[illegible]. CONTROL OF WOOD ROTTING FUNGI

Introduction:

From the early days of civilisation, wood is one of the most important construction material and its importance in human civilisation is still growing. For a long period, man has been trying to find better methods for the preservation of wood against various wood destroying organisms. The fungi, insects and others cause widespread destruction of wood. With the advent of powerful insecticides and fungicides, man has been able to find better methods for the preservation of wood. The search for better wood-preservatives is a continuous process and recently organotin compounds have established themselves as an extremely effective tool to control wood-rotting fungi.

Hymenomycetous fungi like Fomes fomentarius, Lentinus praerigidus, Polyporus sulphureus, Polyporus squamosus, Polyporus versicolor, Lenzites trabea, Poria monticola etc. are the principal organisms that are responsible for the decay of wood[156]. Few others, some belonging to Xylariaceae[157] and a few non-hymenomycetes[158] produce enzymes capable of digesting wood. In advanced countries, works on timber decay by hymenomycetous fungi have not only been extensively done in past but considerable works are still in progress. Along with the western countries, in India, number of workers have done considerable amount of work on the biology of wood rotting fungi, but the measures for controlling wood rotting fungi still merits the better attention of Indian investigators.

Recently much interest has developed in the study of natural resistance of wood decay[159] by wood destroying fungi. In addition to the structural complexity of wood constituents many natural extraneous materials are deposited particularly in heart wood which inhibit wood destroying fungi, thus contributing substantially to the natural durability[160]. But such species are of limited availability and do not always provide the degree of protection desired. Evaluation of decay resistance of any wood may be obtained from laboratory test in which small blocks of wood are exposed in a controlled and highly favourable environment to decay by pure culture of the test fungus. Weight loss in a given period of exposure is taken

as an inverse measure of decay resistance. Considerable variation in decay resistance have been observed not only in same type of host wood, but also between sap-wood and heart-wood. Sap-wood in general, has been found to be much more susceptible to decay than heart-wood[161,162].

The broad aims of wood preservation are to develop principles and practices for prevention of wood from destruction by living organisms with preservatives. According to Cartwright and Findlay[163] an ideal wood preservative is one which when applied to the wood, makes it resistant to attack by fungi. Preservative which may be either a pure chemical compound or mixture of compounds, should be toxic against the wood destroying organisms but harmless to wood. It should not impart any undesirable colour or aroma to the wood and should have no or very little mammalian toxicity. It should not increase the fire hazards for treated woods.

In India, coal-tar being occupied the major position as wood preservative along with some little use of penta-chlorophenol, phenyl mercury acetate, copper-8-quinolinolates, zinc chloride, chromated zinc chloride, fluoride-chromate-arsenate-dinitrophenol mixture, borax and boric acid, acid copper chromate, copper-chromium-arsenic mixture, chromated zinc arsenate, ammoniacal copper arsenate etc. But unfortunately, preservative based on organotin compounds is not used much in India till now.

Van der Kerk and Luijten[13] who were carrying out a systematic investigation into the biocidal properties of organotin compounds, first proposed trialkyltin compounds as wood preservatives. They discovered the high fungicidal property of tributyltin compounds and, by the late 1950's, bis(tributyltin) oxide was already in commercial use as a wood preservative. Richardson[164] made an excellent review of tributyltins in wood preservation upto 1970. Formulations based on tributyltin oxide have proved to be effective against a number of wood rotting fungi and under the U.K. Government Health and Safety Executive's Pesticides Safety Precautions Scheme (PSPS), bis-(tributyltin) oxide is approved for use as a wood preservative in organic solvents at levels of 3% for industrial pretreatment, at 1.5% for professional, and at 1% for domestic timber treatments[165]. The same organotin compounds is also used for wood preservation in many countries.

Organotin compounds have not been much used commercially for wood preservations in the USA[166], although, in UK, there are currently some 60 different formulations containing bis (tributyltin) oxide as one of the active constituents, commercially available for wood preservation[167].

In 1975, British Standard in his "Guide to the choice, Use and Application of Wood Preservatives", suggested that

impregnation of the wood by double-vacuum process is the most effective method of applying the tributyltin preservatives and non-pressure treatments, such as dipping, spraying or brushing, provide only a shallow penetration. But the degree of protection given by the non-pressure methods is quite adequate for many timber usages. Although bis(tributyltin) oxide shows a high protection against various types of wood destroying fungi[160], an organic contact insecticide is usually added to oppose wood boring insect pests, such as the common furniture beetle, Anobium punctatum.

Trimethyltin compounds with a high insecticidal property[169] precludes in wood preservation for their high mammalian toxicity. Bis (tributyltin) oxide combines a high fungicidal property with a moderate mammalian toxicity (acute oral LD_{50} (rats) $\simeq$ 500 mg/kg)[170] and 20 years of industrial use, have no report of serious toxic effects to operators, other than minor skin burns and irritation of the eyes and respiratory tract, which are most commonly associated with the pure compounds or concentrated solutions[164]. Studies by Fish and his co-workers[171,172] on a series of tributyltin compounds in liver microsomal mono-oxygenase systems and in mammals have shown that the compounds are gradually de-alkylated to less toxic di- and monobutyltin derivatives via hydroxybutyltin intermediates. Neuhaus and his associates[173] showed

that both fungal and abiotic degradation of bis(tributyltin) oxide in wood to di- and monobutyltins may also occur under certain conditions.

Richardson[164] suggested that a chemical reaction occurred between bis(tributyltin) oxide and the terminal hydroxyl groups of the wood cellulose, to form 1,4-bis (2 - tributylstannyl) cellulose. Recently Smith[174] with ^{119}Sn Mossbauer spectroscopic studies using Scots Pine have shown that the organotin compound present in treated wood is bis (tributyltin) carbonate, $(Bu_3Sn)_2CO_3$.

Recently workers of International Tin Research Institute have indicated that bis(tributyltin)carbonate which is formed in the treated wood has a very similar activity to bis(tributyltin) oxide against the brown rot fungus Coniophora cerebella. Lower trialkyltins including tributyltins are able to inhibit the mitochondrial oxidative phosphorylation and, therefore, depress general metabolism of both plant and animal cells[175]. The mode of action of the tributyltin compounds against fungi is not definitely known, but the primary action of the toxicant on the fungus appears to be intracellular[176]. By analogy with other living systems, it is likely that the tributyltins are able to bind to amino acids on certain proteins and recent evidence has implicated SH - groups or N-1 of the imidazole ring in histidine as possible

native cities. The di- and tributyltins show a fungi toxicity which is usually lower than that of their tributyltin counterparts[173]. Bis (tributyltin) oxide is a organic solvent based wood preservative. It is interesting to note that the activity of bis(tributyltin) oxide against wood destroying fungi appears to be enhanced in presence of water[177,178]. This is probably due to the ability of the tributyltin compound in aqueous solvents to penetrate into the cell walls of the wood to extent and confer a rather greater protection from fungal enzymatic attack.

In India, Lentinus praerigidus and Poria dunicolus are the common wood rotting fungi inflicting great economic loss by causing decay of stacked logs of Shorea robusta and Melotenia mhoonni respectively. From the available literature it seems that Nandi and his workers[161,162] have done the toxicity test on L.praerigidus and P.dunicolus using a number of wood preservatives, but no work with organotin compounds against these fungi has been done in India. In the present investigation, toxicity test of some selected organotin co-ordination compounds on L.praerigidus and P.dunicolus has been undertaken.

1. Materials and methods:

1(a) Organisms:

1(a)(i) Lentinus praerigidus Berk: The secondary mycelial

culture was collected from the Mycology Division, Forest Research Institute and Colleges, Dehra Dun, India.

1(a)(ii) Fomes durissimus Lloyd: Secondary mycelial culture was collected from the Mycology and Plant Pathology Laboratory, Burdwan University, India.

1(b) Wood

1(b)(i) Shorea robusta Gaertn. f. (the common Sal) : Sap wood blocks of S.robusta were collected from the Government Saw Mill of Siliguri, West Bengal, India.

1(b)(ii) Swietenia mahogani Linn. : Sap wood blocks of S. mahogani were collected from the Burdwan University, West Bengal, India.

1(c) Compounds:

Bis(tributyltin) oxide (TBTO) and 7 organotin co-ordination compounds as bis (triphenyltin) oxalyl bis-N-p-tolyl hydroxamate, triphenyltin dithizonate, tributyltin diphenyl carbazonate tripropyltin diphenyl carbazonate, di-butyltin bis(diphenyl carbazonate), diphenyltin bis(diphenyl carbazonate) and Di-p-tolyl tin bis(diphenyl carbazonate) were used.

1(d) Toxicity test:

Toxicity tests of the compounds against the test fungi were primarily done following the simple American method and

finally by European method on the basis of knowledge gained in American method.

1(d)(i) American agar method:

Acetone solution of suitable quantity of the compound in sterile distilled water was incorporated into melted 8% malt agar so as to get the desired concentration of the compound in the media. Media with desired concentration of compounds were poured in petriplates and after solidification, were inoculated at the centre with uniform discs (7 m.m) of mycelia, punched out with a sterile cork borer from the advancing zone of a culture of test fungus. 3 replications of each test with appropriate control under same conditions were maintained. The petriplates were then incubated at 30±1°C in dark. Linear growth of the fungal discs were measured after a definite period, and the percentage of inhibition over control was calculated following the equation given by Vincent(111).

1(d) (ii) European wood block method:

In this method, standard sized (2" x 1" x ¾") wood blocks were prepared from sap wood of T.robusta and S.mahogani. Sap wood was selected as it was found to be non-resistant against the fungal attack(161,162). Test blocks were properly numbered, dried to constant weight at 60°± 1°C in an oven and thenimpregnated with different concentrations of the compounds.

Concentrations used in this case, were determined on the basis of the results obtained in American method. Compounds showed better inhibitory effect in American method, were selected for wood-block method. The blocks were taken in 1000 ml filtering flask containing the water suspension of the compound and connected with a suction pump for rapid impregnation of the compound under reduced pressure. When the wood-blocks become submerged in the suspension, the pressure was released and allowed to remain in the suspension for 1 hour. The blocks were wiped lightly to remove excess suspension and re-weighed to determine uptake of compound. The actual quantity of compound remained in the wood was finally calculated in terms of µg per cubic inch. Three replicates for each concentration of a compound were used. The blocks similarly treated with distilled water containing appropriate amount of solvent were treated as controls. The impregnated wood-blocks were then sterilised in the usual way and then exposed to the attack of mycelia of test fungus for about 6 months in laboratory condition. At the end of experimental period, the final dry weight of the test-blocks were taken and the difference between the initial and final weight to give the loss in weight due to fungal action.

2. Results:

From the tables 91 and 93 it is evident that among the test compounds, bis(triphenyltin) oxalyl bis-4-p-tolyl hydroxamate, tributyltin diphenyl carbamonate and bis(tributyltin) oxide are the active compounds for growth inhibition of L. praerigidus and P.durissimus.

In the wood-block method (tables 92 and 94), the fungal activity is not totally inhibited even at the highest concentration of the compound used, although that particular concentration has been found to be highly toxic in the agar-medium test which is fully in accordance with the results obtained by Findlay. Bis (tributyltin) oxide is found to be the most effective among the 3 compounds used for both Shorea robusta and Swietenia mahogani where as bis(triphenyltin) oxalyl bis-4-p-tolyl hydroxamate is the least effective one.

3. Discussion:

L.praerigidus and P.durissimus which cause considerable loss to the national economy in India by decaying loss of S.robusta and S.mahogani respectively. Toxicity tests in the laboratory reveal that the sap wood decay can be effectively controlled by using the proper concentrations of the tri-organotin compounds. Of the 3 compounds used, bis(tributyltin)

Table - 21

Effect of some Organotin compounds on [illegible] after 5 days incubation.

Concentration (μg/ml)	Percentage of growth inhibition over control of							
	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8
62.50	100.00	100.00	100.00	100.00	88.23	44.25	77.19	25.92
31.25	100.00	100.00	100.00	100.00	46.73	30.76	50.37	1 .85
15.62	100.00	100.00	90.72	100.00	27.93	15.93	33.33	0.00
12.50	100.00	100.00	85.94	100.00	16.51	7.69	22.00	0.00
6.25	93.40	93.17	77.22	80.29	2.75	0.00	12.23	0.00
3.13	90.60	80.11	63.50	72.22	0.93	0.00	7.50	0.00
1.25	65.60	56.60	45.90	59.50	0.00	0.00	3.50	0.00
0.31	20.90	24.47	23.10	31.00	0.00	0.00	0.20	0.00
0.15	2.03	8.75	7.72	2.15	0.00	0.00	0.00	0.00

C_1 = Bis(tributyltin) oxide

C_2 = Bis(triphenyltin)oxalyl bis-N-p-tolyl hydroxamate

C_3 = Tri-phenyltin dithizonate

C_4 = Tri-butyltin diphenyl carbazonate

C_5 = Tripropyltin diphenyl carbazonate

C_6 = Di-butyltin bis-(diphenyl carbazonate)

C_7 = Di-phenyltin bis-(diphenyl carbazonate)

C_8 = Di-paratolyltin bis-(diphenyl carbazonate)

Table - 32

Effect of some Organotin compounds on F.durissimus after 20 days incubation.

Concentration (µg/ml)	Percentage of growth inhibition over control of							
	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8
62.50	100.00	100.00	96.15	100.00	25.00	16.00	21.27	30.35
31.25	93.16	85.71	76.92	93.15	17.30	2.00	14.89	17.35
15.62	87.50	47.61	56.53	80.76	1.92	0.00	6.38	8.92
12.50	79.16	35.71	14.84	69.23	0.00	0.00	0.00	0.00
6.25	49.66	9.52	0.00	42.30	0.00	0.00	0.00	0.00
3.13	20.83	0.00	0.00	13.16	0.00	0.00	0.00	0.00
1.25	12.50	0.00	0.00	3.84	0.00	0.00	0.00	0.00
0.31	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

C_1 = Bis(tributyltin) oxide

C_2 = Bis(triphenyltin)oxalyl bis-4-p-tolyl hydroxamate

C_3 = Tri-phenyltin dithiocnate

C_4 = Tri butyltin diphenyl carbazonate

C_5 = Tri-propyltin diphenyl carbazonate

C_6 = Di-butyltin bis(diphenyl carbazonate)

C_7 = Di-phenyltin bis-(diphenyl carbazonate)

C_8 = Di-paratolyltin bis-(diphenyl carbazonate)

Table - 98

Toxicity test with sap wood of *S.rubneta* treated with different organotin preservatives and exposed to *L.praemorigidus* for a period of 6 months at 30 ± 1°C

Treatment	Concentration (µg/ml)	Initial dry weight* (g)	Weight after treatment* (g)	Absorption* (g)	Absorption* (µg/inch^3)	Final* dry weight (g)	Loss* (g)	Loss* (%)
Bis(triphenyltin)	31.25	10.992	12.880	1.888	59.000	10.016	0.976	8.88
oxalyl bis-4-o-tolyl hydrazonate	15.62	10.694	12.621	1.927	30.099	9.631	1.013	9.47
	12.50	10.683	12.608	1.920	24.000	9.579	1.104	10.33
Tributyltin diphenyl carbazonate	31.25	10.571	12.152	1.581	49.406	9.861	0.710	6.72
	15.62	10.940	12.631	1.691	26.413	9.997	0.943	8.72
	12.50	10.576	12.180	1.604	20.050	9.576	1.000	9.46
Bis(tributyltin) oxide	31.25	10.244	12.115	1.871	58.463	9.937	0.247	2.41
	15.62	10.698	12.462	1.764	27.553	10.019	0.679	6.35
	12.50	10.483	12.161	1.678	20.975	9.585	0.918	8.76
Control	-	11.542	-	-	-	10.251	1.291	11.18

*Average of 3 replicates.

Table - 94

Toxicity test with sap wood of *S.mahagoni* treated with different Organotin preservatives and exposed to *P.chrysosporium* for a period of 6 months at 30±1°C.

Treatment	Concentration (µg/ml)	Initial dry weight* (g)	Weight after treatment* (g)	Absorption* (g)	Absorption* (µg/inch³)	Final* dry weight (g)	Loss* (g)	Loss* (%)
Bis(triphenyltin)oxalyl bis-N-p-tolyl hydroxamate	200.00	9.373	12.012	2.139	427.800	9.375	0.638	6.04
	100.00	10.171	12.317	2.146	214.600	9.689	0.648	6.31
	62.50	9.768	12.677	2.514	144.625	8.970	0.784	8.03
Tributyltin diphenyl carbonate	200.00	9.392	11.545	2.153	430.600	9.146	0.246	2.62
	100.00	10.365	12.480	2.115	211.500	10.076	0.439	4.65
	62.50	11.621	13.967	2.346	146.625	10.709	0.912	7.85
Bis(tributyltin) oxide	200.00	9.976	12.114	2.138	427.600	9.838	0.138	1.38
	100.00	9.955	12.029	2.074	207.400	9.559	0.396	3.98
	62.50	10.170	12.591	2.421	138.312	9.438	0.632	6.70
Control	-	10.713	-	-	-	9.844	0.869	8.11

* Average of 3 replicates.

oxide is found to be the most effective since the test fungi show least tolerance for this compound. Tributyltin diphenyl carbamate and bis(triphenyltin) oxalyl bis-1-p-tolyl hydrazonate also check the decaying capacity of the test fungi to some extent.