

Preliminary evaluation of certain benzylidine and pyrazole derivatives against wood decay fungi

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ABSTRACT

Three benzylidine derivatives; dibenzylidineacetone (1,5-diphenyl penta-1,4-dien-3-one) (1); dibenzylidine acetylacetone (1,7-diphenyl hepta-1,6-dien-3,5-dione) (2) and benzylidine acetophenone (1,3-diphenyl propen-3-one) (Chalcone) (3) as well as three pyrazole derivatives; 3,5-dimethylpyrazole (4), 3-methyl-1-phenylpyrazol-5-one (5) and 3-methyl-1-(2,4-dinitrophenyl)-pyrazol-5-one (6) were evaluated for their fungitoxic effects against the white rot fungus *Coriolus versicolor* and the brown rot fungus *Gloeophyllum trabeum*. *In vitro* measurements, relationship between the chemical structure and the fungitoxicity of both studied derivatives was conducted. Compound 6 was the most active derivative with IC₅₀ of 19.6 µg/ml against *C. versicolor* and 112.7 µg/ml against *G. trabeum*. The two active compounds (compounds 3 and 6) were tested *in vivo* on poplar wood (*Populus nigra*) and Scots pine sapwood (*Pinus sylvestris*) specimens using mini-blocks technique. Different concentrations of these compounds were examined using *C. versicolor* and *G. trabeum* for leached and unleached wood samples. After six weeks of fungal exposure, compound 6 proved a significant effectiveness against both tested fungi with mass loss ± SE equaled 13.67% ± 0.54 and 14.47% ± 0.34 in case of *C. versicolor* and *G. trabeum*, respectively in the unleached specimens comparing with 41.27% ± 0.43 and 41.53% ± 0.42 in control. These mass loss percentages were 23.87% ± 0.61 and 24.80% ± 0.35 in case of compound 3 in the same array. Their performance in leached samples was detected for both tested compounds with percentage of mass loss exceeded 25% for the two wood specimens under investigation.

Keywords: Benzylidine, pyrazole, wood preservation, *Coriolus versicolor*, *Gloeophyllum trabeum*

INTRODUCTION

Wood as a non homogeneous, fibrous material is the only renewable material resource and one of the first construction materials used. However, because wood is a natural organic material, it is at risk of biodeterioration. To ensure wood soundness and long service life in hot and humid climates or wherever wood comes into contact with the ground or water, wood must be protected from its natural predators. The incidence of fungal infections has increased and spread among different life forms. Wood decay fungi as white-rot fungi, which mainly metabolize lignin and the brown-rot fungi that digest cellulose in plant cell walls are destructive agents of forestry wealth and wood industry. The mechanism of wood degradation by white and brown rot fungi have been demonstrated in many literatures (Xu and Goodell, 2001 and Cohen *et al.*, 2004). In this context, it was found that iron, hydrogen peroxide, biochelators and oxalate play important roles in cellulose degradation by brown-rot fungi. Wang and Gao (2002 and 2003) proved that *G. trabeum*, the brown rot fungus exhibited its effect by a low molecular peptide named Gt chelator that reduces Fe^{+3} to Fe^{+2} . It drives H_2O_2 generation via a superoxide anion intermediate forming hydroxyl radical in the presence of O_2 . It oxidizes the cellulose and disrupts the inter- and intra-hydrogen bonds in cellulose chains making it accessible for further enzymatic hydrolysis. Suzuki *et al.* (2006) added that this process was mediated by two extracellular hydroquinones, 2,5-dimethoxyhydroquinone (2,5-DMHQ), 2,5-dimethoxy-1,4-benzo-quinone (2,5-DMBQ) and 4,5-dimethoxycatechol (4,5-DMC). Several wood decay fungi degraded the used fungicides and compound as wheat straw cultures of the brown rot fungi *G. striatum* and *G. trabeum* degraded 2,4-dichlorophenol and pentachlorophenol (Fahr *et al.*, 1999). Iron-containing liquid cultures of the brown-rot basidiomycete *G. striatum* degraded 2-fluorophenol. (Kramer *et al.*, 2004).

A wide range of synthesized or natural organic as well as inorganic fungicides are currently in use for wood industry preservation (Barnes and Murphy 1995). For instance, *Cinnamomum osmophloeum* Kaneh. is a hardwood possesses significant antifungal activity. Its leaves essential oils of cinnamaldehyde type and cinnamaldehyde/cinnamyl acetate type had an

excellent inhibitory effect against white-rot fungi, *Trametes versicolor* and *Lenzites betulina* and brown-rot fungus *L. sulphureus*. Cinnamaldehyde possessed the strongest antifungal activities with IC₅₀ values of 73, 74 and 73 µg/ml against *C. versicolor*, *L. betulina* and *L. sulphureus*, respectively (Wang *et al.*, 2005 and Cheng *et al.*, 2006) in comparison with other constituents. Octyl gallate antioxidant exhibited an excellent antifungal property with IC₅₀ values of 0.47, 0.16, 0.24 and 0.04 mM against *L. betulina*, *T. versicolor*, *G. trabeum* and *Laetiporus sulphureus*, respectively. It enhanced protection when combined with cinnamaldehyde (Hsu *et al.*, 2007). Boric acid showed a low weight loss in (*Postia placenta* and *Coniophora puteana*) before leaching. It gave synergistic effects when mixed with tall oil derivates and at 2% with the tall oil derivate consisting of 90% free resin acids gave < 3% weight loss (Temiz *et al.*, 2008).

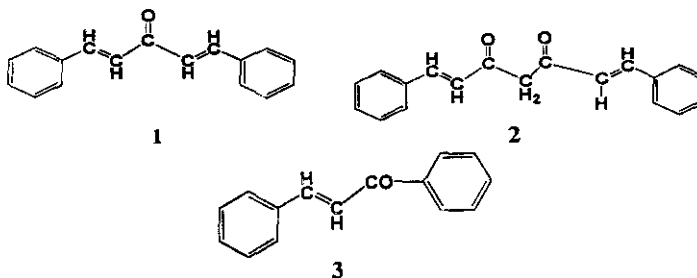
The need for new effective fungicides has been increased and several nitrogen containing heterocyclic compounds have been using for biological activity. Mares *et al.* (2006) showed the antifungal activity of some pyrazole derivatives against the causal agent of rice blast disease, *Magnaporthe grisea*. On the other hand, Abdel-Aty (2007) revealed the fungicidal activity of some prepared pyrazole derivatives against some plant pathogenic fungi.

Due to the importance of the wood decay fungi and the activities of benzylidine and pyrazole derivatives, three benzylidine derivatives and three pyrazole derivatives were studied for *in vitro* and *in vivo* controlling the white rot fungus, *C. versicolor* and the brown rot fungus, *G. trabeum*. as economically wood decay fungi.

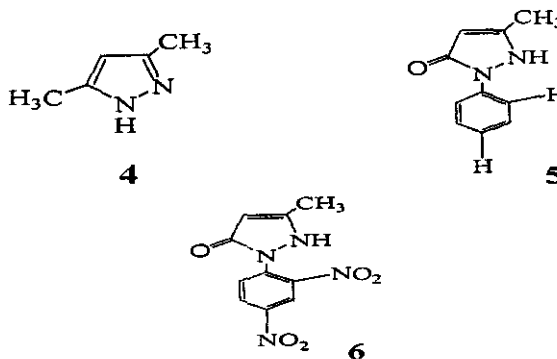
MATERIALS AND METHODS

Tested Compounds: The tested benzylidine derivatives; 1,5-diphenylpenta-1,4-dien-3-one (1), 1,3-diphenyl propen-3-one (Chalcone) (2) and 1,7-diphenylhepta-1,6-dien-3,5-dione (3) were earlier synthesized, purified and confirmation for their structure by Abdel-Aty (2004). The studied pyrazole derivatives (3,5-dimethylpyrazole (4), 3-methyl-1-phenylpyrazol-5-one (5) and 3-methyl-1-(2,4 dinitrophenyl)pyrazol-5-one (6)) were also prepared, purified and identified by Abdel-Aty (2007). These compounds are shown below:

Benzylidene derivatives



Pyrazole derivatives:



Tested fungi: Two wood decay fungi, *Coriolus versicolor* (Linnaeus) Quélet, strain CTB 863 and the brown rot fungus, *Gloeophyllum tarbeum* (Persoo ex Fries) Murrill, strain BAM Ebw. 109 (provided from Laboratory of Wood Technology, Ghent University, Belgium), were used in this study. Fungal cultures were grown and maintained on 90 mm Petri dishes containing 20 ml of an agar medium (Malt Extract Agar (MEA); 4 % malt extract, 2 % agar) at 27°C and 75 % relative humidity (R.H).

Fungitoxic effects:

In vitro antifungal assay: The antifungal assessment of the tested compounds was conducted using a mycelial radial growth inhibition technique (El Ghaouth

et al., 1992) against *C. versicolor* and *G. trabeum* grown on an agar medium. This medium is prepared by dissolving a mixture of 40 g of malt extract and 20 g of agar in 1000 ml of boiling distilled water and stirring until complete dissolving. Growth medium was autoclaved for 15 min at 121 °C and 103.4 kPa (15 psi). Each compound was dissolved in dimethylsulfoxide (DMSO) and finally added to the medium at 10, 50, 100, 200, 300, 500, 1000, 1500, 2000 and 3000 µg/ml. The medium was poured into Petri dishes (90 mm diameter) providing a minimum depth of 3-4 mm and left in a conditioning room at 27 °C for cooling. All the additions were done under aseptic conditions. After solidification, a disc inoculum (7 mm in diameter) of each tested fungus was located in center of the sterile culture medium in the Petri-dish. Control in the presence of the calculated volume of dimethylsulfoxide only to be 1 % as its final concentration was concurrently conducted. All cultures were incubated in a conditioning room at 27°C and 75% R.H. For the mycelial radial growth determination, the results were recorded by measuring the diameter of the hyphal growth in each Petri-dish when the growth of the untreated fungi completely covered the surface of its Petri-dish, the treatments were measured. The percent of inhibition of the hyphal growth were calculated according to Topps and Wain formula (1957). IC₅₀ values (the concentration caused 50% inhibition of the hyphal growth) with corresponding 95% confidence limits were estimated by probit analysis (Finney, 1971). Significance was elucidated through three-way analysis of variance (ANOVA) and Student-Newman-Keuls Test.

***In vivo* antifungal assay:** The most active compounds which exhibited high effect as *in vitro*, were chosen and tested *in vivo* using the Mini block technique at 0.5, 1.0, 5.0 and 10 fold of its IC₅₀ value (determined from the *in vitro* screening test). The compounds were dissolved in 40 % aqueous ethanol. Mini-block specimens (10×5×30 mm) from poplar wood (*Populus nigra*) and Scots pine sapwood (*Pinus sylvestris*) are used. Six blocks were used in each treatment. These wood blocks were oven dried, weighed, and vacuumed for 20 min., then impregnated with the respective toxic solutions. The samples were then wiped with a tissue paper before the weight was recorded for control of uptake. Retention of the applied compounds in the blocks was determined as kg/m³. The treated wood specimens were then conditioned for two weeks. Three of the six wood blocks in each treatment were submerged in distilled

water and subjected to vacuum for 20 minutes to impregnate the blocks followed by soaking two hours in 300 ml of water for the three blocks. This soaking water was changed daily for three successive days. The washed three blocks were used as three replicates for the leached treatment at each concentration and the toxicity was evaluated in both un-leached and leached wood blocks. Upon drying all wood blocks were steam-sterilized and the poplar (hardwood) samples were exposed to the white rot fungus, *C. versicolor* while, Scots pine sapwood (softwood) samples were exposed to the brown rot fungus, *G. trabeum* in the test medium plate as these fungi naturally prefer. Both leached and un-leached wood blocks of the two wood specimens were individually treated. Control (untreated wood blocks) was concurrently conducted. The test samples were incubated at 27°C and 75% relative humidity (R.H). Results of mass loss have been recorded after 6 weeks for treated and untreated samples (Mohareb *et al.*, 2002).

RESULTS AND DISCUSSION

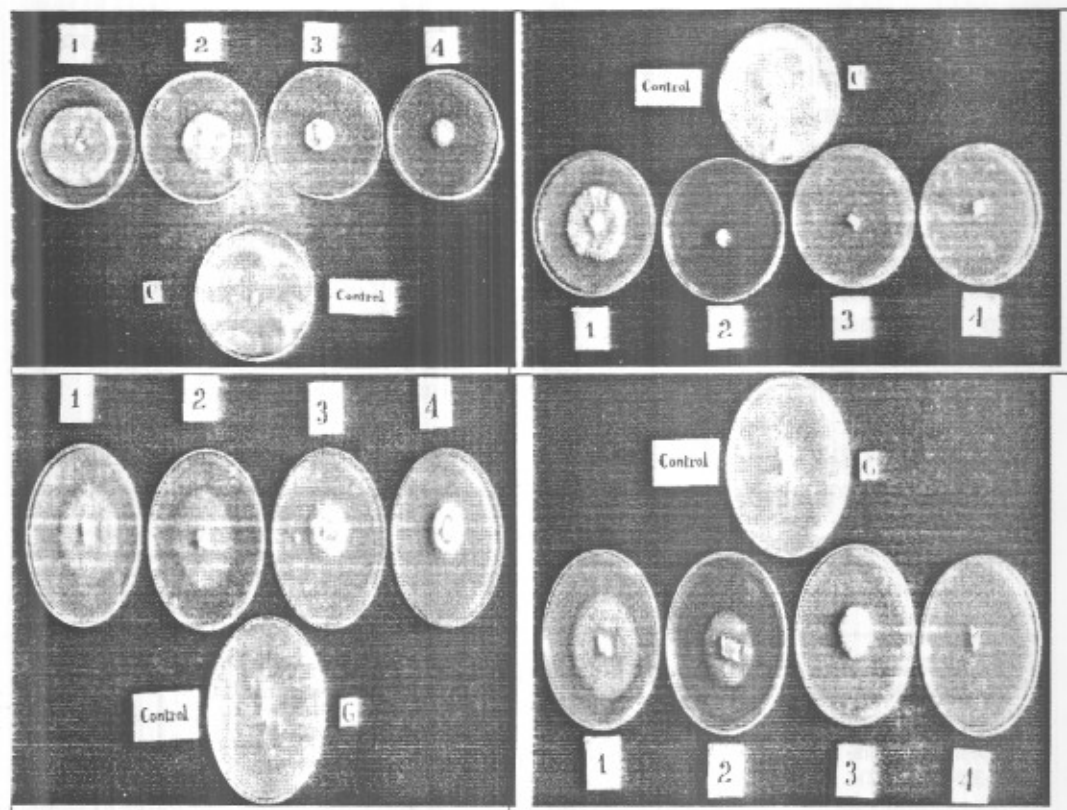
***In vitro* antifungal activity:** Table (1) represents the fungitoxic effects of the tested compounds against the treated fungi shown as IC₅₀ and 95% confidence limit of each compound. These effects were dependent on their concentrations, chemical structures and the treated fungus. Regarding the chemical structure, compound 1 exhibited its fungitoxicity with IC₅₀ of 295.4 and 976.9 µg/ml against *C. versicolor* and *G. trabeum*, respectively. While, the toxicity was diminished due to presence of the CH₂CO- moiety in the compound 2, with IC₅₀ of 317.1 and 1995.4 µg/ml in case of the two studied fungi. Fungitoxicity was more than three times with IC₅₀ of 84.5 µg/ml and more than nine times with IC₅₀ of 103.9 µg/ml against *C. versicolor* and *G. trabeum*, respectively due to absence of the -CH=CH- moiety in the compound 3. The studied pyrazole derivatives affected the two treated fungi differently according also to their structure differences. Compound 4 was less effective against the tested fungi with IC₅₀ values of 867.7 and 944.8 µg/ml against *C. versicolor* and *G. trabeum*, respectively. Substitution with 1-phenyl moiety and changing to the pyrazol-5-one ring in compound 5 enhanced the fungicidal activities with IC₅₀ values of 744.2 and 632.4 µg/ml against the tested fungi. Higher enhancement was achieved by replacing the substituted 1-phenyl ring with 2,4-dinitrophenyl moiety in compound 6 that appeared to be the most active derivative with IC₅₀

of 19.6 µg/ml against the white rot fungi and 112.7 µg/ml against the brown rot fungi. In general, as a descriptive point of importance, significantly *C. versicolor* appeared to be more sensitive than *G. trabeum* as their general mean \pm SE of mycelium growth inhibition percents were 39.18 ± 3.12 and 32.7 ± 2.58 , respectively. Additionally, the most effective compound was compound 6 followed by compound 3, exceeded the boric acid which was used as a standard. They caused mean mycelium growth inhibition percents of 61.0, 46.9 and 40.9%, respectively. While, the other tested compounds were less effective than the standard, their activities were varied as a function of both their chemical structures and the tested fungus. The inhibitory effects of the active compounds against hyphal growth of the tested fungi are shown in Figure (1).

Table (1). Fungicidal effects of certain benzylidine and pyrazole compounds on *Coriolus versicolor* and *Gloeophyllum trabeum* fungi; shown as IC₅₀ in µg/ml

Tested Compound	<i>Coriolus versicolor</i>				<i>Gloeophyllum trabeum</i>			
	IC ₅₀ (95%CL)	Slope \pm S.E	χ^2	df	IC ₅₀ (95%CL)	Slope \pm S.E	χ^2	df
Benzylidine compounds								
1,5-Diphenylpenta-1,4-dien-3-one (1)	295.4 ^c (250-350)	1.51 \pm 0.019	5.9	4	976.9 ^b (796-1198)	1.17 \pm 0.02	6.8	4
1,7-Diphenyl hepta-1,6-dien-3,5-dione (2)	317.1 ^b (263-383)	1.35 \pm 0.02	0.4	4	1995.4 ^a (1452-2747)	0.93 \pm 0.02	6.4	4
1,3-Diphenylpropen-3-one (Chalcone) (3)	84.5 ^e (57.1-124.4)	0.86 \pm 0.01	4.3	4	103.9 ^g (66.6-160.8)	0.73 \pm 0.01	2.5	4
Pyrazole compounds								
3,5-Dimethylpyrazole (4)	867.7 ^a (759-992)	1.96 \pm 0.026	3.0	4	944.8 ^c (784-1138)	1.3 \pm 0.021	2.6	4
3-Methyl-1-phenylpyrazol-5-one (5)	744.2 ^b (655-846)	2.18 \pm 0.028	0.5	4	632.4 ^d (549.6-728)	2.06 \pm 0.026	3.9	4
3-Methyl-1-(2,4-dinitrophenyl)-pyrazol-5-one (6)	19.6 ^f (16.7-22.9)	2.16 \pm 0.028	9.1	4	112.7 ^f (88.9-142.7)	1.17 \pm 0.01	3.9	4
Standard Compound								
Boric acid	252.5 ^d (226-282.3)	2.38 \pm 0.033	2.5	4	189.1 ^e (166.3-215)	2.0 \pm 0.026	7.9	4

Results in the same column with the same superscript are not significantly different ($p < 0.05$).



Upper: Compound 3, 1,3-Diphenylpropen-3-one Chalcone)
Lower: Compound 6, 3-Methyl-1-(2,4-dinitrophenyl)-pyrazol-5-one

C: *C. versicolor*; G: *G. trabeum*; 1: 50 µg/ml; 2: 100 µg/ml; 3: 500 µg/ml; 4: 1000 µg/ml

Figure (1). Inhibitory effects of compounds 3 and 6 against *C. versicolor* and *G. trabeum* hyphal growth.

In vivo antifungal activity: Table (2) summarizes the retention (kg/m^3) and mass loss (%) data of poplar and Scots pine sapwood mini-blocks treated with different concentrations of the tested compounds using *C. versicolor* and *G. trabeum* as examples of wood decay fungi. It is apparent that after six weeks of

exposure to fungal attack, the tested compounds proved their relative biological effectiveness compared to the untreated specimens. The control samples showed an average mass loss of 41.27 and 41.53% for poplar (*P. nigra*) and Scots pine sapwood (*P. sylvestris*), respectively.

Table (2). Average of retention (kg/m³) and mass losses (%) of Poplar (*Populus nigra*) and Scots pine sapwood (*Pinus sylvestris*) mini-blocks treated with compounds **3** and **6** and exposed to *C. versicolor* and *G. trabeum*, respectively.

Treatment	Conc*	<i>Populus nigra</i>			<i>Pinus sylvestris</i>		
		Retention Kg/m ³	Mass loss % ± SE		Retention Kg/m ³	Mass loss % ± SE	
			Un-Leached	Leached		Un-Leached	Leached
Control	0.0	0.0	41.27 ^g ± 0.43	41.27 ^e ± 0.43	0.0	41.53 ^g ± 0.42	41.53 ^g ± 0.42
			30.43 ^f ± 0.77	33.03 ^d ± 0.37		30.83 ^f ± 0.92	34.87 ^f ± 0.37
Compound (3)	0.5	0.022	28.10 ^e ± 0.61	31.07 ^c ± 0.58	0.021	28.10 ^e ± 0.35	32.30 ^d ± 0.15
	1.0	0.044	26.23 ^d ± 0.55	29.33 ^b ± 0.26	0.043	26.4 ^d ± 0.49	31.10 ^c ± 0.51
	5.0	0.194	23.87 ^c ± 0.61	28.10 ^a ± 0.42	0.210	24.80 ^c ± 0.68	29.0 ^b ± 0.21
	10.0	0.447	29.23 ^f ± 0.82	31.13 ^c ± 0.52	0.463	30.0 ^f ± 0.32	33.30 ^e ± 0.38
Compound (6)	0.5	0.004	23.40 ^c ± 0.67	30.13 ^c ± 0.55	0.019	25.57 ^d ± 0.43	31.17 ^c ± 0.15
	1.0	0.009	18.07 ^b ± 0.45	29.40 ^b ± 0.35	0.039	20.87 ^b ± 0.20	29.47 ^b ± 0.55
	5.0	0.042	13.67 ^a ± 0.54	28.63 ^b ± 0.37	0.199	14.47 ^a ± 0.34	27.5 ^a ± 0.58
	10.0	0.089			0.394		

*(IC₅₀ values)

Results in the same column with the same superscript are not significantly different (p < 0.05).

Regarding poplar, treatment with compounds **3** and **6** reduced the mass loss of samples to 30.43% and 29.23% (75% and 71% of control) at the lowest concentration. This effect was significantly increased to reaching 23.87% (57.7% of control) and 13.67 % (33.1% of control) mass losses in systematic arrangement in un-leached samples in case of compound **3** and **6**, respectively at the highest test concentration (10 IC₅₀ value). Leaching of the two

compounds treated samples reduced their antifungal effects to 28.10 % and 28.63% mass loss at the highest concentration with a narrow range of difference with their lowest tested concentration (0.5 IC₅₀) as the mass loss was 33.03% and 31.13% in case of compound **3** and **6**, respectively. Both compounds protected the Scots pine sapwood (*P. sylvestris*) samples in the same manner of the hardwood samples as compound **3** reduced its mass loss to (30.83% - 24.80%) while compound **6** reduced its mass loss to (30.0% - 14.47%) at concentration used in comparison to 41.53% of control samples. Leaching of the used blocks decreased the effect to 34.87% – 29.0% and 33.30% – 27.5%, respectively. From the obtained results, it was found that the difference between the effect before and after leaching of samples treated with compound **6** was higher than that of compound **3** ensuring that the former was easily leached. This may be referred to the hygroscopic nature of compound **6**. In general, the mass loss percentages decreased as the test compounds concentration increased. At the same time, descriptive analysis proved that compound **6** was more significantly effective with general mean of mass loss \pm SE of $25.12\% \pm 2.58$ in comparison to $29.98\% \pm 1.63$ of compound **3** in case of un leached poplar samples, while no significant differences were observed between them in leached poplar samples. In Scots pine sapwood, significant differences appeared in both cases as compound **6** achieved general mean of mass loss \pm SE of $26.49\% \pm 2.44$ and $32.59\% \pm 1.31$ comparing with $30.33\% \pm 1.61$ and $33.76\% \pm 1.16$ of compound **3** in un-leached and leached samples.

Also, differences between the benzylidine derivatives in their fungicidal activity could be referred to the conjugation among the carbonyl groups, the phenyl rings and the double bonds, so compound **2** was less effective due to lack of this conjugation because of CH₂ moiety. Compound **3** was more effective than compound **1** may be due to the lipophilicity as reported by Cheng *et al.* (2006) who proved that derivatives with a conjugated double bond and a length of CH chain outside the ring affect their antifungal properties. They added that, the lipophilicity may play, in part, a crucial role in determining the toxicity of phenylpropenes. The effect of the studied benzylidine derivatives (benzaldehyde derived compounds) was greatly inhibited against *G. trabeum* than *C. versicolor*, which may due to degradation as benzaldehyde and its metabolic intermediates were effectively degraded by *G. trabeum* to 3,4-dihydroxybenzoic acid. This was further metabolized via the decarboxylation

reaction to yield 1,2,4-trihydroxybenzene, which may be susceptible to the ring-fission reaction (Kamada *et al.*, 2002). Leaching reduced the effect of the *in vitro* effective compounds when treated *in vivo* that reflects their easy solubility in water as well as boric acid. (Temiz *et al.*, 2008) proved that boric acid showed no effect after leaching although it exhibited a low weight loss in samples exposed to fungal decay before leaching. It is known that during wood decay, *C. versicolor* produced significant amounts of laccase and lignin peroxidase, carboxymethyl cellulase, and avicelase (Tanaka *et al.*, 1999). These enzymes may play as sites of action in wood decay fungi. Quinone reductases (QRDs) are needed to maintain the intracellular pool of these metabolites in the reduced form giving their importance in *G. trabeum* metabolism, so quinone reductases QRDs could be proved as useful targets for new wood preservatives Cohen *et al.* (2004).

Regarding the retention of the two active compounds in wood which presented as kg/m³, compound **3** was retained approximately in the same amount in both wood specimens at all tested concentrations. The retained amount of compound **6** in *P. sylvestris* was more than four times of that retained in *P. nigra*. On the other hand, compound **6** was retained in about only one fifth of compound **3** in *P. nigra*, although it appeared more effective. So it could be concluded that compound **6** was found to be more effective than compound **3** in all cases and it was more toxic against *C. versicolor* than against *G. trabeum*. Moreover, these compound need to be applied at higher concentrations to enter wood preservatives clique.

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تقييم بعض مشتقات البنزليدين والبيرازول على الفطريات المحللة للخشب

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يتناول هذا البحث تقييم ثلاثة مشتقات من مركبات البنزليدين وثلاثة مشتقات أخرى من مركبات البيرازول التي تم تحضيرها مسبقاً والتأكد من تركيبها الكيميائي على نوعين من الفطريات المحللة للأخشاب وهى فطر *Coriolus versicolor* (من مجموعة فطريات العفن الأبيض) وفطر *Gloeophyllum trabeum* (من مجموعة فطريات العفن البنى) حيث تم اختبار هذه المركبات *vitro* على سلسلة من التركيزات وقد أثبت المركب رقم (6) وهو مركب 3-methyl-1-(2,4-dinitrophenyl)-pyrazol-s-one أنه الأكثر فاعلية بين المركبات المختبرة بتركيز مثبط لـ 50% من النمو الهيفى قدره 19.6، 112.7 ميكروجرام/ملى ضد كل من *C. versicolor* و *G. trabeum* على الترتيب، كما أثبت المركب رقم (3) وهو مركب 1,7-diphenylhepta-1,6-dien-3,5-dione أنه أكثر مشتقات البنزليدين فاعلية، وكلا المركبين الفعالين تم اختبارهما داخلياً *In vivo* على نوعين من الخشب هما خشب الحور *Poplar* (*Populus nigra*) (من صالادات الأخشاب) وخشب الصنوبر *Pinus* (*Pinus sylvestris*) (من الأخشاب اللينة) والتي سبق عدوتهما بالفطريات السابق ذكرها حيث تمت العدوى باستخدام فطر العفن الأبيض *C. versicolor* لعينات من خشب الحور أما فطر العفن البنى *G. trabeum* فقد استخدم لعمل العدوى لعينات خشب الصنوبر وذلك قبل الغسيل وبعد الغسيل لكلا نوعى الخشب الفعالة بالمركبات المعامل .

أثبت المركب رقم (6) ملحوظاً نشاطاً ضد نوعى الفطريات تحت الدراسة حيث كانت نسبة الفقد فى الوزن $13.67 \pm 0.54\%$ ، $14.47 \pm 0.34\%$ ، فى حالة فطر العفن الأبيض والعفن البنى على الترتيب مقارنة بالكنترول والتي بلغت نسبة الفقد فى الوزن فيه $41.27 \pm 0.43\%$ ، $41.53 \pm 0.42\%$ فى حالة نوعى الفطريات تحت الدراسة على الترتيب. بينما بالنسبة للأخشاب المعالجة بالمركب رقم (3) فبلغت نسبة الفقد فى الوزن بها $23.87 \pm 0.61\%$ و $24.8 \pm 0.35\%$ لكلا نوعى الفطريات المختبرة على الترتيب وذلك فى حالة الأخشاب الغير مغسولة، أما فى حالة الأخشاب التي أجريت عليها معاملة الغسيل بعد المعالجة فقد تبين انخفاض تأثير هذه المركبات نتيجة تأثرها بالغسيل حيث ارتفعت نسبة الفقد فى الوزن إلى 25 % مقارنة بالكنترول.