Evaluation of Inner Bark Extract of Barberry Stem and Its Synergy with Propiconazole, EDTA, BHT, and their Combinations against the White-rot Fungus *Trametes versicolor*

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The synergistic action of water-methanol (1:1 v/v) inner bark extract of barberry (IBEB) stem and biocide, propiconazole (PCZ), and non-biocidal additives, EDTA, BHT, and their combinations with various concentrations (50, 150, 250, 350, and 450 ppm) against the white-rot fungus, *Trametes (Coriolus) versicolor* was investigated. Results obtained herein demonstrated that IBEB by itself did not exhibit antifungal property, and enhanced protection was further observed by combining it with PCZ. BHT and PCZ showed inhibition percentages of 54.3% and 43.6%, respectively, against the growth of *T. versicolor*, which reached 75% with BHT at 50 ppm. A synergistic effect was found by introducing PCZ to IBEB when tested against *T. versicolor in vitro*, with an inhibition percentage of 62% at 150 ppm. No synergistic action was exhibited from the combination treatment of IBEB+EDTA+BHT, and the activity was enhanced by introducing PCZ to this combination treatment (50% at 350 ppm). Significant synergistic action between each of factors of IBEB+PCZ, EDTA+PCZ, and BHT+PCZ was found with inhibition percentages of 49.7%, 48.6%, and 52.7%, respectively. In conclusion, it is advised that IBEB and PCZ be used clinically at the same time.

Keywords: Trametes (Coriolus) versicolor; Barberry inner bark extract; Propiconazole; EDTA; BHT

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INTRODUCTION

The extracts from different parts of the barberry (*Berberis vulgaris* L., family Berberidaceae) plant, including the roots, bark, leaves, and fruit, have been studied for their antibacterial and antifungal activities (Jain and Kar 1971; McCartney 1989; Parekh and Chanda 2005; Ghareeb *et al.* 2013; Mahmoudvand *et al.* 2014), are used extensively for medicinal purposes, and are known for possessing antiarrhythmic and sedative effects in Iranian traditional medicine (Fatehi *et al.* 2005; Javadzadeh and Fallah 2012).

Berberine (BER) is an isoquinoline plant alkaloid with a bright yellow color that is usually found in the stem bark, rhizomes, and roots of the herb *Berberis* (Kulkarni and Dhir 2010). BER notably inhibits the growth of a wide range of *Candida* species (Park *et al.* 1999; Park *et al.* 2001; Bian *et al.* 2006). A synergistic interaction of BER and fluconazole was found in fluconazole-resistant clinical isolates of *Candida albicans* (Quan *et al.* 2006; Iwazaki *et al.* 2009).

These plants are used medicinally in many traditional medical systems, and they have a history of usage in Chinese and Korean medicine dating back at least 3,000 years. BER extract, when used as a crude drug, has been demonstrated to have significant antimicrobial activity against bacteria, viruses, protozoans, fungi, and yeast (Sun *et al.* 1988; Nakamoto *et al.* 1995; Soffar *et al.* 2001; Freile *et al.* 2003; Cordero *et al.* 2004; Kulkarni and Dhir 2010), chlamydia and helminths (worms) (Hawrelak 2003) under *in vitro* conditions. The drug has been used in Chinese and Indian medicines for the treatment of bacterial diarrhea, intestinal parasitic infections, and ocular trachoma infections (Cecil *et al.* 2011).

Several azoles are widely used to treat human fungal infections. Increasing resistance to these azoles has prompted exploration of their synergistic antifungal activities when combined with other agents (Shrestha *et al.* 2015). Tebuconazole or tebuconazole in combination with propiconazole (PCZ) is the major non-arsenical, water-based wood preservative for above- and ground-contact used in European and Asian countries (Schultz and Nicholas 2003).

Treatment of *Aspergillus fumigatus* infections mainly involves azole derivatives, a class that includes itraconazole (ICZ), which has historically been the front-line drug in these treatments (Limper *et al.* 2011). However, the pharmacological profile of azole drugs is determined and restricted by their liver toxicity, metabolic elimination, and pharmacokinetic drug-drug interactions involving CYP3A4 metabolic inhibition (Billaud *et al.* 2010).

Lei *et al.* (2011) have shown that the minimum inhibitory concentration (MIC) ranges of BER and ICZ were 4-256 and 0.031-0.250 μg/mL, respectively. In addition, against *A. fumigatus* IFM 40808 strain, the MIC50 values of BER and ICZ were 8 and 0.125 μg/mL. Using this strain, they compared the giant colonies with or without BER, and concluded that BER could restrain *A. fumigatus* mycelial growth and conidial pigment production. In conclusion, it is not advised that BER and ICZ be used clinically at the same time. Their results indicated that BER may inhibit *A. fumigatus* through the ergosterol biosynthesis pathway, like ICZ.

The combining of BHT with various organic biocides resulted in enhanced effectiveness against decay caused by fungi as compared to using the organic biocide alone (Schultz and Nicholas 2000, 2002). Also, strongly significant synergy was observed by using systems of chelator EDTA as reported by Mabicka *et al.* (2005). Lin and Fung (1983) reported that BHT did not inhibit the growth, sporulation, or toxigenesis of six strains of aspergilli, but the produced aflatoxin (B_1, B_2, G_1, and G_2) in the presence of butylated hydroxyanisole (BHA), tertiary butylhydroquinone (TBHQ), and a combination of BHA and TBHQ was reduced significantly ($P < 0.05$). On the other hand, the synergistic effect was not observed for the combination of natural antifungal compound cinnamaldehyde with BHT (Hsu *et al.* 2007). Additionally, the combination of caffeine (1,3,7-trimethylxanthine) and PCZ is effective against wood-destroying basidiomycetes (Lekounougou *et al.* 2007).

As reported previously, extracts of heartwood have biocidal, antioxidant, and metal chelating properties, all of which may influence natural durability (Green and Schultz 2003). In laboratory tests, an antioxidant or metal chelator alone often has little or no protective effect (Kerem *et al.* 1999; Green *et al.* 1997), but when combined with a biocide enhanced synergistic efficacy can be observed (Green and Schultz 2003).

Basidiomycete's white-rot fungi are responsible for the most extensive biodegradation of lignin (Wu *et al.* 2005). The produced extracellular enzymatic complex

have a powerful ability to depolymerize this aromatic polymer into lower molecular weight compounds (Bajpai, 2004). *Trametes* (*Coriolus*) *versicolor* is a basidiomycete, and during wood decay it produces significant amounts of ligninolytic enzymes (laccase and lignin peroxidase, carboxymethyl cellulase, and avicelase) and it has an efficient degradation capacity of lignin, polycyclic aromatic hydrocarbons, a polychlorinated biphenyl mixture and a number of synthetic dyes (Tanaka *et al.* 1999; Novotný *et al.* 2004). Induction of ligninolytic enzymes expression and activity increases have been reported for copper (Collins and Dobson 1997), xylidine (Rancaño *et al.* 2003), veratryl alcohol (Dekker and Barbosa 2001), and a phenolic mixture (Tavares *et al.* 2005). The hydroxyl radical produced by the low-molecular-weight substance secreted by *T. versicolor* results in new phenolic substructures on the lignin polymer, making it susceptible to attack by laccase or manganese peroxidase is suggested (Tanaka *et al.* 1999).

There have been no reports on the synergistic interactions between inner bark extracts from *B. vulgaris*, biocides, and non-biocidal additives against plant and fungal pathogens. This novel approach was used to investigate the synergistic effects between the inner bark extracts of *B. vulgaris* stem and the most active biocide and non-biocidal additives in the agar plate test.

EXPERIMENTAL

Plant Materials

Fresh stems of *B. vulgaris* were collected from Siahbishe, Chalous, and Mazandaran, Iran in May of 2013. The plant material was identified by Khosrow Ashrafi, Assistant Professor, Department of Wood Science and Paper Technology and Karaj Branch, Islamic Azad University, Karaj, Iran, and a voucher specimen was deposited in the Herbarium College of Agricultural and Natural Resources, Karaj Branch, Islamic Azad University, Karaj, Iran. The inner bark was separated from the stems and air-dried to achieve 8.0% moisture content.

Biocide and non-biocidal additives

The biocide propiconazole (PCZ, Syngenta, Swiss), and the non-biocidal additives butylhydroxytoluene (BHT, SIGMA-ALDRICH, USA) and ethylenediamine-tetraacetic acid, disodium salt dehydrate (EDTA, New Jersey, USA) were used.

Methods

Preparation of B. vulgaris extract

The inner bark of *B. vulgaris* stems was cut into small pieces and chopped using a laboratory electrical rotary mill to get bark flour. The flour size was between 40 and 60 mesh. Approximately 50 g of this flour was placed into the 5 extraction thimbles and then extracted in a Soxhlet-type apparatus (Fig. 1) for 6 to 8 h using an acetone-based solvent (300 mL in a 500-mL balloon) according to the TAPPI standard (T204 om-88 (1988)).

The combined extracts were concentrated using a Heidolph Laborota 4001 rotary-evaporator apparatus (at 40 °C to reach total solvent evaporation) for approximately 15 min. Then, the extracts were collected, dried over anhydrous sodium sulphate, and stored at 4 °C until further analysis. The solid extractive weight was 2.72 g. Subsequently, 2.0 g of the solid extractives were dissolved in water:methanol (1:1 *v/v*) and poured into a separatory funnel, followed by the addition 50 mL of *n*-hexane. The mixture was shaken

by hand for 10 min and the water:methanol was separated and concentrated under reduced pressured, while the *n*-hexane fraction was discarded.

Antifungal Activity Assay

To evaluate its toxicity, 22.5 mg of IBEB solid water:methanol extract was dissolved into the 50 mL water and then by syringe was passed from the 0.45 μm Microsolve filter and was poured into the glass vial. The media were sterilized in an autoclave at 120°C. About 25 mL of the media was poured into the every Petri plates and by micro-sampler 20 μL of extract solution was added at different concentrations (50, 150, 250, 350, and 450 ppm on 3 antibiogram discs as replicates for every concentration) to media containing malt extract agar (MEA) and were poured into the one Petri plates.

The plates were cooled in a sterile hood and were inoculated with 0.50 cm plugs of *Trametes versicolor* fungus mycelia introduced into the center of the Petri plate. Inoculated plates were incubated at 23°C and 75% relative humidity without light. Three replicate antibiogram discs were used per treatment. Fungus was also grown on non-biocide MEA (*i.e.*, with no biocides) as a control. Fungal growth was monitored daily by measuring the percentage of area that was covered by fungus in the plates.

The percentage of fungal growth was plotted against the extract concentrations, and the toxic level was determined by the extract concentration at which the fungal growth was completely inhibited in accordance with the methods of Hosseini Hashemi *et al.* (2008) and Hosseini Hashemi and Jahan Latibari (2011). EDTA, BHT, and PCZ were prepared individually, as well as in combinations with the extract at different concentrations (50, 150, 250, 350, and 450 ppm) to study their synergistic effects and their antifungal activity (Table 1).

Table 1. Treatments and Concentrations

Treatment	Code	Concentration, ppm
Water:methanol extract	Ex	50, 150, 250, 350, and 450
EDTA	Ed	50, 150, 250, 350, and 450
BHT	B	50, 150, 250, 350, and 450
PCZ	P	50, 150, 250, 350, and 450
Water:methanol extract+EDTA	Ex+Ed	50, 150, 250, 350, and 450
Water:methanol extract+BHT	Ex+B	50, 150, 250, 350, and 450
Water:methanol extract+PCZ	Ex+P	50, 150, 250, 350, and 450
EDTA+PCZ	Ed+P	50, 150, 250, 350, and 450
BHT+ PCZ	B+P	50, 150, 250, 350, and 450
Water:methanol extract+EDTA+BHT	Ex+Ed+B	50, 150, 250, 350, and 450
Water:methanol extract+EDTA+ PCZ	Ex+Ed+P	50, 150, 250, 350, and 450
Water:methanol extract+BHT+ PCZ	Ex+B+P	50, 150, 250, 350, and 450
EDTA+BHT+ PCZ	Ed+B+P	50, 150, 250, 350, and 450
Water:methanol extract+EDTA+BHT+ PCZ	Ex+Ed+B+P	50, 150, 250, 350, and 450

The fungal growth (colony diameter) was measured and percentage inhibition was calculated according to the formula,

$$\text{Percentage inhibition} = [(C\text{-}T)/C] \times 100 \quad (1)$$

where C is the colony diameter (mm) of the control and T is the colony diameter (mm) of the test plate.

Statistical analysis

Percentage inhibition was calculated and an analysis of variance for the different treatments was conducted using Duncan's test.

RESULTS AND DISCUSSION

Statistically, there was a significant difference between the treatments and their concentrations on the growth of *Trametes versicolor* (Table 2).

Table 2. Univariate Test Results for Percentage Inhibition of *Trametes versicolor* Growth by Treatment Solutions with Various Concentrations at 7th Day

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	127076.007	69	1841.681	24.372	0.000
Intercept	191572.495	1	191572.495	2535.227	0.000
Treatment	110491.692	13	8499.361	112.479	0.000
Concentration	2063.094	4	515.773	6.826	0.000
Treatment * Concentration	14521.220	52	279.254	3.696	0.000
Error	10578.993	140	75.564		
Total	329227.494	210			
Corrected Total	137655.000	209			

Effect of Individual Treatment of Water:methanol of IBEB, EDTA, BHT and PCZ on *Trametes versicolor*

Table 3 shows that at the end of the 7 days of incubation for all the studied concentrations, no inhibitions were found for the individual treatments of the water: methanol of IBEB and EDTA against the growth of *Trametes versicolor*. On the other hand, BHT and PCZ treatments showed good inhibition against the growth of *T. versicolor* with inhibition percentage of 54.31% and 43.65%, respectively (Fig. 1). For the individual treatments, the best active concentrations were 50 ppm and 450 ppm from BHT with inhibition percentage values of 75% and 72%, respectively, and the concentration of 450 ppm from PCZ treatments (49%) (Fig. 2).

Synergistic Action between Each Two Factors of IBEB, EDTA, BHT, and PCZ on *T. versicolor*

Table 3 shows that at the end of the 7 days of incubation for all the studied concentrations, there was good synergistic action between each of factors of IBEB+PCZ, EDTA+PCZ, and BHT+PCZ with inhibition percentage of 49.69%, 48.62%, and 52.71%, respectively (Fig. 1). On the other hand, no synergistic action was found for each treatment of IBEB+EDTA and IBEB+BHT (Fig. 1). On the other hand, the antifungal activity of IBEB alone was good, and this means that the introducing of EDTA or BHT in combinations with IBEB had reduced the antifungal activity. The best active concentrations were 450 ppm, 150 ppm, and 450 ppm from BHT+PCZ, IBEB+PCZ, and EDTA+PCZ, which gave rise to inhibition percentage values of 64%, 62%, and 54%, respectively (Fig. 2).

Synergistic Action among Each Combination of Three Treatments of IBEB, BHT, EDTA, and PCZ on *T. versicolor*

At the end of the 7 days of incubation for all of the studied concentrations, Table 3 shows that there was a good synergistic action among the treatment combination of IBEB+BHT+PCZ with an inhibition percentage of 50.58% (Fig. 1), and the best effective concentration was 50 ppm with an inhibition percentage of 64% (Fig. 2).

Combination treatments of IBEB+EDTA+PCZ and EDTA+BHT+PCZ showed moderate activity against *T. versicolor* (Table 3) with inhibition percentages of 37.24%, and 39.56% (Fig. 1), with the most effective concentrations of 450 ppm (45%) and 350 ppm (48%), respectively, as shown in Fig. 2. On the other, there was no synergistic action of the combination treatment of IBEB+EDTA+BHT.

Table 3. Mean ± SD Value of Mycelial Growth (mm) of *Trametes versicolor* in Response to Five Treatment Solutions per 25 cm^3 of Malt Extract Agar (MEA) on the 7th Day

Treatments	Concentrations (ppm)				
	50	150	250	350	450
IBEB	37.50 ± 0.00 ^{l}	37.50 ± 0.00 ^{l}	37.50 ± 0.00 ^{l}	37.50 ± 0.00 ^{l}	37.50 ± 0.00 ^{l}
EDTA	37.50 ± 0.00 ^{l}	37.50 ± 0.00 ^{l}	37.50 ± 0.00 ^{l}	37.50 ± 0.00 ^{l}	37.50 ± 0.00 ^{l}
BHT	9.33 ± 0.58 ^{a}	9.67 ± 2.31 ^{a}	27.67 ± 17.03 ^{hijk}	28.33 ± 15.88 ^{ijk}	10.67 ± 0.58 ^{ab}
PCZ	23.67 ± 0.58 ^{fghij}	20.67 ± 0.58 ^{cdefgh}	21.67 ± 1.15 ^{defghi}	20.67 ± 2.08 ^{cdefgh}	19.00 ± 1.00 ^{cdef}
IBEB+EDTA	37.50 ± 0.00 ^{l}	37.50 ± 0.00 ^{l}	37.50 ± 0.00 ^{l}	37.50 ± 0.00 ^{l}	37.50 ± 0.00 ^{l}
IBEB+BHT	37.50 ± 0.00 ^{l}	37.50 ± 0.00 ^{l}	37.50 ± 0.00 ^{l}	37.50 ± 0.00 ^{l}	37.50 ± 0.00 ^{l}
IBEB+PCZ	27.00 ± 0.00 ^{ghijk}	14.33 ± 0.58 ^{abcd}	20.33 ± 0.58 ^{cdefg}	17.00 ± 4.36 ^{bcdef}	15.67 ± 1.15 ^{abcde}
EDTA+PCZ	23.33 ± 0.58 ^{fghij}	18.67 ± 0.58 ^{cdef}	19.00 ± 0.00 ^{cdef}	18.00 ± 0.00 ^{cdef}	17.33 ± 0.58 ^{bcdef}
BHT+PCZ	20.67 ± 3.21 ^{cdefgh}	18.00 ± 0.00 ^{cdef}	18.33 ± 0.58 ^{cdef}	18.33 ± 1.15 ^{cdef}	13.33 ± 0.58 ^{abc}
IBEB+EDTA+BHT	37.50 ± 0.00 ^{l}	37.50 ± 0.00 ^{l}	37.50 ± 0.00 ^{l}	37.50 ± 0.00 ^{l}	37.50 ± 0.00 ^{l}
IBEB+EDTA+PCZ	33.00 ± 1.00 ^{l}	21.33 ± 1.15 ^{defghi}	21.33 ± 1.15 ^{defghi}	20.67 ± 0.58 ^{cdefgh}	21.33 ± 1.15 ^{defghi}
IBEB+BHT+PCZ	21.00 ± 1.00 ^{defghi}	19.67 ± 0.58 ^{cdef}	20.67 ± 1.15 ^{cdefgh}	21.33 ± 0.58 ^{defghi}	17.33 ± 0.58 ^{bcdef}
EDTA+BHT+PCZ	29.00 ± 1.00 ^{jk}	23.33 ± 0.58 ^{fghij}	20.00 ± 0.00 ^{cdefg}	19.33 ± 1.15 ^{cdef}	21.67 ± 0.58 ^{defghi}
IBEB+EDTA+BHT+PCZ	22.00 ± 1.00 ^{efghi}	20.00 ± 0.00 ^{cdefg}	20.33 ± 0.58 ^{cdefg}	18.67 ± 0.58 ^{cdef}	19.33 ± 1.15 ^{cdef}

Means with the same letter are not significantly difference according to Duncan's test

Synergistic Action among the Combination Treatment of IBEB+BHT+EDTA+PCZ on *T. versicolor*

Results presented in Table 3 indicate that the combination treatment of IBEB+EDTA+BHT+PCZ showed good synergistic action against the growth of *T. versicolor* with an inhibition percentage of 46.5% (Fig. 1), which reached 50% at the concentration of 350 ppm (Fig. 2).

It has been shown here that barberry inner bark extract did not have antifungal effects on the growth of *T. versicolor*. A synergic effect was found *in vitro* by introducing PCZ to IBEB when tested against *T. versicolor*, where the percentage of inhibition reached 62% at 150 ppm (Fig. 2). Also, there was no synergistic action of the combination treatment of IBEB+EDTA+BHT, but the activity was enhanced by introducing PCZ to this combination of treatment (50% at 350 ppm) (Fig. 2).

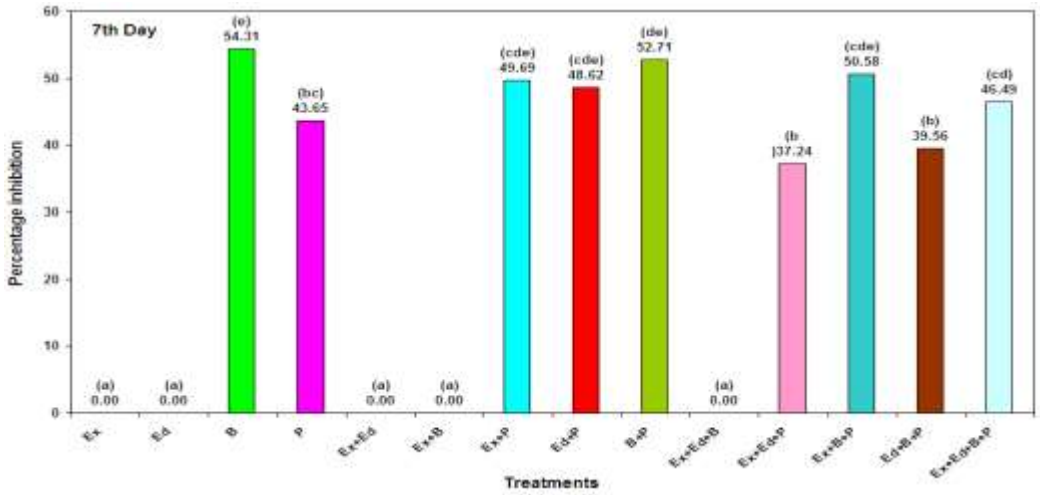

Fig. 1. Percentage inhibition of *Trametes versicolor* growth by treatment solutions with various concentrations on the 7th day

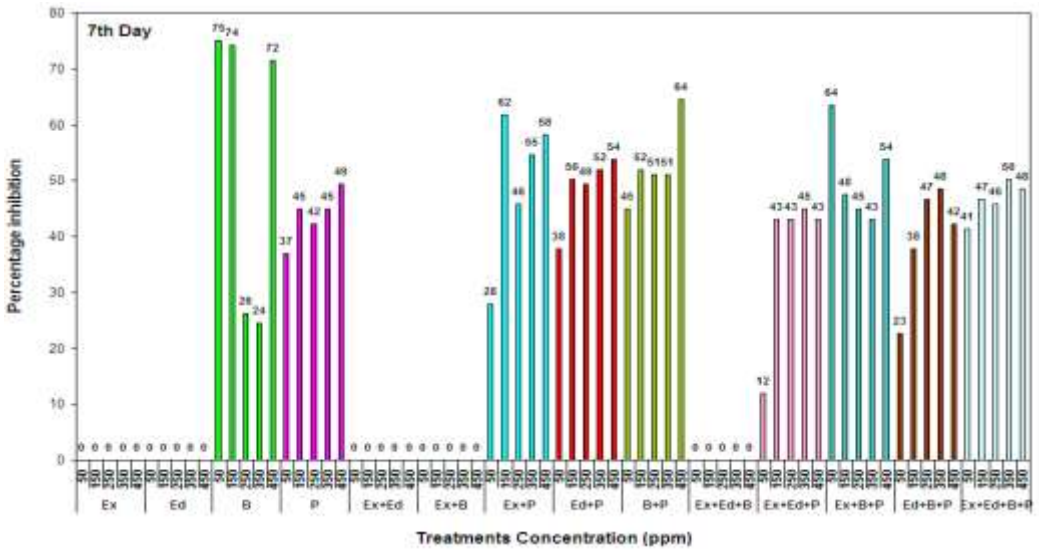

Fig. 2. Percentage inhibition of *Trametes versicolor* growth by different treatment solutions with 50, 150, 250, 350, and 450 ppm concentrations on the 7th day

Under *in vitro* conditions used in previous reports, berberine has significant antifungal activity (Park *et al.* 2006; Kim *et al.* 2007; Iwazaki *et al.* 2009; Park *et al.* 2010). Berberine and itraconazole are not synergistic *in vitro* against *Aspergillus fumigatus* isolated from clinical patients (Lei *et al.* 2011).

Propiconazole (PCZ) is used in wood protection chemicals and has shown good antifungal effectiveness (Buschhaus and Valcke 1995). PCZ is very resistant to weathering (leaching and evaporation) and does not break down in solvent-borne and water-borne formulations when exposed to various temperatures; its toxic value against *Coriolus (Trametes) versicolor* is 0.25 to 0.33 kg a.i./m^3 wood (Valcke 1989). The synergistic action was previously investigated for two triazoles, PCZ and tebuconazole, and the results showed that by combining both triazoles a better balanced spectrum of activity can be achieved against the white-rot fungus *C. versicolor* (Buschhaus and Valcke 1995). In the presence of PCZ, there was a strong induction of chitinases at the beginning of wood colonization by *Trametes versicolor*. The addition of caffeine, a pleiotropic drug and chitinase inhibitor, together with PCZ resulted in synergistic inhibition of the fungal growth (Lekounougou *et al.* 2008).

According to the fundamental knowledge of wood-degrading mechanisms, it is well known that wood-degrading fungi employ metal-mediated reactions (Henry 2003; Rodriguez *et al.* 2003) to generate free radicals that attack the wood chemical components (Goodell 2003; Reading *et al.* 2003). From the previous results, it can be seen that IBEB and BHT are not synergistic *in vitro* against *T. versicolor*, or in other meaning the introducing of EDTA and/or BHT in combination with IBEB was reduced the activity of IBEB against the fungal growth. Schultz and Nicholas (2002) reported that EDTA is very soluble, so they selected an oil-borne metal complexing agent for outdoor exposure tests. BHT is a common antioxidant, and employing it as additives showed an increasing to the organic biocide's efficacy and/or reduces biodegradation (Schultz and Nicholas 2008) also enhanced efficacy with both decay fungi and termites when BHT is co-added was observed.

Also, PCZ enhanced the activity of IBEB but it was reduced by the addition of EDTA in the combination, which means that EDTA has a significant effect in the reduction of the activity against the fungal growth. The comparative proteomic study in two fluconazole-resistant *C. albicans* cell strains treated or untreated with fluconazole and/or berberine was studied by Xu *et al.* (2009). Most of the differentially expressed proteins were related to energy metabolisms and a series of functional analysis revealed that fluconazole + berberine combination treatment augmented endogenous reactive oxygen species (ROS) production by enhancing the tricarboxylic acid cycle and inhibiting ATP-synthase activity. This biochemical process might be the synergistic antifungal mechanism of fluconazole + berberine.

CONCLUSIONS

1. The results of evaluation of synergistic effects showed that PCZ enhanced the activity of IBEB but it was reduced by the addition of EDTA in the combination, which revealed that EDTA has a negative significant effect in the activity reduction against the fungal growth.

2. No inhibitions were found by the individual treatments of the water:methanol of IBEB and EDTA against the growth of *Trametes versicolor*. Also, there was no synergistic action of the combination treatment of IBEB+EDTA+BHT, IBEB+BHT, and IBEB+EDTA.
3. BHT and PCZ treatments showed significant inhibition against the growth of *T. versicolor* with inhibition percentages of 54.31% and 43.65%, respectively, and an inhibition percentage of 75% was reached with BHT at 50 ppm.
4. There was a significant synergistic action between each of factors of IBEB+PCZ, EDTA+PCZ, and BHT+PCZ with inhibition percentages of 49.69%, 48.62%, and 52.71%, respectively.
5. A synergistic action was found with the treatment combination of IBEB+BHT+PCZ, with an inhibition percentage of 50.58%, and the best effective concentration was 50 ppm with an inhibition percentage of 64%. But this percentage was lower than the effect of BHT as individual treatment.
6. No synergistic action was demonstrated with the combination treatment of IBEB+EDTA+BHT, but IBEB+EDTA+BHT+PCZ showed a synergistic action against the growth of *T. versicolor* with an inhibition percentage of 46.49%, which reached 50% at the concentration of 350 ppm, but only PCZ had the similar percentage inhibition.

ACKNOWLEDGMENTS

The authors would like to thank Mr. Jalali (Head of Wood Anatomy, Biology, and Protection Laboratory) and Mr. Namvar (Head of Plant Diseases Laboratory) of Islamic Azad University, Karaj Branch for the use of their laboratory equipment, and give a special thanks to Mr. Mohammadi (Head of Research Laboratory and all Laboratories) for helping with the stock preparation of treatment solutions.

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Article submitted: October 15, 2015; Peer review completed: December 8, 2015; Revised version received and accepted: December 11, 2015; Published: December 17, 2015.
DOI: 10.15376/biores.11.1.1505-1517