Studies on wood preservatives (Ⅵ)
Investigation of screening methods

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木材防腐剤に関する研究（第6報）
スクリーニング方法の検討

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Introduction

The Japanese Industrial Standard (JIS) A 9302, test for wood-rotting fungi is a lengthy method and requires a large quantity of preservatives. It is thus not suitable as an initial screening method. The agar dilution method has frequently been used as a first screen, but there are few interrelation between the results of this method and JIS A 9302. No test piece is used in the agar dilution method. We have adopted a method using wood or similar test pieces, and molds found on the surface of industrial material. Wood preservatives effective against both molds and wood-rotting fungi are often in demand and the test period is required to be comparatively short.

Methods

1. Test for prevention of mold growth

Ten molds were tested, as follows: Three 9cm diameter paperfilters were sterilized in a Petri dish, and then, 5ml of sterilized distilled water was poured on to the filters. Two test pieces of *Fagus crenata* Bl. sapwood (20×20mm in cross section and 2mm in length, sterilized at 110±2℃ for 1 hour) were placed on the paperfilters. One drop of spore suspension of

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a mold previously cultivated on potato dextrose agar was placed on the center of the surface of the test piece, and then cultured at 25±2℃ for 7 days. Ten molds that grew comparatively well were selected, and tested for resistance against chemicals. Structures of the chemicals used in this investigation are shown in Table 1.

Test pieces were dipped in each chemical solution (Nos. 1, 5, 6, 10 and 11, concentration: 0.5%, 1% and 5%) for 5 seconds, then air-dried and sterilized at 110±2℃ for 1 hour before testing with molds.

2. Decaying test

The method described in JIS A 9302 was applied to this test. Sapwood of *Cryptomeria japonica* D. Don. (20×20mm in cross section and 10mm in length, sterilized at 110±2℃ for 1 hour) were used in a test against wood-rotting fungi. Chemical solutions (Nos. 1, 2, 3, 4, 6, 7, 8, 9, 10, 12, 13, 14 and 15, concentration: 0.1% and 0.05%) were impregnated into the test pieces by vacuum, and after drying (60±2℃ for 48 hours), the pieces were exposed to ten cycles of a weathering procedure: leaching out by running water (1～2／min., 25±3℃, 1 hour), then, dried (60±2℃, 23 hours). Wood-rotting fungi used were *Coriolus versicolor* and *Thyromyces palustris.* Decaying period was for 60 days. After that, weight loss of the test pieces were measured.

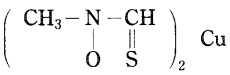
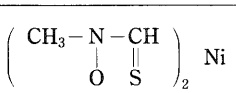
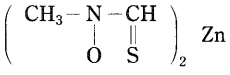
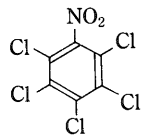
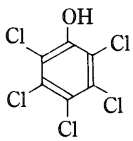
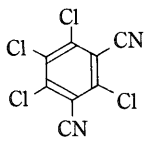
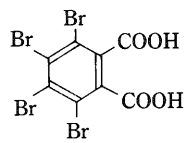
3. The influence of different woods and weathering method on the protective effects against molds.

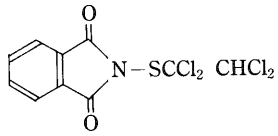
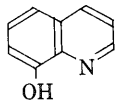
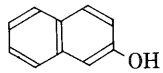
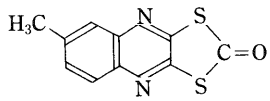
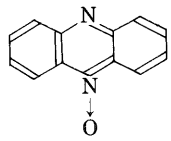
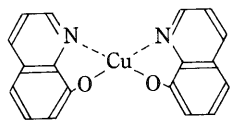
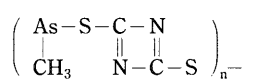
Test pieces of beech (*Fagus crenata* Bl.) Mousou-Chiku (*Phyllostachys pubescence* Mazel) and Ramin (*Gonystylus* sp.) were dipped in six kinds of chemical solutions containing 1% of chemicals (Nos. 1, 6, 9, 10, 14 and 15, solvent: dimethylformamide) for five seconds. After air drying, some of the test pieces were exposed to one or three cycles of a weathering procedure: leaching out by running water for 1 hour, air-dried for 24 hours and then heated at 60±2℃ for 24 hours. The pieces were then tested as described in section 1 above.

4. The influence of different kinds of test methods on the protective effects against molds.

Three test methods were compared as follows; ① the method described in JIS Z 2911 in which a spore suspension is dropped on test pieces treated with chemicals. ② test pieces treated with chemicals were placed on the mycelium of test fungi previously cultured on agar medium containing 2% of malt extract. ③ test pieces treated with chemicals were placed on the feeder block of wood previously prepared using test fungi. In all cases mentioned above, test pieces consisted of Mousou-Chiku were dipped in chemical solutions containing

Table 1 Structure of chemicals

No.	chemical structure
1	 bis (Dimethylthiocarbamoyl) disulfide
2	 Copper (methylthiomethyloxyiminide)
3	 Nickel (methylthiomethyloxyiminide)
4	 Zinc (methylthiomethyloxyiminide)
5	 Pentachloronitrobenzene
6	 Pentachlorophenol
7	 Tetrachloroisophthalonitril
8	 Tetrabromophthalic acid

No.	chemical structure
9	 N-Tetrachloroethylthiophthalimide
10	 8-Hydroxyquinoline
11	 β-Naphthol
12	 6-Methylquinoxaline-2, 3-dithiocarbonate
13	 Phenazine-N-oxide
14	 Copper-8-quinolinolate
15	 Polymeric methyldithiocyanato arsine

1% of chemicals (Nos. 1, 6, 9, 10, 14 and 15) for 5 seconds and air-dried, then sterilized at 110±2℃ for 1 hour.

Results and Discussion

As shown in Table 2, *Rhizopus nigricans* and *Aspergillus flavus* showed comparatively strong resistance to preservatives among the molds. To get severe results from screen of

Table 2 Resistance of molds against preservatives

species	concentration (%)	growth of mold				
		1*	5	6	10	11
Aspergillus flavus	0.5	+	+	+	−	+
	1	+	+	+	−	+
	5	−	+	−	−	−
Aspergillus niger	0.5	−	+	+	−	+
	1	−	+	−	−	+
	5	−	+	−	−	−
Penicillium luteum	0.5	−	+	+	−	+
	1	−	+	−	−	+
	5	−	+	−	−	−
Rhizopys nigricans	0.5	+	+	+	+	+
	1	+	+	−	−	+
	5	−	+	−	−	+
Aureobasidium pullulans	0.5	−	+	+	−	+
	1	−	+	+	−	+
	5	±	−	−	−	−
Chaetomium japonica	0.5	−	+	+	+	+
	1	−	+	−	−	+
	5	−	+	−	−	−
Fusarium moniliforme	0.5	−	+	+	+	+
	1	−	+	−	−	+
	5	−	+	−	−	−
Fusarium oxysporum	0.5	−	+	±	−	+
	1	−	+	−	−	+
	5	−	+	−	−	−
Alternaria citri	0.5	−	+	−	−	+
	1	−	+	−	−	+
	5	−	+	−	−	−
Tricoderma T-C	0.5	±	+	+	−	+
	1	−	+	−	−	+
	5	−	+	−	−	−

*Number of preservatives shown in Table 1.
+: grew, ±: grew sligtly, −: inhibited

preservatives, the molds which showed strong resistance should be used. So *Rhizopus nigricans* and *Aspergillus flavus* were selected first of all. In JIS Z 2911, the molds were classified in five clusters, *Rhizopus nigricans* was classified in the third cluster and *Aspergillus flavus* was classified in the first cluster. Beside these two molds, *Aureobasidium pullulance* (the forth cluster), *Fusarium moniliforme* (the fifth cluster) and *Penicillium luteum* (the second cluster) were selected to represent other three clusters.

Relation between preventive effect against molds and effect against wood-rotting fungi is shown in Table 3. Effective value against wood-rotting fungi was calculated by following formula.

Table 3 Relation between preventive effect against molds and effect against wood-rotting fungi

chemicals No.	effect						effective value		
	concentration (%)	molds					concentration (%)	wood-rotting fungi	
		A	B	C	D	E		C.v.	T.p.
2	1	−	−	−	−	−	0.1 0.05	100 100	− −
9*	1	−	−	−	−	−	0.1 0.05	100 100	100 100
14	1	−	−	−	−	−	0.1 0.06	99.6 99.3	100 100
15	1	−	±	−	−	−	0.1 0.05	100 88.1	100 28.9
1	1	−	+	−	+	−	0.1	4.4	0
3	1	+	+	+	+	+	0.1	22.1	−
4	1	+	+	+	+	+	0.1	19.8	−
6	1	−	−	−	−	−	0.1	23.3	100
7	1	+	−	+	+	+	0.1	6.2	−
8	1	+	+	+	+	+	0.1	0	32
10	1	−	−	−	−	−	0.1	71.1	68.2
12	1	+	+	+	+	+	0.1	15.1	−
13	1	+	+	+	+	+	0.1	2.3	−

*Number of chemicals shown in Table 1.
A: *Aspergillus flavus*,
B: *Penicillium luteum*,
C: *Rhizopus nigricans*,
D: *Fusarium moniliforme*,
E: *Aureobasidium pullulans*,
C.v.: *Coriolus versicolor*,
T.p.: *Tyromyces palustris*,
+: grew, ±: grew sligtly, −: inhibited

$$\text{Effective value} = \frac{\left[\begin{array}{c}\text{weight loss ratio of}\\ \text{untreated test piece}(\%)\end{array}\right] - \left[\begin{array}{c}\text{weight loss ratio of}\\ \text{treated test piece}(\%)\end{array}\right]}{\left[\begin{array}{c}\text{weight loss ratio of}\\ \text{untreated test piece}(\%)\end{array}\right]} \times 100$$

$$\text{weight loss ratio}(\%) = \frac{\left[\begin{array}{c}\text{weight of test piece}\\ \text{before decaying}(g)\end{array}\right] - \left[\begin{array}{c}\text{weight of test piece}\\ \text{after decaying}(g)\end{array}\right]}{\left[\begin{array}{c}\text{weight of test piece}\\ \text{before decaying}(g)\end{array}\right]} \times 100$$

As shown in Table 2, most of the chemicals which showed preventive effect against molds also showed preventive effect against wood-rotting fungi. So it can be considered that the mold prevention test is a valuable method for presuming the effect of chemicals against wood-rotting fungi.

Concerning the influence of wood species of test pieces on preventive effect against molds, as shown in Table 4, the order of preventive effect of each wood species was as follows; Ramin (*Gonystylus* sp.) > Beech (*Fagus crenata*) > Mousou-Chiku (*Phyllostachys*

Table 4 Influence of wood species and weathering procedure on preventive effect against molds

chemicals No.	weathering (cycles)	number of inhibited molds*			absorption of chemical (%)		
		F. crenata	*G.*sp.	*P. pubescence*	*F. crenata*	*G.*sp.	*P. pubescence*
1	0	3	5	0	9	12	6
	1	2	4	0			
	3	0	3	0			
6	0	5	5	3	11	26	5
	1	5	4	2			
	3	5	4	0			
9	0	5	5	3	14	24	7
	1	5	5	2			
	3	2	2	0			
10	0	4	5	5	14	23	5
	1	3	5	3.5			
	3	1	5	1			
14	0	5	5	5	12	13	6
	1	5	5	5			
	3	5	4.5	5			
15	0	5	5	5	12	23	6
	1	5	5	5			
	3	2	5	0			

*Count of inhibited molds to five test molds.

Test molds: *Aspergillus flavus, Penicillium luteum, Rhizopus nigricans, Fusarium moniliforme, Aureobasidium pullulans*

F. crenata: *Fagus crenata* Bl.

G. sp.: *Gonystylus* sp.

P. pubescence: *Phyllostachys pubescence* Mazel

pubescence). To get severe result from screen of preservatives, test pieces which showed poor preventive effect against molds should be used. So Mousou-Chiku was most suitable of the three.

In JIS A 9302, method for testing effectiveness of wood preservatives against decay is described. According to the test method, one cycle of weathering procedure is composed of leaching (1 hour) and drying (23 hours), and test pieces impregnated with chemicals must undergo ten cycles of this procedures. Therefore, it takes 10 days to accomplish only weathering procedure. To reduce the test period, dipping as treating method with chemicals and one or three times of leaching as weathering procedure were adopted. As shown in Table 3, preservative effect against molds decreased in the case with weathering procedure. By three times of weathering procedure, almost all treated test pieces lost effects of chemicals, and so resistance of chemicals against weathring could not be compare each other. But in the case that weathering procedure was carried out only once, almost all treated test pieces showed some effects of chemicals and so comparison of resistance against weathering was possible. Therefore, the weathering procedure should be carried out only once. Concerning wood spieces, preventive effects against molds were least in the case using Mousou-Chiku. The reason seemed to be that Mousou-Chiku had comparatively little absorption of chemicals. Absorption of chemical shown in Table 3 was calculated by following formula.

$$\text{absorption of chemical}(\%) = \frac{\left[\begin{array}{l}\text{weight of test piece}\\ \text{after impregnation(g)}\end{array}\right] - \left[\begin{array}{l}\text{weight of test piece}\\ \text{before impregnation(g)}\end{array}\right]}{\left[\begin{array}{l}\text{weight of test piece}\\ \text{before impregnation(g)}\end{array}\right]} \times 100$$

Difference of effect against molds caused by testing methods is shown in Table 5. In the case that spore suspension was dropped on the test pieces (test method ①), preservative effects against molds was higher than in the cases that test pieces were placed on mycelium previously cultured (test methods ② and ③). It seemed to be because the difference of effects were caused that the spore had to use their own nutrients to grow (test method ①), while mycelium could get nutrients from agar medium or feeder block (test methods ② and ③). So it can be considered that the test method placing test pieces on mycelium is suitable for severe screening.

Table 5 Difference of effect against molds caused by testing methods

chemicals No.	method	effect to mold				
		A	B	C	D	E
1	①	+	+	+	+	+
	②	+	+	+	+	+
6	①	+	+	−	−	−
	②	+	+	+	+	+
	③	+	+	+	+	+
9	①	+	−	+	+	−
	②	+	+	+	+	+
	③	+	−	+	+	+
10	①	−	−	−	−	−
	②	+	+	−	+	+
	③	+	+	±	+	+
14	①	−	−	−	−	−
	②	−	−	+	+	±
	③	−	−	+	±	−
15	①	−	−	±	−	−
	②	+	+	+	+	+
	③	+	+	+	+	±

A: *Aspergillus flavus*, B: *Penicillium luteum*, C: *Rhizopus nigricans*,
D: *Fusarium moniliforme*, E: *Aureobasidium pullulans*
+: grew, −: inhibited
Test pieces: Mousou-Chiku
Testing metod
①: Spore suspension was dropped on treated test pieces.
②: Treated test pieces were placed on mycelium of the test fungi previously cultured on agar medium.
③: Treated test pieces were placed on feeder block of Mousou-Chiku previously prepared using test fungi.

Conclusion

Mold prevention test is a valuable method to presume the effect of chemicals aginst wood-rotting fungi. And, placing treated test pieces on mycelium previously cultured is a suitable method as the mold prevention test.

Reference

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要旨

15種の抗菌性化合物を用い，木材に対する防カビおよび防腐効力について検討を行った。その結果，良好な防カビ効力を示す化合物は木材腐朽菌に対しても有効なことが明らかになった。しかし，防腐効力は認められてもカビに有効でない化合物が認められた。また，防カビ効力試験において，胞子懸濁液を滴下する方法および菌糸を繁殖させた餌木上に置く方法に比較して，あらかじめ培養した菌叢上に処理試験体をのせる方法が良好なことが明らかになった。