J. Korean Wood Sci. Technol. 2016, 44(4): 526~538 pISSN: 1017-0715 eISSN: 2233-7180
http://dx.doi.org/DOI:10.5658/WOOD.2016.44.4.526

Original Article

Evaluation on Biological Sensitivity of Three Fumigants Used for Conservation of Wooden Cultural Property[1]

Si Hyun Kim[2,3] · Dae Woon Kim[4] · Hyun Ju Lee[3] ·
Byung Ho Lee[5] · Bong Su Kim[5] · Yong Jae Chung[3,†]

ABSTRACT

Fumigants are used worldwide for control of biological agents that damage wooden cultural property. To establish a policy for fumigant use, biological evaluation of insects and microorganisms considering many factors is required. This study was performed to evaluate biological sensitivity and wood penetration of three fumigants applied for control of biological agents that damage wooden cultural properties in Korea. Among these, methyl bromide and ethylene oxide can control insects and fungi when exposed directly. However, they were unable to completely control biological agents within deeper parts of wood. Ethanedinitrile, which was developed as an alternative fumigant, exhibited outstanding wood penetration and biocidal efficacy. Further research involving various environmental conditions is warranted.

Keywords : fumigant, wooden cultural property, biological sensitivity, methyl bromide, ethylene oxide, ethanedinitrile

1. INTRODUCTION

Organic substances in cultural properties can be broadly classified into plant materials, such as paper, wood, and textile; and animal materials, such as fur, leather, and silk. They lose material characteristics due to damage as a habitation or food source of insects and fungi. Fumigation using chemical fumigants is the most frequent method of controlling biological damage of organic materials. Being active in gaseous a state at room temperature, a fumigant, a type of agricultural pesticide, is widely applied to movable cultural properties and wooden architecture as a biocide.

In Korea, the first fumigation treatment of cultural properties was conducted using a mixture (product name: Ekibon) of 86% methyl

[1] Date Received March 10, 2016, Date Accepted June 19, 2016
[2] Research Division of Architectural Heritage, National Research Institute of Cultural Heritage, Dae-jeon, 34122, Republic of Korea
[3] Department of Heritage Conservation & Restoration, Graduate School of Convergence Cultural Heritage, Korea National University of Cultural Heritage, Bu-yeo, 33115, Republic of Korea
[4] Department of Biology, College of Natural Science, Kongju National University, Gong-ju, 32588, Republic of Korea
[5] Dongbu ARI, Dongbu Farm Hannong Co., Ltd., Non-san, 33006, Republic of Korea
[†] Corresponding author: Yong Jae Chung (e-mail: iamchung@nuch.ac.kr)

bromide (MB) and 14% ethylene oxide (EO) of Gongsunyeongeung monument (Historical Site No. 205) in 1982 (Cultural Heritage Administration, 2009). Since then, 159,446 movable cultural properties in large storage facilities were fumigated up to March 2015 (Cultural Heritage Administration, 2015).

Pursuant to the Montreal Protocol, the use of MB, an ozone layer-depleting substance, was prohibited in 2015. Since then, various alternative fumigants and eco-friendly methods have been evaluated, such as heat treatment, irradiation, and modified atmosphere treatment (Gilberg, 1989; Elert and Maekawa, 1997; Ackery *et al*., 2004; Sonoda and Hidaka, 2008; Gilman *et al*., 2015). Though fumigants are toxic to human health, they are effective for control of biological agents. Recent studies of fumigants have focused on evaluation of the stability of various materials (Lee *et al*., 2002; Chae *et al*., 2004; Sophie, 2004; Kang, 2009b; Kang, 2009c; Rika *et al*., 2011; Kim *et al*., 2015; Robbiola *et al*., 2015). However, no research on the biological sensitivity of species damaging to wooden cultural properties has been conducted.

The efficiency of fumigants varies dependent upon environmental conditions, such as temperature and humidity, and the concentration and treatment duration (Nayak and Colins, 2008). Furthermore, sensitivity is subject to the growth stage of insect (Winks and Waterford, 1986). Thus, application of fumigants should involve the optimum exposure condition, stage of growth, and other environmental conditions such as temperature and humidity (Schortemeyer *et al*., 2011).

Accordingly, this research evaluated the degree of sensitivity to biology of three fumigants, two of which have been used previously for fumigation in conservation science. Ethanedinitrile (EDN), which was developed as an alternative to MB, was also evaluated. Our findings will facilitate development of a standard fumigation treatment for cultural properties.

2. MATERIALS and METHODS

2.1. Fumigants

The fumigants used were MB 14% + EO 86%, used for fumigation for cultural properties from 1982 until recently; EO 15% + HFC 134a 85%, which is currently used for fumigation of cultural properties in Japan and Korea as an alternative to MB, and EDN, which has recently been used for quarantine and as an alternative to MB. The chemical compositions of fumigants are shown in Table 1.

2.2. Insects and fungi

Three test insect species were used to evaluate biological sensitivity to fumigants: termites (*Reticulitermes speratus kyushuensis*), which damage wooden historical buildings, cigarette beetles (*Lasioderma serricorne*), which damage all types of organic substance, and rice weevil (*Sitophilus oryzae*). Termites were collected from a hill near Korea National University of

Table 1. Chemical composition of three fumigants

Fumigant	Chemical composition	
Methyl Bromide + Ethylene Oxide	Methyl bromide 86% + Ethylene Oxide 14%	$CH_3Br + C_2H_4O$
Ethylene Oxide	Ethylene Oxide 15% + HFC 134a 85%	$C_2H_4O + CH_2FCF_3$
Ethanedinitrile	Ethanedinitrile	C_2N_2

Table 2. List of test insect and fungi

type		species	feature
Insect	Termite (Isoptera)	termite (*Reticulitermes speratus kyushuensis*)	adult (worker 95%, soildier 5%)
	Beetle (Coleoptera)	cigarette beetle (*Lasioderma serricorne*)	adult
		rice weevil (*Sitophilus oryzae*)	egg, larva, adult standard test insect in fumigation of cultural heritage
Fungi	Mold	*Aspergillus niger*	standard test fungi in fumigation of cultural heritage
		Penisillium chrysogenum	
		Trichoderma koningii	
	Wood rot fungi	*Trametes versicolor*	white-rot fungi
		Fomitopsis palustris	brown-rot fungi

Cultural Heritage in Buyeo-gun city (E 126.92°, N 36.31°) and cultivated in a darkroom at 22 ± 3℃ with a relative humidity of 50 ± 10%. Only adult termites including 5% soldiers were separated and used. Only active adult cigarette beetles were used. Egg, larva, and adult rice weevils were used to evaluate sensitivity to fumigants according to growth stage. After putting 100 adult rice weevils per 100 g rice into an insect breeding bottle and mating them for 3 days, cultivation was conducted in a darkroom at 22 ± 2℃. Rice cultivated for 2 and 4 weeks were used to evaluate eggs and larvae, respectively (Lee *et al*., 2004).

Three molds and two wood rot fungi were used. The molds were *Trichoderma koningii* (KACC No. 40779), *Penisillium chrysogenum* (Provided from Institute of Preventive Conservation for Cultural Heritage in Korea National University of Cultural Heritage), and *Aspergillus niger* (KACC No. 43547), which are standard species for fumigation. The wood rot fungi used were *Trametes versicolor* (IPCCH), which is a white rot fungus, and *Fomitopsis palustris* (IPCCH), which is a brown rot fungus.

2.3. Direct exposure evaluation

To evaluate the sensitivity to fumigants of test insects and fungi, direct exposure was conducted. Fifty adults of test insects, 50 g of rice containing eggs and larvae of rice weevil and the edge of fungus colonies stripped off

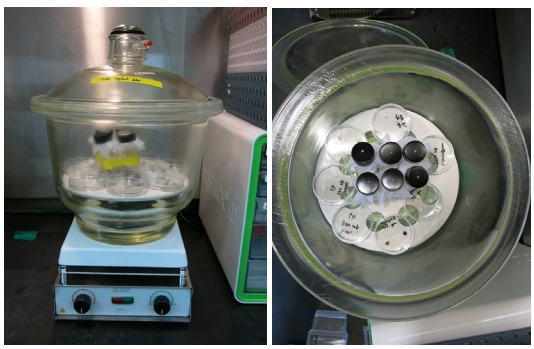

Fig. 1. Direct-exposure method (left: front side, right: upper side).

using a cork borer were placed in a glass desiccator with a capacity of 7.8 ℓ. The desiccator was sealed with grease (Dow Corning high vacuum grease, USA), and fumigants were injected using a syringe (100 mℓ, Hamilton, USA). The treatment concentrations of MB+EO were 50, 100, and 200 g/m^3, and those of EO+HFC were 100, 200, and 400 g/m^3 which based on that recommended by the manufacturer. The treatment times were 3, 6, 12, and 24 h. EDN was treated for 3, 6, 12, and 24 h at 2.5, 5, 10, and 20 g/m^3. The temperature within the treatment space was maintained at 22 ± 3℃ and its relative humidity was maintained at 60 ± 10%. A small fan inside the desiccator distributed the agents homogenously (Fig. 1).

The biocidal effectiveness against test adult insects was evaluated by determining the mortality rate after being exposed to air for 3 days after treatment. Eggs and larvae of rice weevil were evaluated by determining their emergence rate compared to the non-treated control after 6 and 4 weeks, respectively. Fungicidal efficacy was evaluated as − (no growth), + (slow growth rate), and ++ (normal growth rate) after culture for 5 days at 27℃ in Potato Dextrose Agar medium after treatment.

2.4. In−direct exposure evaluation

An indirect exposure evaluation was conducted to mimic the habitation of wood-damaging biological agents in the deeper parts of wood (Choi, 2014). The wooden test apparatus (10 cm diameter and 6.5 cm height) used in the wood penetration test was produced from a pine tree (*Pinus densiflora S. et Z.*). It has a cylindrical space (3 cm diameter and 0.5 cm height) in which test insects and fungi are placed, and a loop was hung on both sides. After production, it was dried for 3 weeks within air-circulating chamber. The test insects and fungi were placed in the empty space, the wooden test apparatus was fixed with loops, and the cross-section where the two test apparatus met was sealed using fabric tape.

Adults of rice weevil and *A. niger* were used in the evaluation. Fifty adult test insects were selected and treated under the same conditions as the direct exposure test by placing them in an empty space inside the wooden test apparatus along with ∼20 grains of rice. The edge of a fungus colony stripped off using a cork borer was placed in the space within the wooden test ports and treated using the same method as for direct exposure (Fig. 2). The temperature and relative humidity within the treatment space was maintained at 22 ± 3℃ and 60 ± 10%. A small fan inside the desiccator distributed the

Table 3. Insecticidal Activity of MB+EO Following Direct Exposure (Death Rate: %)

Test insect (growth stage)	Conc. (g/m^3)	Time (h)			
		3	6	12	24
S. oryzae (adult)	50	100%	100%	100%	100%
	100	100%	100%	100%	100%
	200	100%	100%	100%	100%
L. serricorne (adult)	50	100%	100%	100%	100%
	100	100%	100%	100%	100%
	200	100%	100%	100%	100%
R. speratus kyushuensis (adult)	50	100%	100%	100%	100%
	100	100%	100%	100%	100%
	200	100%	100%	100%	100%

Table 4. Insecticidal Activity of MB+EO Following Direct Exposure (Number of Emergences)

Test insect (growth stage)	Conc. (g/m^3)	Time (h)			
		3	6	12	24
S. oryzae (egg)	50	3.2 (5.2%)*	0	0	0
	100	0	0	0	0
	200	0	0	0	0
S. oryzae (larva)	50	0	0	0	0
	100	0	0	0	0
	200	0	0	0	0

* Emergence ratio between the test insect and control

Fig. 2. Indirect-exposure method.

agents homogenously. Biocidal efficacy was evaluated by determining the mortality rate and growth status.

3. RESULTS and DISCUSSION

3.1. MB+EO

Direct exposure to MB+EO for 3 h at 50 g/m^3 resulted in death of all test insects. However, 5% of eggs emerged to adults, so sensitivity differed according to growth stage (Tables 3, 4). The two species of wood rot fungi

Table 5. Fungicidal Activity of MB+EO Following Direct Exposure

Test fungi		Conc. (g/m^3)	Time (h)			
			3	6	12	24
Wood rot fungi	*T. versicolor*	50	++	++	-	-
		100	-	-	-	-
		200	-	-	-	-
	F. palustris	50	++	++	-	-
		100	+	-	-	-
		200	-	-	-	-
Molds	*T. koningii*	50	++	++	+	-
		100	++	++	-	-
		200	++	-	-	-
	P. chrysogenum	50	++	++	++	-
		100	++	++	-	-
		200	++	-	-	-
	A. niger	50	++	++	++	-
		100	++	++	-	-
		200	++	++	-	-

* Activity evaluation: no growth (-), slow growth rate (+), normal growth rate (++)

Table 6. Biocidal Activity of MB+EO Following Non-direct Exposure

Hour (h) \ Conc. (g/m^3)	Insecticidal activity*			Fungicidal activity**		
	50	100	200	50	100	200
12	90%	94%	100%	++	++	++
24	92%	100%	100%	++	++	+

* Death rate (%)
** Activity evaluation: no growth (-), slow growth rate (+), normal growth rate (++)

were removed following direct exposure for 3 h to 200 g/m^3 or 6 h to 100 g/m^3; however, the three species of molds were removed following direct exposure for > 12 h at 100 g/m^3 (Table 5).

Indirect exposure for 12 h to 200 g/m^3 or 24 h to 100 g/m^3 resulted in the death of all test insects. Indirect exposure for 24 h to 200 g/m^3, which were the maximum time and concentration applied in this research, some of the insects and fungi grew. Thus, the insecticidal and fungicidal effects were incomplete (Table 6). Therefore, fumigation may not always control insects or fungi in wood due to the high enclosure ratio within the treatment space. In outdoor environments, these problems occur more frequently due to leakage of fumigant through soil.

Table 7. Insecticidal Activity of EO+HFC Following Direct Exposure (Death Rate: %)

Test insect (growth stage)	Conc. (g/m^3)	Time (h)			
		3	6	12	24
S. oryzae (adult)	100	83%	100%	100%	100%
	200	92%	100%	100%	100%
	400	100%	100%	100%	100%
L. serricorne (adult)	100	83%	100%	100%	100%
	200	85%	100%	100%	100%
	400	100%	100%	100%	100%
R. speratus kyushuensis (adult)	100	100%	100%	100%	100%
	200	100%	100%	100%	100%
	400	100%	100%	100%	100%

Table 8. Insecticidal Activity of EO+HFC Following Direct Exposure (Number of Emergences)

Test insect (growth stage)	Conc. (g/m^3)	Time (h)			
		3	6	12	24
S. oryzae (egg)	100	16.5 (26.7%)*	11.3 (18.3%)*	0	0
	200	7.5 (12.2%)*	0	0	0
	400	0	0	0	0
S. oryzae (larva)	100	23.6 (28.6%)*	9.8 (11.9%)*	0	0
	200	13.1 (15.9%)*	0	0	0
	400	0	0	0	0

* Emergence ratio between the test insect and control

3.2. EO+HFC

Direct exposure to EO+HFC for 6 h at 100 g/m^3, resulted in the death of all adult test insects. The frequency of egg and larva emergence was 18.3% and 11.9%, respectively, compared to the non-treated control group (Tables 7, 8). The two species of wood rot fungi were removed by treatment for 12 h at 100 g/m^3. However, the three species of molds were removed by treatment for 24 h at 200 g/m^3 (Table 9).

Indirect exposure for 12 h at 400 g/m^3 resulted in the death of all test insects. After treatment for 48 h at 400 g/m^3, the maximum time and concentration, fungi exhibited active growth (Table 10). Similar to MB+EO, fungi actively grew inside the wood in the presence of a concentration twofold that recommended by the manufacturer. Thus, penetration deep inside the wood under outdoor conditions may be problematic.

3.3. EDN

Direct exposure to EDN for 3 h at 10 g/m^3 or 6 h at 5 g/m^3 resulted in the death of adult test insects. Treatment for 6 h and 3 h at 20

Table 9. Fungicidal Activity of EO+HFC Following Direct Exposure

Test fungi		Conc. (g/m^3)	Time (h)			
			3	6	12	24
wood rot fungi	*T. versicolor*	100	++	++	-	-
		200	++	-	-	-
		400	+	-	-	-
	F. palustris	100	++	++	-	-
		200	++	+	-	-
		400	++	-	-	-
Molds	*T. koningii*	100	++	++	++	+
		200	++	++	+	-
		400	++	++	++	-
	P. chrysogenum	100	++	++	-	-
		200	++	++	-	-
		400	++	-	-	-
	A. niger	100	++	++	+	+
		200	++	++	+	-
		400	++	++	-	-

*Activity evaluation: no growth (-), slow growth rate (+), normal growth rate (++)

Table 10. Biocidal Activity of EO+HFC Following Non-direct Exposure

Hour (h) \ Conc. (g/m^3)	Insecticidal activity*			Fungicidal activity**		
	100	200	400	100	200	400
12	41% (20.5/50)	82% (41/50)	100% (50/50)	++	++	++
24	69% (34.5/50)	95% (47.5/50)	100% (50/50)	++	++	++

*Insect: death rate (%)
**Fungi: activity evaluation: no growth (-), slow growth rate (+), normal growth rate (++)

g/m^3 resulted in the death of all eggs and larvae, respectively (Tables 11, 12). This indicates a greater difference in sensitivity according to growth stage than the other two fumigants. The two species of wood rot fungi were removed by treatment for 3 h at 5 g/m^3 and the three species of molds were removed by treatment for 3 h at 10 g/m^3 or 6 h at 5 g/m^3, similar to the other two fumigants (Table 13).

Indirect exposure for 12 h at 5 g/m^3 resulted in the death of all test insects, and the fungi were removed by treatment for 24 h at 20 g/m^3 (Table 14). As EDN has not been applied as a fumigant for cultural property to date, the optimum concentration and treatment duration are unclear. Thus, treatment at 20 g/m^3 for 24

Table 11. Insecticidal Activity of EDN Following Direct Exposure (Death Rate: %)

Test insect (growth stage)	Conc. (g/m^3)	Time (h)			
		3	6	12	24
S. oryzae (adult)	5	59%	100%	100%	100%
	10	100%	100%	100%	100%
	20	100%	100%	100%	100%
L. serricorne (adult)	5	100%	100%	100%	100%
	10	100%	100%	100%	100%
	20	100%	100%	100%	100%
R. speratus kyushuensis (adult)	5	100%	100%	100%	100%
	10	100%	100%	100%	100%
	20	100%	100%	100%	100%

Table 12. Insecticidal Activity of EDN Following Direct Exposure (Number of Emergences)

Test insect (growth stage)	Conc. (g/m^3)	Time (h)			
		3	6	12	24
S. oryzae (egg)	5	47.1 (65.3%)	14.6 (23.7%)	0	0
	10	17.7 (28.7%)	16.2 (26.2%)	0	0
	20	8.1 (13.1%)	0	0	0
S. oryzae (larva)	5	53.6 (65.0%)	21.6 (26.2%)	0	0
	10	15.3 (18.6%)	0	0	0
	20	0	0	0	0

*Emergence ratio between test insect and control

h can be used to establish such a standard. It have to consider the characteristics of the material, diverse field conditions, such as temperature, enclosure ratio, and treatment capacity.

3.4. Discussion

The biocidal effects of fumigants are in proportion to the concentration time product (CT Product), which is the product of the treatment concentration and duration (Su *et al*., 1989). The CT Product for control of adult rice weevil by direct exposure to MB+EO was ≤ 150 g · h/m^3 at 22℃ with 60% relative humidity, compared to 1200-2400 g · h/m^3 for indirect exposure. This indicates that the amount of agents penetrating wood decreased rapidly.

MB does not penetrate and spread in the deeper parts of wood (United Nations Environment Programme, 2006), and the pine (*Pseudotsuga menziesii*) wood penetration of MB was significantly lower than that of SF, PH_3 and EDN. Moreover, the equilibrium concentration was not achieved after 50 h at 5, 10, and 15 cm depths within the wood (Ren *et al*., 2011).

Table 13. Fungicidal Activity of EDN Following Direct Exposure

Test fungi		Conc. (g/m^3)	Time (h)			
			3	6	12	24
Wood rot fungi	*T. versicolor*	5	-	-	-	-
		10	-	-	-	-
		20	-	-	-	-
	F. palustris	5	-	-	-	-
		10	-	-	-	-
		20	-	-	-	-
Molds	*T. koningii*	5	++	-	-	-
		10	-	-	-	-
		20	-	-	-	-
	P. chrysogenum	5	-	-	-	-
		10	-	-	-	-
		20	-	-	-	-
	A. niger	5	+	-	-	-
		10	-	-	-	-
		20	-	-	-	-

*Activity evaluation: no growth (-), slow growth rate (+), normal growth rate (++)

Table 14. Biocidal Activity of EDN Following Non-direct Exposure

Hour (h) \ Conc. (g/m^3)	Insecticidal activity*			Fungicidal activity**		
	5	10	20	5	10	20
12	100% (50/50)	100% (50/50)	100% (50/50)	++	++	++
24	100% (50/50)	100% (50/50)	100% (50/50)	++	+	-

*Insect: death rate (%)
**Fungi: activity evaluation: no growth (-), slow growth rate (+), normal growth rate (++)

EO+HFC was first used in Japan to replace MB+EO, and is less effective on various materials (Kang, 2009b; Kang, 2009c; Jeong *et al*., 2015). The CT Product required for control of adult rice weevils was 600-1,200 $g \cdot h/m^3$. However, the corresponding value for indirect exposure was ～4800 $g \cdot h/m^3$. Thus, the quantity of agent penetrating the wood decreased significantly.

EO remains for a long period when adsorbed to rubber or plastic (Abbey Publication, 1982). If the fumigant does not affect the material immediately after treatment, deterioration of the material may occur due to the effect of the residual gas (Kang, 2009a). Since EO mutagenic and carcinogenic (National Institute for Occupational Safety and Health, 1981), long-term ventilation after treatment would be

required.

EDN could be applied at a low dosage and exhibited an excellent fungicidal effect. It can likely control insects and fungi inside wood following treatment for 24 h at 20 g/m^3 at 22℃, as it shows outstanding wood penetration. Therefore, it has various advantages as a fumigant. However, discoloration of metal and paper can occur (National Research Institute of Cultural Heritage, 2008; Robbiola *et al*., 2015); thus, this agent should be applied to cultural properties with caution.

4. CONCLUSION

We evaluated the sensitivity of biological agents and wood penetration of fumigants used to control insects and fungi damaging to wooden cultural properties. MB+EO and EO+HFC did not completely control insects and fungi in the deeper parts of wood. The biocidal effect of fumigants is in proportion o the accumulated quantity of fumigant through breathing (Liu, 2008), and the environmental condition significantly affects the spread of fumigant and the breathing of biological agents. Thus, the treatment conditions must take into consideration these factors. Fumigants applied to wooden buildings located outdoors can leak through soil. Thus, a biocidal effect may require additional agent during the treatment period.

Numerous studies on eco-biocidal treatments, such as heat treatment and low oxygen treatment, have been conducted (Oh *et al*., 2012; Jang *et al*., 2014; Oh and Choi, 2014; Oh *et al*., 2014), and these methods are applied to management of museum collections. These methods cannot be applied to outdoor wooden architecture. However, fumigants can be used outdoors, in closed rooms, and in large spaces. Thus, fumigants can complement eco-friendly methods for controlling organisms, which will require additional research and product development.

ACKNOWLEDGEMENTS

This study was supported by the National Research Institute of Cultural Heritage (Project No. NRICH-1307-B15F).

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