10040674

Narender P, Srinivas U, Gangadasu B, Biswas S, Jayathirtha RV (2005) Anti-malarial activity of Baylis-Hillman adducts from substituted 2-chloronicotinaldehydes. Bioorg Med Chem Lett 15(24):5378–5381. https://doi.org/10.1016/j.bmcl.2005.09.008

Peng Y, Li SJ, Yan J, Tang Y, Cheng JP, Gao AJ, Yao X, Ruan JJ, Xu BL (2021) Research progress on phytopathogenic fungi and their role as biocontrol agents. Front. Microbiol. 12:670135. https://doi.org/10.3389/fmicb.2021.670135

Pinto E, Pina-Vaz C, Salgueiro L, Gonçalves MJ, Costa-de-Oliveira S, Cavaleiro C, Palmeira A, Rodrigues A, Martinez-de-Oliveira J (2006) Antifungal activity of the essential oil of *Thymus pulegioides* on Candida, *Aspergillus* and *Dermatophyte species*. J Med Microbiol 55:1367–1373. https://doi.org/10.1099/jmm.0.46443-0

Sa MM, Ferreira M, Lima ES, Santos ID, Orlandi PP, Fernandes L (2014) Antimicrobial activity of allylic thiocyanates derived from the Morita–Baylis–Hillman reaction. Braz J Microbiol 45(3):807–812. https://doi.org/10.1590/S1517-83822014000300007

Saremi H, Rostami A, Saremi H (2018) Fusarium oxysporum a possible agent for biological control of *Papaver somniferum* in the Middle East. Crop Prot 114:187–194. https://doi.org/10.1016/j.cropro.2018.08.031

Skouri-Gargouri H, Gargouri A (2008) First isolation of a novel thermostable antifungal peptide secreted by *Aspergillus clavatus*. Peptides 29(11):1871–1877. https://doi.org/10.1016/j.peptides.2008.07.005

Sousa SCO, Rocha JC, Keesen TSL, Silva EP, Assis PAC, Oliveira JPG, Capim SL, Xavier FJS, Marinho BG, Silva FPL, Lima-Junior

CG, Vasconcellos MLAA (2017) Synthesis of 16 new hybrids from tetrahydropyrans derivatives and Morita-Baylis-Hillman adducts: in vitro screening against *Leishmania donovani*. Molecules 22(2):207–221. https://doi.org/10.3390/molecules22020207

Srivastava S, Bhargava A, Pathak N, Srivastava P (2019) Production, characterization and anti bacterial activity of silvernano particles produced by *Fusarium oxysporum* and monitoring of protein-ligand interaction through in-silico approaches. Microb Pathog 129:136–145. https://doi.org/10.1016/j.micpath.2019.02.013

Sun F, Sun S, Zhu L, Duan C, Zhu Z (2019) Confirmation of *Fusarium oxysporum* as a causal agent of mung bean wilt in China. Crop Prot 117:77–85. https://doi.org/10.1016/j.cropro.2018.11.017

Tabanca N, Demirci B, Crockett SL, Başer KHC, Wedge DE (2007) Chemical composition and antifungal activity of *Arnica longifolia Aster hesperius*, and *Chrysothamnus nauseosus* essential oils. J Agric Food Chem 55(21):8430–8435. https://doi.org/10.1021/jf071379c

Tullio V, Nostro A, Mandras N, Dugo P, Banche G, Cannatelli MA, Cuffini AM, Alonzo V, Carlone NA (2007) Antifungal activity of essential oils against filamentous fungi determined by broth microdilution and vapour contact methods. J Appl Microbiol 102(6):1544–1550. https://doi.org/10.1111/j.1365-2672.2006.03191.x

Weidenborner M, Encyclopedia of food mycotoxins; Springer*:* Berlin, 2001. https://www.springer.com/gp/book/9783540675563

Xiao J, Zhang Q, Gao YQ, Tang JJ, Zhang AL, Gao J-M (2014) Secondary metabolites from the endophytic *Botryosphaeria dothidea* of *Melia azedarach* and their antifungal, antibacterial, antioxidant, and cytotoxic activities. J Agric Food Chem 62(16):3584–3590. https://doi.org/10.1021/jf500054f