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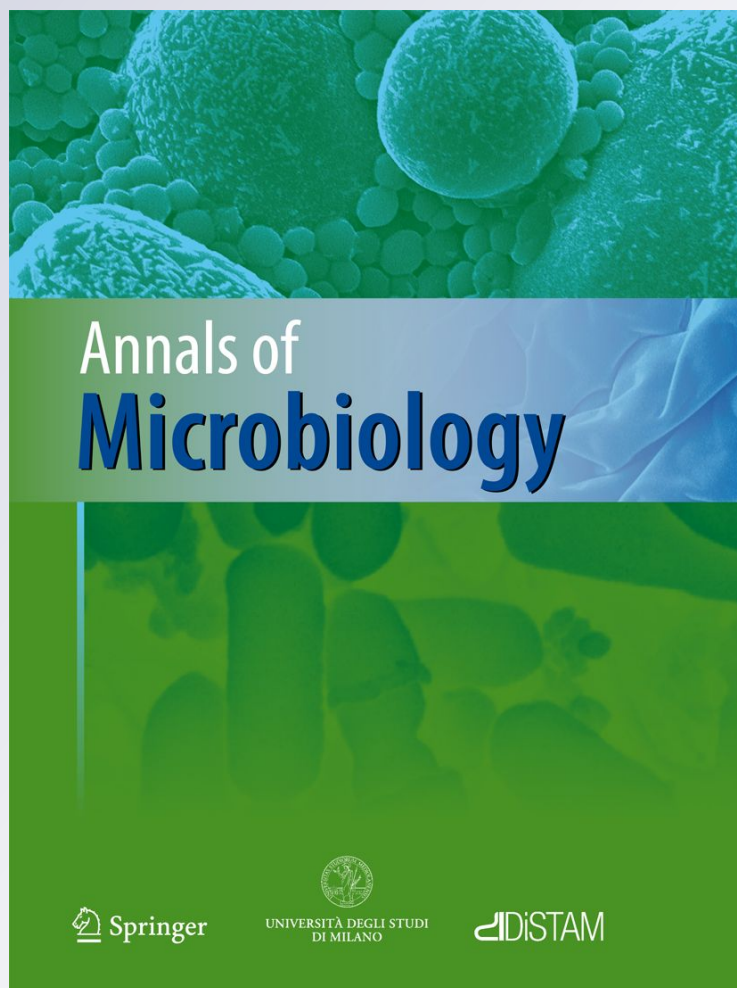
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Biological control of sapstain fungi in Egyptian wood stores and infected trees

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Abstract Using microorganisms or their secondary products, especially volatile organic compounds (VOCs), to control wood infection by fungi has been exploited in recent years. A total of 17 local microbial isolates were screened for growth inhibition of wood-pathogenic fungi on solid artificial medium. *Lactobacillus plantarum* was selected as the most active VOC producer since it reduced the growth of sapstain fungi by 60–90% and that of wood-degrading fungi by 20–60%. During the interaction between the majority of the tested wood-pathogenic fungi and VOCs emitted by *L. plantarum* on wood blocks, both hyphal growth and conidial formation were inhibited. It was also observed that loss of wood dry weight and wood strength due to the action of pathogenic fungi was decreased to negligible values by exposing wood blocks to VOCs emitted by *L. plantarum*. The VOCs produced were trapped using a simple method and quantified by GC/MS. The main constituents of the VOC profile were 1,2-benzenedicarboxylic acid, dibutyl ester, isopropyl myristate, n-nonadecane, octadecane and n-eicosane. This study also evaluated using gamma radiation to enhance microbial production of VOCs.

Keywords Volatile organic compounds · Wood control · Sapstain fungi · Gamma radiation

Introduction

Cairo is one of the most crowded cities in the world. The modern manner of building, together with the continuously increasing population, greatly affect the size of green areas in Cairo. Although public gardens are the only “breathing lungs” for citizens living in the capital, their number remains almost the same and they have not kept pace with the population increase. In addition, many trees growing in such parks suffer from wood-pathogenic fungi that cause wood decay. Intensive wood decay has an adverse effect on tree strength.

At the same time, the furniture industry in Egypt has grown intensively in recent years, and has become highly competitive in the most important export countries in this field. According to economic reports issued by the Egyptian Chamber of Commerce in 2008, furniture specialists have increased dramatically in the last 5 years by more than 175% compared to the number reported in 1995. American and European Union markets represent the most important consumers of Egyptian furniture—American exports grew to US \$20.9 million in 2003 in comparison with US \$7.2 million in 2002. Egypt now has a strong industrial base in this field, including excellently trained workers, modern equipment, and art technology; hence, wood is imported in huge quantities and kept in big stores. In these stores, wood may lay for periods of more than 6 months. Humidity and poor environmental conditions in big stores favor fungal growth.

Certain classes of the phyto-pathogenic fungi that attack wood cause permanent discoloration of the wood. Such discoloration is called sapstain and the causal fungi are known as sapstain fungi. The most common colorations are

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black, grey, brown or blue, each color showing different intensities depending on the nature of the melanin pigment produced by the fungus. Sapstain fungi have a serious impact on the pulp and paper industry since they colonize and disfigure freshly felled material prior to drying, which results in significant economic losses (Bruce et al. 2003).

Many approaches to combat sapstaining problems have been evaluated. Chemical fungicides were the first solution used by timber producers and paper manufacturers (Moita et al. 2005). However, employing synthetic fungicides on a large scale can cause some environmental and health concerns, especially when used in the open air in spaces such as public gardens. Biological control strategies, which are safer and more eco-friendly, are thus recommended. Many researchers have used a variety of microorganisms—bacteria (Wei-wei et al. 2008), mold (Wheatley et al. 1997; Schubert et al. 2008) and yeast (Payne and Bruce 2001)—to act as biocontrol and bioprotectant agents. Furthermore, many authors have described microbial secondary metabolites as effective biocontrol agents against sapstain fungi. Such agents have included crude culture filtrates (Ejechi and Akpomede 1998; Moita et al. 2005) and, more recently, volatile organic compounds (VOCs) (Vanneste et al. 2002; Bruce et al. 2003; Wei-Wei et al. 2008; Evans et al. 2008).

Isolated microbial volatiles can be used against wood-pathogenic fungi in physical situations such as in the case of wood stores, or loaded onto a suitable carrier to treat infected trees in public gardens or pulp paper paste. In addition, identification of microbial volatiles will reveal novel chemical compounds that may be used as antagonists to wood-pathogenic fungi.

The objectives of this study were: (1) to isolate wood-pathogenic fungi from infected trees located in one of the most famous public gardens in Egypt; (2) to study the emission of volatiles from microbial isolates that have been demonstrated to be antagonists for wood-pathogenic fungi, and select the most active volatile producer; (3) to evaluate the effectiveness of volatile compounds emitted from the selected microorganism in inhibiting the growth and pigmentation of isolated pathogenic fungi growing on wood blocks; (4) to study the effect of ionizing radiation as a stimulating factor for volatiles produced by the selected microorganism; and (5) to trap and identify (by GC-MS) the volatile compounds emitted from the irradiated microorganism.

Materials and methods

Wood-pathogenic fungi

Infected wood samples were selected from infected trees located in El-Orman Garden, Giza, Egypt, and kept inside

sterilized plastic bags at 4°C until use. Wood-pathogenic fungi were isolated from infected wood samples and purified using standard isolation and purification methods. Three standard fungal strains known as wood-pathogenic fungi, i.e., *Aureobasidium pullulans* AUMC 101, *Botryodiplodia theobromae* AUMC 102 and *Polyporus squamosus* AUMC 133, were kindly provided by the Mycological Center, Faculty of Science, Assuit University, Egypt.

Volatile producers

A total of 17 local microbial isolates was selected from a preliminary study due to their antagonistic properties against pathogenic fungi. They were isolated from different sources, including soil, plant and waste oil and screened individually for VOCs production. They were categorized as follows:

- 1 Three bacteria: NC-B, NC-F and NC-S, isolated from soil, lettuce and bean, respectively.
- 2 Eleven yeasts: NC-A, NC-D, NC-E, NC-G, NC-H, NC-I, NC-M, NC-N, NC-O, NC-P and NC-R, isolated from wheat, garlic, soil, garlic, medical plant, guava, medical plant, soil, waste oil, grass and bean, respectively.
- 3 Three mold isolates: *Trichoderma atroviride* and *T. viride* were isolated from soil, whereas *Trichoderma* sp. was isolated from waste oil.

Identification of the most active VOC producer

After morphological characterization, the most active volatile producer was identified using the biochemical tests described in *Bergey's Manual of Systematic Bacteriology, vol.2* (Kandler and Weiss 1986) at the Micro Analytical Center, Cairo University, Egypt.

Volatile inhibition of wood-pathogenic fungi on artificial medium

The effect on the radial growth of wood pathogenic fungi of volatiles emitted from 17 antagonists was studied individually according to the procedure recommended by Bruce et al. (2003).

Volatile inhibition of wood-pathogenic fungi on wood blocks

Wood blocks (2 x 2 x 2 cm) were cut from the same timber of a common tree (*Delonix regia*) that exists abundantly in Egypt and dried at 70°C overnight. Dried blocks were sterilized by autoclaving at 121°C for 20 min, weighed and kept under vacuum conditions until use. Sterilized wood blocks were immersed in conidial suspensions of tested

wood-pathogenic fungi approximately 2×10^5 CFU/ml, aseptically reweighed, inoculated with a selected volatile producer and incubated at 30°C. After 3 weeks of incubation, inoculated wood blocks were evaluated for fungal growth by visual observation (Payne and Bruce 2001) and re-weighed using a sensitive balance (0.01 mg, AL104 Mettler Toledo®, Greifensee, Switzerland). The microbial load on the inoculated wood blocks was also determined before and after incubation, and the difference percentage was calculated. Control uninoculated wood blocks were used to determine the natural strength by means of a tensometer (Ejechi and Akpomedaye 1998), and the loss of strength in terms of resistance to compression of the treated blocks was also determined for blocks treated either with the selected volatile producer or uninoculated medium.

Irradiation studies

To study the effect of increasing doses of gamma radiation on VOC production, bacterial cells of *Lactobacillus plantarum* were irradiated at various dose levels (0, 0.5, 1.0, 1.5, 2.0, 2.5 kGy) using a Cobalt-60 model Indian gamma cell located at the National Center for Radiation Research and Technology, Atomic Energy Authority of Egypt. The dose rate at the time of the experiment was 4 kGy/h. The D_{10} value of the selected bacterium was also determined using bacterial cells irradiated up to 5 kGy.

Trapping and identification of volatiles emitted by the most active volatile producer (*Lactobacillus plantarum*)

VOCs produced by the most active producer (*Lactobacillus plantarum*) were collected using a setup suggested by Fernando et al. (2005) with slight modifications. The volatile trap was made of a glass plate (5 cm) containing 250 mg activated charcoal and sterilized at 350°C for 24–36 h before use. The bacterial isolate was smeared on a large glass plate (25 cm) with the volatile trap placed inside it. Emitted volatiles were then eluted using 0.5 ml methylene chloride.

VOCs were analyzed by GC (HP6890) interfaced with MS (HP5973) using the Wiley275.L database for MS identification of the GC components (Wiley-VCH, Weinheim, Germany). The column used was TR-5MS 5% phenyl polysil phenylene siloxane (internal dimension 30 m×0.25 mm×0.25 μm) with a MSD detector. The injector and detector temperatures were 250°C. The oven was temperature-programmed from 50 to 180°C at a constant rate of 5°C min⁻¹, with a final isothermal period of 10 min. The carrier gas was helium at a flow rate of 1 ml min⁻¹. The mass spectra of the unknown compounds were compared with those in the Wiley 275 L and NBS75KL libraries.

Statistical analysis

The significance of the data with respect to growth inhibition percentage of wood-pathogenic fungi and loss of wood blocks weight and strength was evaluated using one-way analysis of variance ANOVA. Differences were considered significant at a probability of $P < 0.05$.

Results and discussion

Wood is a valuable material in terms of both structure and micro-nutrients, and can be considered a good culture substrate for fungal attack. Using biological control agents in place of synthetic chemical compounds to prevent fungal colonization has become very acceptable in recent years. Several microorganisms, such as bacteria, yeasts and fungi, can be exploited as effective biological control agents. There is less risk associated with using microbial secondary metabolites, including soluble antibiotics, lytic enzymes and VOCs, rather than living microorganisms to control pathogenic fungi.

Any successful strategy for wood preservation must take into consideration the variation among fungi that attack wood. Some pathogenic fungi cause wood decay but others stain wood with permanent colors. This study focused on the seven wood-pathogenic fungi listed in Table 1. Three *Alternaria* spp. (W5, W16 and W18) were isolated from

Table 1 Origin of wood-pathogenic fungi tested in the present study

Origin	Wood-pathogenic fungus
Isolated from Mexican blue palm (<i>Brahea armata</i>), El-Orman Garden, Giza, Egypt	<i>Alternaria</i> sp. (W5)
Isolated from desert fan palm (<i>Washingtonia filifera</i>), El-Orman Garden, Giza, Egypt	<i>Alternaria</i> sp. (W16)
Isolated from Cabbage palm (<i>Sabal palmetto</i>), El-Orman garden, Giza, Egypt	<i>Alternaria</i> sp. (W18)
Provided kindly by Mycological Center, Faculty of Science, Assuit University, Egypt.	<i>Aureobasidium pullulans</i> AUMC 101
Provided kindly by Mycological Center, Faculty of Science, Assuit University, Egypt.	<i>Botryodiplodia theobromae</i> AUMC 102
Provided kindly by Mycological Center, Faculty of Science, Assuit University, Egypt.	<i>Polyporus squamosus</i> AUMC 133
Isolated from desert fan palm (<i>W. filifera</i>), El-Orman Garden, Giza, Egypt	Unidentified isolate (W15)

Brahea armata, *Washingtonia filifera* and *Sabal palmetto*, respectively. An unidentified isolate (W15) that was isolated from *W. filifera* as well as the standard strain *Polyporus squamosus* AUMC 133 have the ability to decay wood, while the remaining wood-pathogenic fungi tested are considered sapstain fungi.

Volatile inhibition of wood-pathogenic fungi on artificial medium

The present experiment aims to select a promising microbial candidate able to control wood-pathogenic fungi by VOC emission. To achieve this aim, 17 microbial local isolates were screened; the results of this screening experiment are presented in Figs. 1, 2 and 3. Figure 1 shows that the VOCs produced by *Trichoderma atroviride* had the greatest effect on wood-pathogenic fungi compared to other antagonist molds (*T. viride* and *Trichoderma* sp.). The radial growth of most tested wood-pathogenic fungi was reduced by less than 30% when exposed to volatiles emitted by *T. viride*. *Botryodiplodia theobromae* AUMC 102 was the fungal strain most resistant to VOCs emitted by the three tested molds, with growth inhibition percentages not exceeding 2%.

The results illustrated in Fig. 2 indicated that VOCs emitted by bacterial antagonists (NC-S) inhibited the radial growth of the tested wood-pathogenic fungi by more than 50%, with the inhibition ratio in some cases reaching nearly 90%. Figure 2 also clarified the pattern of specificity of volatiles against the wood-pathogenic fungi under investigation; hence, VOCs emitted by NC-S showed no inhibition effect on the growth of *Polyporus squamosus* AUMC 133, and VOCs produced by NC-F had a negative effect on growth of *Aureobasidium pullulans* AUMC 101 and the unidentified wood-pathogenic fungal isolate (W15).

Chaurasia et al. (2005) noted that the VOCs of many microorganisms have different effects on multiple different members of the ecological community. In vitro, these interactions range from almost complete inhibition of

mycelium growth to only small reductions in growth as well as mycelial and conidial morphological abnormalities. In the present study, the effect of VOCs on wood-pathogenic fungi was visualized as a reduction in their radial growth on artificial medium (tryptone soy agar). In the same manner, Wheatley et al. (1997) examined the growth characteristics of four wood-decaying fungi belonging to basidiomycetes when exposed to VOCs from *Trichoderma pseudokoningii* (T64) and *T. viride* (T60). They recorded no obvious morphological changes in the colonies exposed to VOCs. Out of 14 bacterial isolates screened for VOC production, 12 isolates completely inhibited the mycelial growth and sclerotial germination of *Sclerotinia sclerotiorum* SS33 (Fernando et al. 2005). On the other hand, Bruce et al. (2003) noted that growth of *Ophiostoma piceae* H & P Syd IMI 391990 and *Aureobasidium pullulans* (de Pary) Arnaud IMI 145194 increased significantly due to the presence of VOCs from some of the antagonists, but that growth stimulation was modest and did not exceed 15%. Exploiting VOCs as an additional nutrient source is interesting feature of bacilli in their ecological niche. Growth promotion of microorganisms and plants by the action of VOCs is a very active field of research (Ryu et al. 2004).

Of the seven wood-pathogenic fungi, *Polyporus squamosus* AUMC 133 was the most sensitive to volatiles emitted from yeast isolates (Fig 3). The growth inhibition percentage by VOCs produced by yeast isolate (NC-M) reached nearly 85%. Figure 3 also shows that VOCs emitted by the 11 yeast antagonists tested cannot be used to control *Botryodiplodia theobromae* AUMC 102 and the unidentified wood-pathogenic fungal isolate (W15). Bruce et al. (2003) noted that yeasts were generally less effective in producing growth inhibition through the action of their VOCs against wood-pathogenic fungi in comparison with bacteria.

Figure 3 also confirms the specificity of volatiles for wood-pathogenic fungi. During screening experiments, selectivity of VOCs action against the wood-pathogenic

Fig. 1 Growth inhibition percentages of wood pathogenic fungi exposed to volatile organic compounds (VOCs) produced by mold antagonists

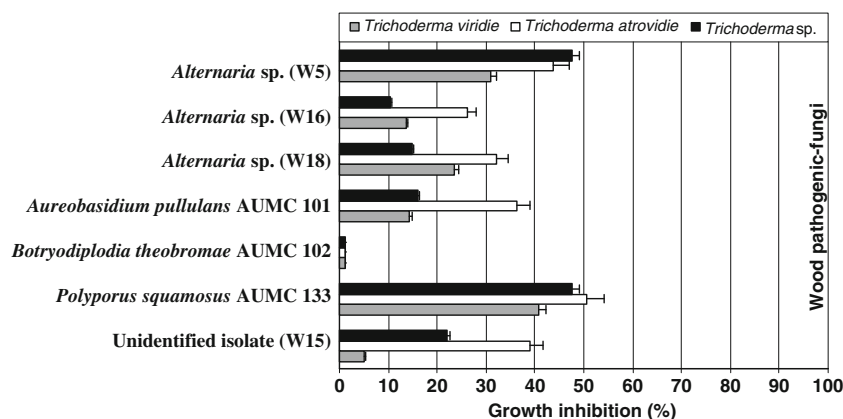


Fig. 2 Growth inhibition percentage of wood pathogenic fungi exposed to VOCs produced by bacterial antagonists

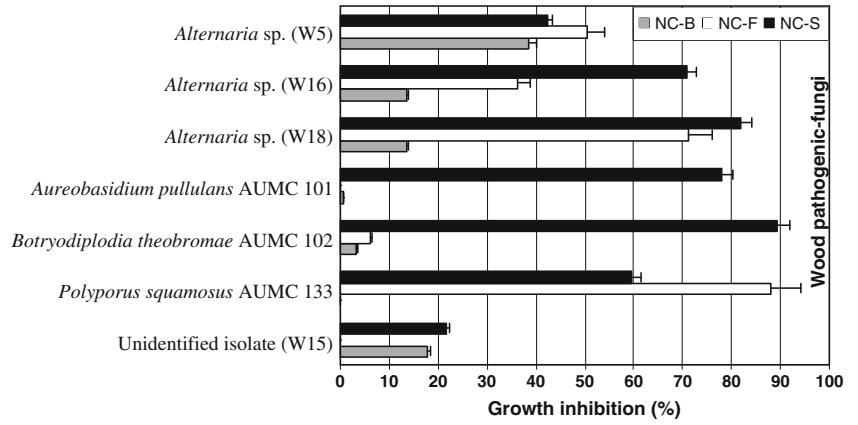
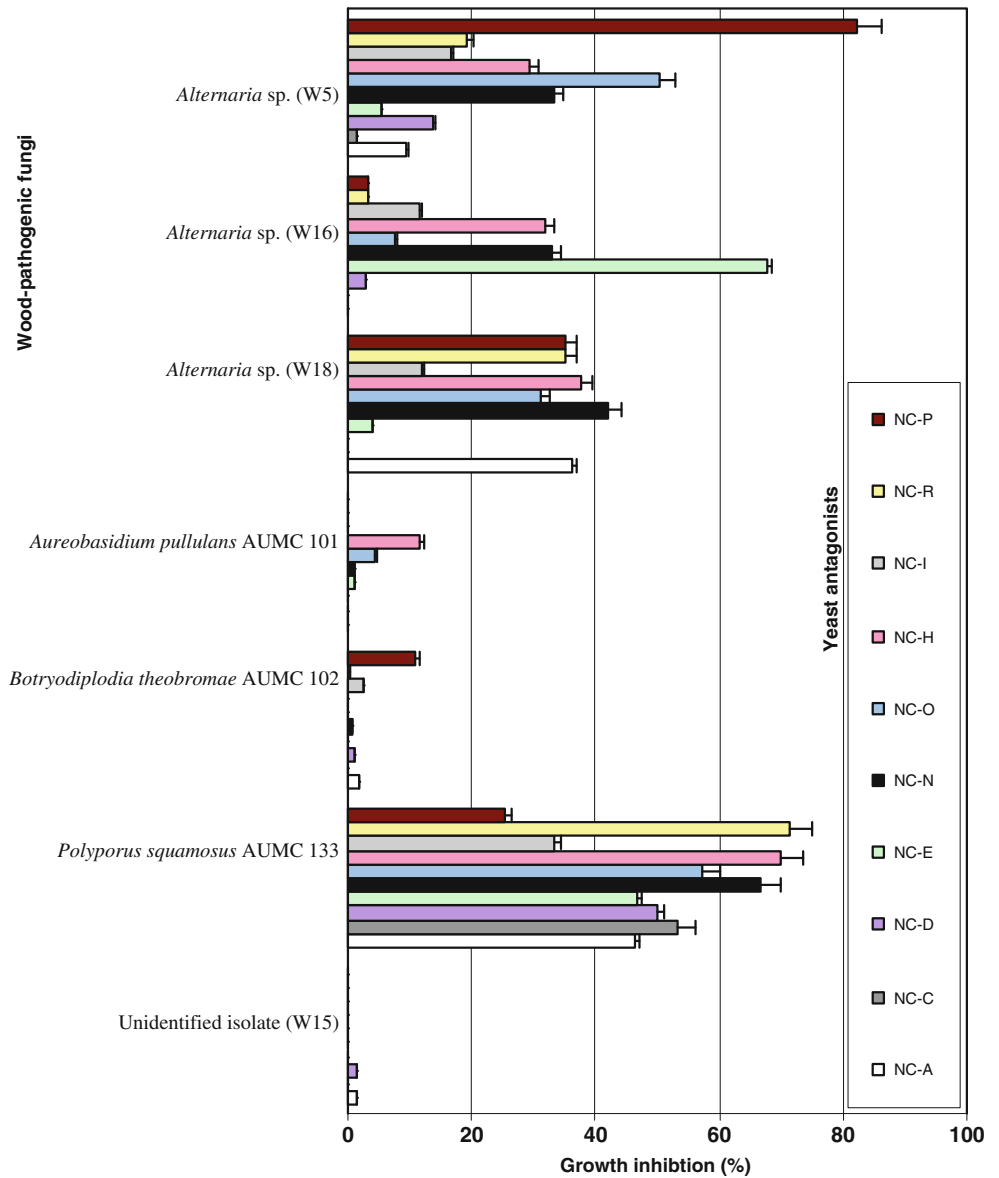


Fig. 3 Growth inhibition percentage of wood pathogenic fungi exposed to VOCs produced by yeast antagonists



fungi tested was very clear. Similarly, Wei-wei et al. (2008) noted species-specificity among different tested fungi. VOCs emitted from *Bacillus subtilis* G₈ inhibited the growth of *Botrytis cinerea* and *Cercospora kikuchii* chupp by more than 75%, while inhibition of *Rhizoctonia solani* was less than 46%. Bruce et al. (2003) showed that each sapstain fungus was inhibited by VOCs from at least one of the antagonists. VOCs produced by bacterial isolate B127 were much more selective in their action, causing more than 90% inhibition of *Sclerophoma pithyophila* (Corda) v. Höhn IMI 269217, but had little effect against *Aureobasidium pullulans* (de Pary) Arnaud IMI 145194 or either of the *Ophiostoma* spp. when the bacterium was grown on tryptone soya agar. This variation in antifungal activity reflects different sites of action and the diverse ability of fungi to detoxify secondary metabolites including VOCs.

Regarding pigmentation, volatiles produced by different antagonists (bacteria, mold and yeast) had no significant effect on pigmentation of wood-pathogenic fungi except *Botryodiplodia theobromae* AUMC 102 strain, the grey color of which turned to white when exposed to VOCs emitted from all tested antagonists, and *Aureobasidium pullulans* AUMC 101, whose reverse pigmentation was intensified in some cases. Bacterial isolate NC-S was selected as the most active VOC producers and was identified as *Lactobacillus plantarum*.

Volatile inhibition of wood-pathogenic fungi on wood blocks

Identical sterilized wood blocks were inoculated individually with the wood-pathogenic fungi under investigation. Table 2 shows the change in configuration between inoculated wood blocks exposed either to VOCs of the selected bacterial isolate *L. plantarum* (Treated) or to uninoculated medium (Control). It is worth mentioning that treated wood blocks were exposed to VOCs 2 days before being inoculated with pathogenic fungi since Schubert et al. (2008) noted that establishment of the antagonist in the wood substrate before a pathogen arrives appears to be a good strategy for a successful protection treatment.

The data in Table 2 show clearly that VOCs were very effective in inhibiting the growth of all tested wood-pathogenic fungi when grown on wood blocks, except those infected by *Botryodiplodia theobromae* AUMC 102 and *Aureobasidium pullulans* AUMC101. The growth of former fungus was inhibited to a lesser extent while the growth of latter fungus was clearly stimulated. In a similar experiment, Payne and Bruce (2001) used *Debaromyces hansenii* Y7036 cells to control Swan *Pinus sylvestris* timber. They found that *D. hansenii* cells reduced disfiguration caused by *Penicillium brevicompactum* Dierckx (IMI 321324), *Aspergillus niger* v. Tieghem (IMI 329399), and

Table 2 Changes in configuration of wood blocks estimated by visual observation after 21 days of incubation

Wood pathogenic fungi	Visual observation ^a	
	Treated ^b	Control ^c
<i>Alternaria</i> sp. (W5)	0	5
<i>Alternaria</i> sp. (W16)	0	5
<i>Alternaria</i> sp. (W18)	0	5
<i>Aureobasidium pullulans</i> AUMC 101	3	5
<i>Botryodiplodia theobromae</i> AUMC 102	5	4
<i>Polyporus squamosus</i> AUMC 133	1	5
Unidentified isolate (W15)	1	5

^a Visual observation was based on classification score of Payne and Bruce (2001): 5 severe disfiguration over the majority of the wood block surface; 4 substantial disfiguration intensity over a large part of the wood block surface; 3 moderate disfiguration intensity over at least 20% of wood block surface; 2 slight production of pigmented spores or hyphae over more than 10% of the block surface; 1 slight production of pigmented spores or hyphae over less than 10% of the block surface; 0 clean to the naked eye, unaffected by fungal growth

^b Wood blocks inoculated by pathogenic fungi and exposed to VOCs

^c Wood blocks inoculated by pathogenic fungi and not exposed to VOCs

Cladosporium herbarum (Pers.) Link (IMI 299105) added to autoclaved wood blocks and by fungi present naturally in undried unsterilized blocks in comparison to control blocks.

It should be noted that there was a difference in the growth inhibition pattern of wood-pathogenic fungi between those inoculated on solid artificial medium and those inoculated on wood blocks by the action of volatiles; this difference was attributed to the variation in nutrient content between enriched artificial medium and wood, which has a low nutrient content.

Obvious fungal growth appeared around all the control wood blocks (inoculated by wood-pathogenic fungi but not exposed to VOCs). When hyphal mycelia of *Alternaria* spp. were removed from control wood blocks, the blocks were clearly stained with black melanin pigment (data not shown). Similarly, Schubert et al. (2008) mentioned that the reduction in growth of wood-degrading fungi is associated mainly with inhibition of melanin synthesis, since melanin is the primary defense system of pathogenic fungi against poor environmental conditions. On the other hand, the efficiency of VOCs in controlling tested wood-pathogenic fungi could be attributed to changes in the organic environment of the wood cell, which appeared to be less conducive to the development of decay fungi (Brown and Bruce 1999).

The data shown in Table 3 illustrate the great variation in dry weight between wood blocks treated by volatiles emitted from *L. plantarum* and control blocks. Wood blocks inoculated with *Alternaria* spp. (W5, W16 and

Table 3 Change in dry weight percentage of wood blocks

Wood pathogenic fungus	Change in dry weight%	
	Treated ^a	Control ^b
<i>Alternaria</i> sp. (W5)	-3.00±0.3	-4.67±0.2
<i>Alternaria</i> sp. (W16)	-2.30±0.2	-34.67±2.2
<i>Alternaria</i> sp. (W18)	-2.33±0.2	-26.68±1.5
<i>A. pullulans</i> AUMC 101	+7.67±0.4	+30.67±3.1
<i>B. theobromae</i> AUMC 102	-2.67±0.2	-2.66±0.1
<i>P. squamosus</i> AUMC 133	-2.40±0.3	-36.00±2.4
Unidentified isolate (W15)	+4.00±0.1	+49.33±4.1

^a Wood blocks inoculated by pathogenic fungi and exposed to VOCs

^b Wood blocks inoculated by pathogenic fungi and not exposed to VOCs

W18) as well as *P. squamosus* AUMC 133 showed a loss in dry weight. The loss percentage reached nearly 30% in control blocks but did not exceed 5% in treated blocks. Similarly, Schubert et al. (2008) stated that wood decay fungi have distinctive differences in their potential to decompose wood; *Kretzschmaria deusta* (Hoffm.) P.M.D. Martin 271098.1 caused the highest mean dry weight loss (11.7%), followed by the *Ganoderma* species (8.2%), *P. squamosus* (Hud.:Fr.) Fr. 291101.2 (5%), whereas *Inonotus hispidus* (Bull.:Fr.) Karsten. 200792.1 caused the lowest mean weight loss (3.6%). Only negligible weight loss (1.6%) was recorded from wood samples (Münchh.) that were merely treated with *Trichoderma* spp. i.e. treatment of wood samples with conidial suspensions of *Trichoderma* spp. significantly reduced the mean dry weight loss from all wood decay fungi. The dry weight loss could be explained partially by the degradation of readily accessible carbohydrates within parenchyma cells and pits (Schubert et al. 2008).

On the other hand, wood blocks inoculated with *L. pullulans* AUMC101 and unidentified bacterial isolate (W15) showed an increase in dry weight. The increases in control blocks were 30.67% and 49.33%, respectively, but less than 8% in treated blocks. This increment could be attributed to intensive fungal growth on control wood blocks.

Enumeration of conidia dislodged from wood surfaces by careful scratching and shaking for a long time (3 h) provided an acceptable indication of total viable counts present on the surface of wood blocks. The number of conidia dislodged from control wood blocks was represented as N_0 , while those dislodged from treated wood blocks was represented as N . Figure 4 revealed the $\log N/N_0$ for seven tested wood-pathogenic fungi. The $\log N/N_0$ for *L. pullulans* AUMC101 and unidentified bacterial isolate (W15) were -0.50 and -0.52, respectively. On the other hand, $\log N/N_0$ for the remaining tested pathogenic fungi were all approaching -5.0. Exposing wood-

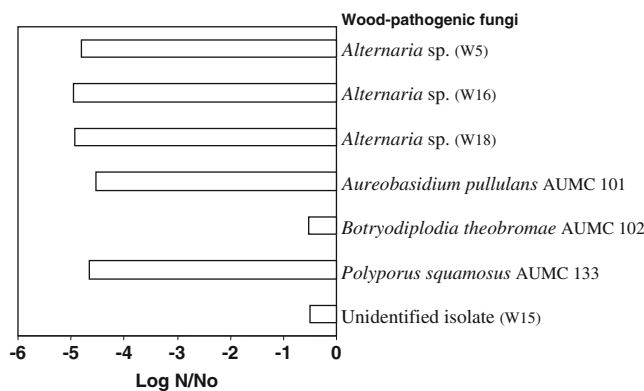


Fig. 4 Effect of VOCs on the viability of wood-pathogenic fungi inoculated on wood blocks

pathogenic fungi to VOCs emitted by *L. plantarum* in this experiment created poor environmental conditions for fungal survival. Ejechi and Akpomedaye (1998) reported that every microbial strain has a repair mechanism to overcome hostile environments. When microorganisms use such repair systems, they exhausted their energy supply, which can lead to death. On the other hand, Wheatley et al. (1997) concluded that the protective effect of VOCs on wooden structures might be produced by their direct actions on the degradative enzymes of the pathogenic fungi rather than their fungal toxicity.

Regarding loss of wood strength percentage, control wood blocks showed a greater loss of wood strength compared to untreated blocks (Fig. 5). The loss in wood strength was between 20% and 40% for wood blocks not exposed to VOCs emitted by *L. plantarum* while wood strength decreased by only 5% in treated wood blocks. A similar reduction in wood strength by the activity of wood-deteriorating fungi *Gloeophyllum sepiarium*, *Gloeophyllum* sp., *Trametes* sp. and *Pleurotus* sp. isolates, was previously recorded by Ejechi and Akpomedaye in 1998 using culture filtrate of *Proteus* sp.

The decrease in wood strength due to the degrading action of pathogenic fungi is caused by the rapid depolymerization of hemicellulose and cellulose—the principle structural carbohydrates of wood. This depolymerization is

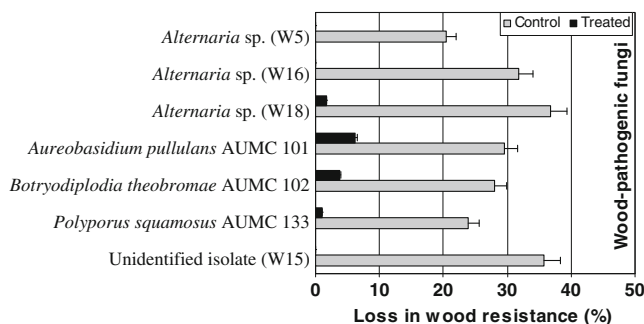


Fig. 5 Effect of VOCs on wood-strength (compressive strength)

thought to be a non-enzymatic, chemical process involving the generation of highly reactive, low-molecular weight oxidative agents such as oxalic acid (Micales 1997).

Effect of gamma irradiation on VOC production by *Lactobacillus plantarum*

Irradiation studies showed that the D_{10} value for the most active volatile producer *L. plantarum* was 1.04 kGy (Data not shown). Volatiles emitted from *L. plantarum* cells previously exposed to different doses of gamma radiation (0.5, 1.0 and 1.5 kGy) inhibited the growth of the tested pathogenic fungi at nearly same percentage achieved by VOCs emitted from unirradiated *L. plantarum* cells (Fig. 6). The optimal radiation dose, causing the maximum growth inhibition for the majority of tested wood-pathogenic fungi ($P < 0.05$), was found to be 2 kGy. The increase in growth inhibition was nearly 10%. Thereafter, growth inhibition was retarded clearly when using VOCs emitted from *L. plantarum* cells previously irradiated with 2.5 kGy. Volatiles emitted from *L. plantarum* cells previously irradiated with 0.5 kGy and 2.0 kGy showed the maximum growth inhibition percentages for *Alternaria* sp. W18 (95.18%) and *Botryodiplodia theobromae* AUMC 102 (92.08%), respectively.

To the best of our knowledge, this is the first study to test the effect of radiation on microbial volatiles. For plant volatiles, some studies have reported increase activity due

to the action of radiation; others note that there was no systematic change in volatile profile when plants were irradiated by different doses of gamma radiation, although some investigators report a negative effect of radiation on plant volatiles (Piggott and Othman 1993; Alcarde et al. 2004; Seo et al. 2007).

Trapping and identification of volatiles emitted by the most active volatile producer (*Lactobacillus plantarum*)

The GC-MS analysis of volatile compounds eluted from charcoal traps and subsequent database search yielded 13 compounds, which included a range of esters, ketones, aldehydes, aliphatic alkanes and phenols (Table 4). Five major organic compounds were identified as 1,2-benzenedicarboxylic acid, dibutyl ester; isopropyl myristate; n-nonadecane; octadecane and n-eicosane. Their intensity in volatiles profile was 31.42%, 25.30%, 7.77%, 7.64% and 7.0%, respectively (Fig. 7).

Isopropyl myristate is a fatty acid ester that exhibits high toxicity against mainly Gram-negative microorganisms (Cardoso et al. 2006). Hao et al. (2004) isolated the thermophilic bacterium strain TH-2 from oil field in East China that had the ability to produce volatile fatty as 1,2-benzenedicarboxylic acid-bis ester.

It should be noted that the volatile identification accomplished in this study does not reflect the complete spectrum of volatiles emitted from the selected bacterium

Fig. 6 Growth inhibition percentage of wood pathogenic fungi exposed to VOCs produced by *Lactobacillus plantarum* cells previously irradiated with increasing doses of gamma radiation

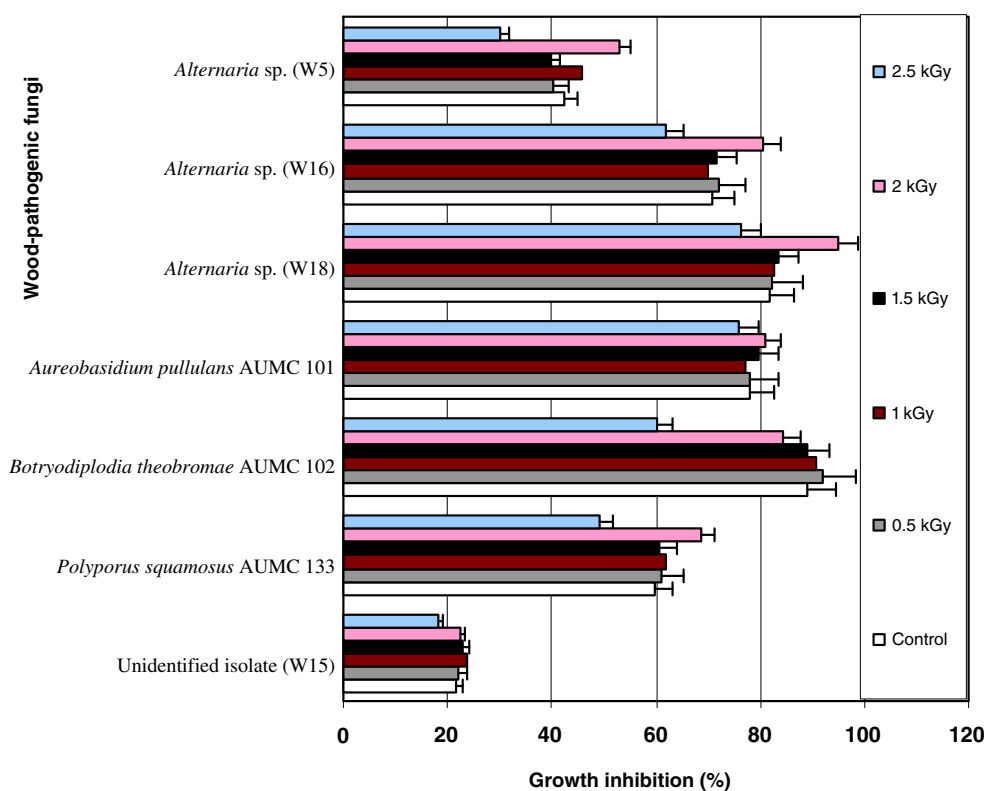


Table 4 Profile of organic compounds produced by *L. plantarum* cells previously irradiated by 2 kGy, tapped in activated charcoal and identified by GC/MS

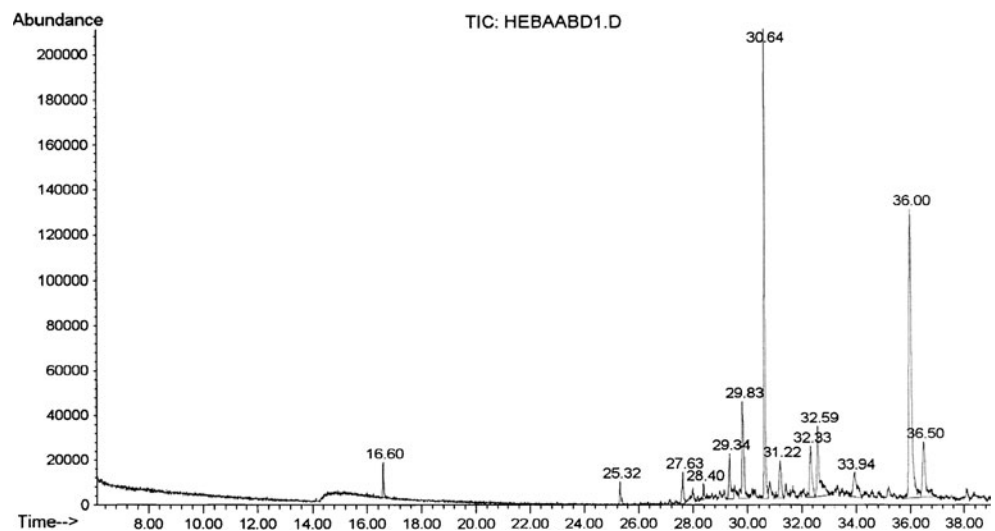
Peak No.	RT (min)	Intensity %	Name of compound
1	16.60	1.46	Goitrin
2	25.30	1.16	Propionic acid, 2-methyl-, 1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester
3	27.63	1.97	Hexadecane, 2,6,10- trimethyl
4	28.39	1.17	2,4-Diphenyl-4-methyl-2 (Z)- pentene
5	29.34	2.94	2-Decanone
6	29.83	7.64	n-Octadecane
7	30.64	25.30	Isopropyl myrstate
8	31.22	3.67	2-Isopropyl-5-oxohexanal
9	32.33	4.68	1,2-Benzenecarboxylic acid, bis ester
10	7.77	32.59	n-Nonadecane
11	33.94	3.83	8-Pentadecanone
12	36.00	31.42	1,2-Benzenedicarboxylic acid, dibutyl ester
13	36.50	7.00	n-Eicosane

Lactobacillus plantarum. This is could be due to one of several reasons:

- 1 The aeration system used during the study.
- 2 Volatile composition can change greatly during the incubation period: one volatile compound can be converted to another, or consumed, with increasing incubation period. Ryu et al. (2004) noted that butanediol and acetoin are typically synthesized and excreted by bacilli upon growth on simple carbon sources as glucose. After glucose consumption, bacilli are able utilize butanediol and acetoin as additional sources of carbon, i.e., these volatile compounds serve as external nutrient sources.
- 3 The type and structure of trapping system.

- 4 Identification of small minor volatile compounds with low molecular weight (< 50) can be interfered by the solvent during GC separation.
- 5 The present study evaluated VOCs composition of *L. plantarum* using one GC-MS analysis system, i.e., the analysis process was performed using only one column type (TR-5MS); other analytical columns may be able to separate other compounds and reveal other profiles.
- 6 VOCs vary greatly in chemical composition; they may belong to different classes such as alkanes, alkenes, alcohols, esters, ketones and acids. Each class can be identified by different programmed gas chromatography.

Many previous works suggested tens of VOCs that have a determinant effect on pathogenic fungi. Five organic

Fig. 7 Organic compounds produced by *L. plantarum* cells previously irradiated by 2 kGy

compounds—2-propanone, 2-methyl-1-butanol, heptanal, octanal and decanal—were produced by *Trichoderma* spp. isolates in significantly higher proportions when grown on yeast malt medium and therefore may be responsible for fungal growth inhibition (Wheatley et al. 1997).

Schöller et al. (1997) detected VOCs emitted from five Gram-negative organisms. For *Pseudomonas putida* KT 2442 and *P. aeruginosa* ATCC 10145, the major volatile compound determined from relative peak areas was dimethyl disulfide, whereas isoprene and 1-undecene were dominant for *P. fluorescens* R2F; isoprene and 2-nonanone for *Lactobacillus plantarum* liquefaciens SM 1302; and isoprene, dimethyl disulphide and 3-methyl-1-butanol for *Enterobacter cloacae* SM 639. Such results indicate that the three examined *Pseudomonas* spp. isolates produced only hydrocarbons and organosulfur compounds but not oxygenated VOCs. Analysis of volatiles samples emitted from *E. cloacae* SM 639 and *L. plantarum* liquefaciens SM 1302 evaluated oxygenated derivatives including branched alcohols from *K. cloacae* SM 639 and methyl ketones from *L. plantarum* liquefaciens SM 1302. Humphris et al. (2001) separated heptanal and octanal as anti-fungal chemicals and confirmed that they were particularly toxic to wood decay fungi even at very low concentrations.

Kai et al. (2007) noted the particularity of VOCs produced by *Serratia* spp. β -phenylethanol, trans-9-hexadecen-1-ol, dimethyl trisulfide and benzyl nitrile were the major compounds identified from *Serratia* spp. Gu et al. (2007) found that phenol, 2-octanol, 2-nonanone and 2-undecanone displayed 100% nematocidal activities to both the free-living nematode *Panagrellus redivivus* and the pine wood nematode *Bursaphelenchus xylophilus*. Wei-wei et al. (2008) suggested that *Bacillus subtilis* G₈ emitted more than one bioactive volatile compound and that they belong to alkyls, alcohols, esters, ketones, acids, amine, oxime, phenols and heterocycling compounds. These compounds can be applied to control of pathogens in greenhouses.

Conclusion

Microbial volatiles have been used to control wood-pathogenic fungi in huge forests located in England, Ireland and New Zealand. To the best of our knowledge, this is the first report to evaluate microbial volatiles to control wood-pathogenic fungi in Egypt. This study succeeded in identifying new classes of VOCs (1, 2-benzenedicarboxylic acid, dibutyl ester and isopropyl myrstate) emitted from local microbial isolates that are especially effective against sapstain fungi in their natural habitat. These volatiles could be used as fumigants to protect wooden structures in big stores. Enhancement of volatiles production by gamma radiation is

a very promising result, and gives a good indication that other physical methods could also be used to achieve this aim.

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