European Journal of Science and Theology, March 2011, Vol.7, No.1, 25-34

CINNAMALDEHYDE, A POTENTIAL ACTIVE AGENT FOR THE CONSERVATION OF WOOD AND STONE RELIGIOUS ARTEFACTS

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(Received 1 June 2010, revised 30 November 2010)

Abstract

Amongst the religious artefacts, the exposed churches, crucifixes and crosses made usually of wood and stone are very often subjected to the micro-/macro-biological attack and colonization. The conservation problems of the cultural/religious heritage affected by this type of deteriorations remain still opened. In this sense, many studies are in progress for finding the most suitable methods, materials and products, with an effective and efficient applicability in the conservation of various monuments and objects with cultural value. In this study the efficacy of a natural derivative from cinnamon against some common biological agents of cultural goods made of wood and/or stone was tested. Five types of organisms (an alga, a cyanobacteria, an imperfect fungus, a macromycete and an insect) were chosen for this research in order to assess the cinnamaldehyde action in for a broad use. The results have shown that phototrophs (*Chlorella* sp., *Chroococcus* sp.) and the microfungus (*Torula* sp.) were more sensitive to cinnamaldehyde with respect to the brown rot fungus (*Coniophora puteana*), while *Hylotrupes bajulus* seems to be resistant to this product.

Keywords: cinnamaldehyde, biological agents, phototrophs, basiodiomycete, boring insect, cultural heritage

1. Introduction

The human beings have used the best materials from the beginning to represent, preserve and transmit forward their spiritual values. Therefore Christians have built churches and other objects with spiritual significance (e.g. crosses, icons, religious furniture) using mostly wood and stone as materials. The first churches were built in wood, being more accessible and easier to

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process, while stone churches, more durable, were built later, with the evolution of art and techniques of stone processing, where the economic situation allowed it. Thus, the religious monuments and artefacts have both a spiritual and historical significance, bearing over time the specific creative fingerprint of a people.

Many of the religious artistic objects or monuments exposed to improper environmental conditions are hazarded to biological colonization, especially when high humidity, poor ventilation and bad maintenance are present. Chemical products (various types of biocides), commonly used to control biological attacks are not so appropriate for indoor applications due to the health risks. Natural alternatives, friendly and non toxic to humans, are desirable. Essential oil compounds and their derivatives are considered to be a possible substitute for controlling different types of biological settlement. Many natural and flavouring substances were tested especially for the antimicrobial and/or antifungal activity in food industries [1, 2], agriculture [3-5] and few recent studies were developed in the building materials field [6, 7] as well. Recent investigations confirm that some plant essential oils not only repel insects, but have contact and fumigant action against larvae and adults of many harmful insects which cause pests [8-11].

Cinnamaldehyde (CI), the natural component that was tested in this study, is a major component of cinnamon essential oils, a yellow oily liquid that can be isolated by steam distillation from cinnamon bark and leaves of the genus *Cinnamon*. It has been proven to interfere with the cell-density regulation system of the prokaryotes (bacteria, cyanobacteria) preventing therefore the biofilm development [12-15]. Recent studies have shown that cinnamaldehyde has a strong antifungal activities against wide variety of moulds [3, 4, 6, 7] and wood decay fungi [16-19], being a potential candidate for effective and environmentally-safe wood preservatives, while literature related with the influence of cinnamaldehyde on wood boring insects was not found. In this paper we have evaluated the cinnamaldehyde efficiency against five types of organisms that are common colonizers of wood and/or stone materials: an alga (*Chlorella* sp.), a cyanobacteria (*Chroococcus* sp.), an black mould (*Torula* sp.), a basidiomycete (*Coniophora puteana*) and a coleoptera (*Hylotrupes bajulus*).

2. Experimental

2.1. Materials

Cinnamaldehyde (*trans-3-phenyl-2-propenal*) (CI) and solvents used in this research were purchased from Sigma-Aldrich Chemical Co.. CI was used as methanol, ethanol or linseed oil solutions in the concentrations reported in Table 1 for various types of organisms.

Table 1. Operational conditions of cinnamaldehyde efficiency screening tests

Conc. CI (v/v)	Evaluation method	Solvent	Retention (g/m^2)		Tested organism
			product	CI	
0.5 %	Diffusion method	Methanol	-	-	*Chlorella* sp., *Chroococcus* sp., *Torula* sp.
1%	Standard EN-46-1	Ethanol (1 layer)	13.52	0.27	*Hylotrupes bajulus*
1.5 %	Diffusion method	Methanol	-	-	*Chlorella* sp., *Chroococcus* sp., *Torula* sp.
		Ethanol	-	-	*Coniophora puteana*
2%	Standard EN-46-1	Ethanol (1 layer)	11.56	0.28	*Hylotrupes bajulus*
3 %	Diffusion method	Methanol	-	-	*Chlorella* sp., *Chroococcus* sp., *Torula* sp.
		Ethanol	-	-	*Coniophora puteana*
4%	Standard EN-46-1	Ethanol (1 layer)	11.3	0.32	*Hylotrupes bajulus*
		Linseed oil (1/2 layers)	55.04/ 81.85	2.20/ 3.27	
4.5%	Diffusion method	Ethanol	-	-	*Coniophora puteana*
6%	Standard EN-46-1	Linseed oil (2 layers)	75.78	4.54	*Hylotrupes bajulus*

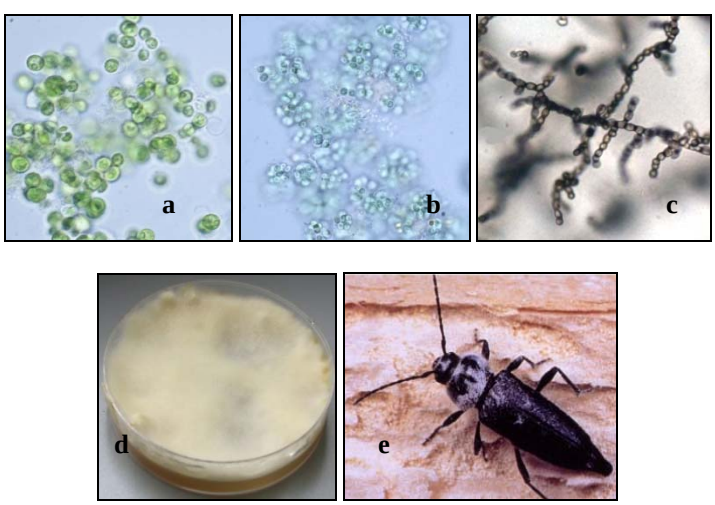

Figure 1. The biological material used in this study for testing the efficiency of cinnamaldehyde: (a) *Chlorella* sp., (b) *Chroococcus* sp., (c) *Torula* sp., (d) *Coniophora puteana* and (e) *Hylotrupes bajulus*.

2.2. Tested organisms

Two types of phototrophic microorganisms (a green alga *Chlorella* sp. and a cyanobacteria *Chroococcus* sp.), two types of fungi (an imperfect fungus, the black mould *Torula* sp. and a basidiomycete, the brown rot fungus *Coniophora puteana*) and a Coleoptera (newborn larvae of *Hylotrupes bajulus,* the common old house borer) have been used for the screening tests in this study (Figure 1). The chosen microorganisms (*Chlorella* sp., *Chroococcus* sp., *Torula* sp.) can inhabit both wood and stone materials that are found in dump conditions, forming green biofilms or promoting the lichens development. The cellar fungus *Coniophora puteana* and house longhorn beetle *Hylotrupes bajulus* are among the most dangerous wood destroying organisms, leading to the loosing of mechanical resistance of wood.

2.3. Diffusion method

A set of 3 sterilized Petri dishes (90 Ø) with agarized specific culture medium (BG11 for the cyanobacteria; BG11 modified and diluted 1:1 for the alga; PDA for the mould strain and MEA for the brown rot fungus) were used for each type of organism. The microorganisms were uniformly distributed in Petri dishes with the appropriate culture medium, using 0.25ml inoculum with a concentration of ≈57 x 10^5 cells/ml for the cyanobacteria, ≈38 x 10^5 cells/ml for the alga and ≈15 x 10^5 cells/ml for the black mould. The inoculation of the dry rot fungus was done placing small parts of the mycelium at equal distance from the source of cinnamaldehyde. Sterile small cylinders (≈4 mm diameter) were sunk into the inoculated culture medium and filled with 0.1 ml of the CI solution at different concentrations, as reported in Table 1. Then, the Petri dishes with phototrophs were incubated in continuous low artificial light at photosynthetic photon flux density of 10 μmol photon $m^{-2}.s^{-1}$ in conditioned room at 28ºC, while the ones with fungi were placed in darkness at 22±2°C and 70±5% relative humidity. The evaluation was done by visual inspection at different time intervals (10 days for both fungi and 60 days for phototrophs).

2.4. Insect attack test

The preventive action of cinnamaldehyde at different concentrations (Table 1) against *Hylotrupes bajulus* (Linnaeus) was performed in accordance with the European Normative UNI EN 46-1 (2005) [20] with some modification of the established evaluations intervals. This normative specifies the method to assess the preventive action of a product applied as a superficial treatment on wood. The method consists in placing the recently hatched larvae in direct contact with treated wood specimens. The evaluation of the efficacy is made in four weeks after the start of the test, determining how many larvae were capable of tunnelling after boring trough the treated surface and how many of them were dead. If all the larvae on the treated specimens died the test is finished, otherwise

the test has to continue for eight more weeks. The test is considered valid if at least 70% of the larvae placed on control specimens and on those treated only with solvent survived. The evaluations during this experimentation were effectuated after four and eight weeks.

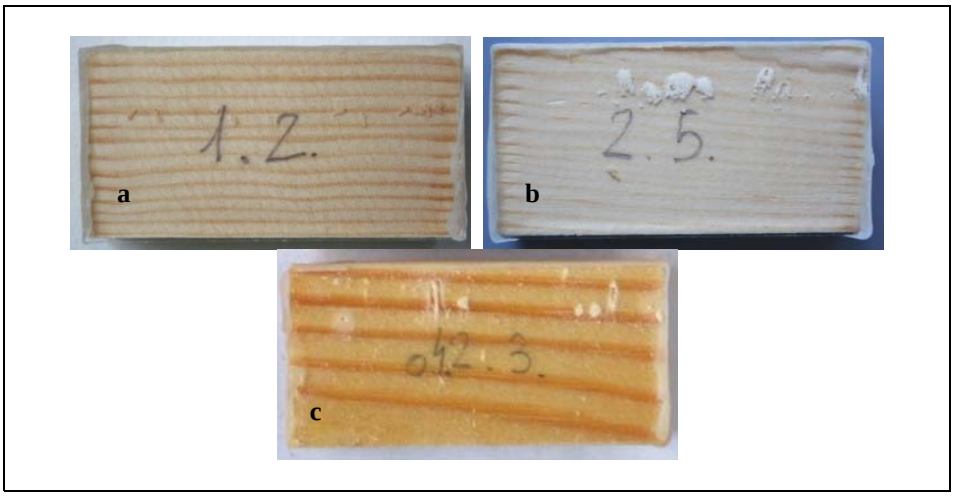

Figure 2. The visual observations of some treated specimens with CI in ethanol (1% (a), 2% (b) - one layer for each) and CI in linseed oil (4% (c) - two layers): (a) new hatched larvae placed on the wood surface, at the beginning of the experiment; (b) after 2 days the larvae started to bore and to make tunnels (b); after eight weeks sawdust and superficial tunnels and holes can be seen on the surface.

Three pine specimens (*Pinus sylvestris* L.) for each concentration and control were used in our experiment. The dimensions of each specimens were 50 x 25 x 15 mm^3 with the longitudinal faces parallel to the direction of the grain. The transversal section of specimens were sealed with paraffin wax in advance, while on the faces of test specimens, except untreated reference samples, the solutions of CI (see Table 1) in ethanol (E) or in linseed oil (LO) were applied by brushing in one or two layers. Ten recently hatched larvae of *Hylotrupes bajulus* were placed on each test specimens (Figure 2a) and covered with glass plates, fixed with paraffin wax in order to prevent the lateral slit of the larvae, avoiding any squashing risk. The wood test specimens were kept in a grow chamber at 22±2°C and 70±5% relative humidity. The evaluation of the efficacy was performed after 4 weeks by naked-eye observations and after 8 weeks by visual examination and X-ray radiography. Survival, mortality and unrecovered rate (%) of the larvae were calculated at the end of the experiment for each specimen, according to the following equations:

- Survival rate (%) = (the number of live recovered larvae / the initial number of larvae) *100 (%);
- Mortality rate (%) = (the number of dead recovered larvae (the sum of not having tunnelled and having tunnelled) / the initial number of larvae) *100 (%);

- Unrecovered rate (%) = (the number of not recovered larvae/the initial number of larvae) *100 (%).

The X-ray radiographies were performed using Agfa Structurix D7 DW radiographic films, exposing the tested wood specimens in the following conditions: 28 kV voltage, 5 mA anodic current; at 700 mm distance between X-ray tube and object; 3 min exposure time.

3. Results and discussion

3.1. Diffusion method

The visual evaluation of CI efficiency for each tested organism was done in correspondence with references (Figure 3). CI showed a good inhibitory effect against all tested organisms at all tested concentrations (Table 2) while the solvents, by themselves, did not show to prevent the biological growth.

Table 2. The efficiency of CI against three microbial strains (an alga, a cyanobacteria and a fungus) and a basidiomycete (wet rot fungus). Key to symbols: '+' efficient; '-' non efficient, '+/-' uncertain.

Conc. (v/v)		**(A) green alga (*Chlorella* sp.)**	**(B) cyanobacterium (*Chroococcus* sp.)**	**(C) black mould (*Torula* sp.)**	**(D) wet rot fungus (*C. puteana*)**
Cinnamaldehide	4%	Not tested	Not tested	Not tested	+
	3%	+	+	+	+
	1.5%	+	+	+	+
	0.5%	+	+	+	Not tested
Solvent	100%	Not tested	Not tested	Not tested	+/-
	30%	+/-	+/-	+/-	Not tested
	15%	-	-	-	Not tested
	5%	-	-	-	Not tested

3.2. Insect attack test

The assessment of CI efficiency against *Hylotrupes bajulus*, either using ethanol (E) and linseed oil (LO) as a solvents, revealed that the larvae were tunnelled all the treated specimens. The test was considered valid because 70% of larvae exposed to untreated control test specimens were survived. The average values of the replicates specimens for each type of treatment were calculated and reported in Table. 3.

In Figure 4 can be clearly seen that the survival rate of the treated specimens was generally up to 50% for almost all concentrations tested, not so much lower with respect to the controls, with an average of 74% for the survival

rate. The samples treated with 2% CI in ethanol (E) showed a 40% survival rate, but with a significant difference for unrecovered rate (46%) with respect to the other treatments. The reason of unrecovered larvae rate could be cannibalism, being found this behavioural trait in wide variety of animals and insects, as reported by some authors [21, 22].

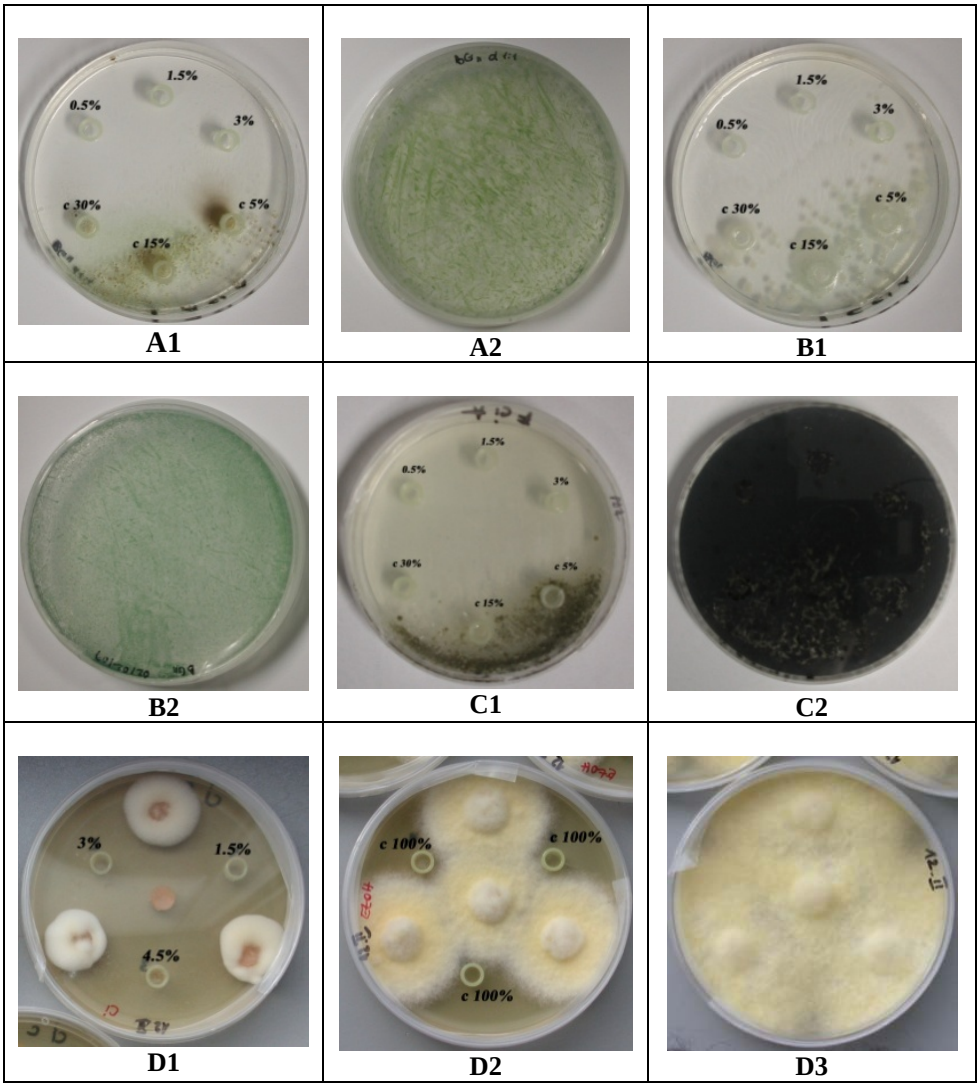

Figure 3. Cinnamaldehyde efficiency at different tested concentrations against different types of organisms: (A) green alga *Chlorella* sp., (B) cyanobacteria *Chroococcus* sp., (C) black mould *Torula* sp. and (D) cellar fungus *Coniophora puteana* beside the references for each of them (A2, B2, C2 and D3 respectively). Controls with solvents at different concentrations are symbolized with c 5%; c 15%; c 30% and c 100%.

Table 3. Survival, mortality and unrecovered rate of each treatment.

Treatment			Survival rate	Mortality rate	Unrecovered rate
CI in ethanol		**1%**	59%	7%	34%
		2%	40%	14%	46%
		4%	66%	3%	31%
CI in linseed oil		**6% - 2 layers**	67%	13%	20%
		4% - 1 layer	57%	7%	36%
		4% - 2 layer	77%	20%	3%
Solvent	**Ethanol (E)**	**100%**	70%	13%	17%
	Linseed oil (LO)	**100%**	73%	17%	10%
Untreated specimens			80%	13%%	7%

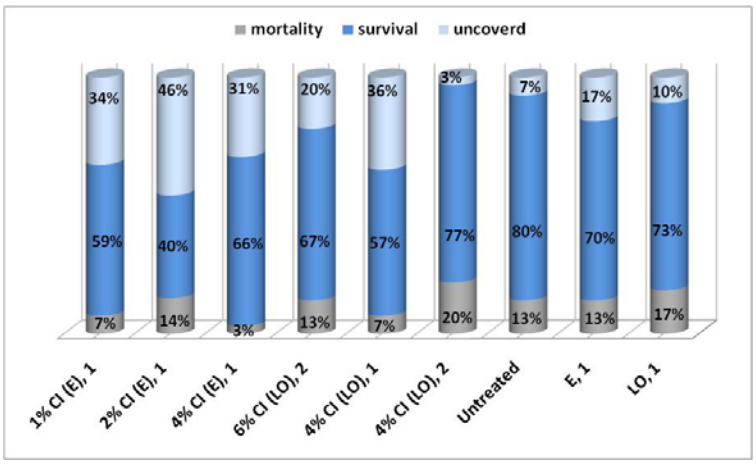

Figure 4. Values of survival, mortality and unrecovered rate for each treatment after eight weeks (1 or 2 represent the number of applied layers).

3.2.1. Naked-eye observations

The larvae were very active from the beginning of the experiment, some of them have trying to escape few minutes after their contact with all the treated wood surfaces, being therefore necessary to replace them. After only 2 days was noticed that the larvae bored into almost all treated specimens (Figure 2b), and after four and eight weeks (Figure 2c), the naked-eye observations revealed the presence of holes, tunnels and sawdust on controls and treated specimens as well. The intensity of the attack was only a bit lower on the specimens treated with CI, with respect to the untreated specimens or the ones treated only with the solvents.

3.2.2. X-ray Radiography observation

After eight weeks, even the larvae are still tiny, their image and tunnels aspect were captured by X-ray radiography (Figure 5.) and carefully observed with magnification in order to check their presence/absence in the whole sample. When specimens are enough infested, it is very easy to see the larvae and damage in wood using this method.

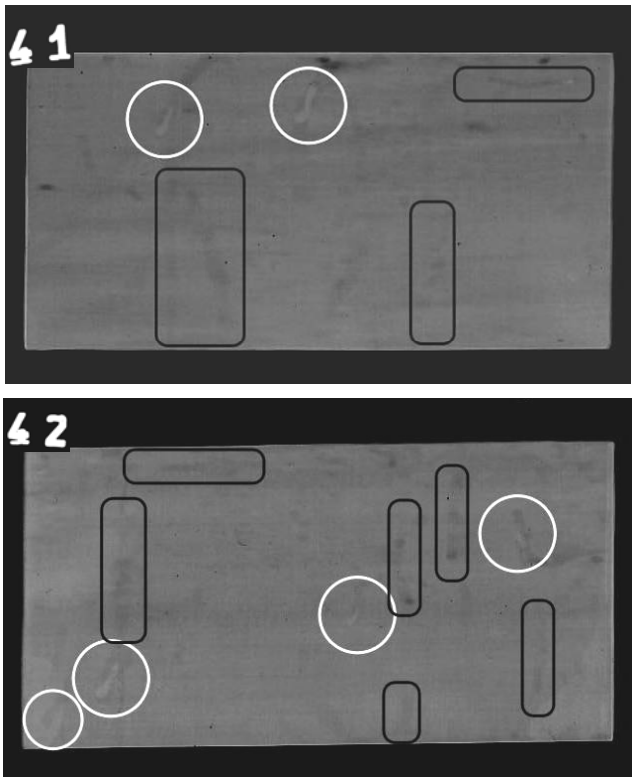

Figure 5. The X-ray radiography of wood specimens treated with CI in linseed oil (4%, 1 and 2 layers, respectively). Dark tones identify woodworm tunnels (black rectangles), light tones identify larvae (white circles).

4. Conclusions

Cinnamaldeyhe (CI) seems to be effective especially against microorganisms while the more complex organisms were more resistant to this product. The positive effect against phototrophs and fungi could be due to the volatile property of CI. This hypothesis raised up by comparing the methods that have been used in this study for CI efficacy testing. Diffusion method that was used for algal, cyanobacterial and both fungal strains have been allowed the holding of CI vapours inside of the Petri dishes, while in case of the insect attack test, the active agent (CI) have been evaporated in the air, the product being

applied by brushing and afterward let to dry. The results obtained in this study are quite encouraging for the use of CI against microbial colonization and brown rot fungus. Further detailed studies to use CI as an alternative for biological control must take into account the problems related with its volatility, poor water solubility and aptitude for oxidation in order to define the optimal concentrations, most suitable type of applications and the best conditions for a long time lasting action.

Acknowledgements

Part of this work has been made in the frame of the EPISCON Project (MEST-CT-2005-020559-European PhD in Science for Conservation)**.**

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