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Introduction:

Recently, many developing countries have achieved considerable increase in the production of food grains through use of high yielding crop varieties along with improved agricultural practices. But such efforts are not accompanied by a corresponding improvement in technology of post-harvest protection against parasites.

India, too, has increased her food grain production tremendously through improved agricultural practices in recent years. Rice is, however, the most important cereal crop produced, reaching around 60 million tons a year. Sanikkar[431] stated that in India rice grains was more subject to deterioration than that of any other cereal crops, for it was grown and stored under humid and often primitive conditions. Prevailing fairly high day temperature and high relative humidity in

atmosphere cause rise in moisture content of the grains above the dangerous level of 14% resulting thereby in growth of micro-organisms not only in private storages but also in government owned FCI (Food Corporation of India) godowns. The present day capacity of FCI godowns is far less than the production and hence, a large portion of the grains harvested are either kept open or stored in private houses in an unscientific manner. In such cases the grain health is often affected by the atmospheric conditions that cause the moisture level to rise and thus facilitate store fungi to grow.

According to FAO (Food and Agricultural Organisation) a large amount of food grains harvested is lost before reaching the consumer. It has also been estimated that in some of the South American countries, certain parts of the Indian subcontinent and Africa, nearly 30-35% of the annual food production are lost by different agencies and a substantial share of these losses are due to fungi. In 1947 Padmanabhan[132] reported the presence of 61 fungal species with rice grains only. These fungi have been ecologically classified into field fungi and store fungi[133,134] although the classification is not taxonomically valid. Storage fungi are those that grow on stored products. Most of them are able to grow without free water and on media with a high osmotic pressure. They include the species of Aspergillus and Penicillium. There are no exact data on the world loss of food grains and other seeds

by store fungi. A rough and probably conservative estimate (FAO) put it at 5% of all harvested food grains during storage which in India may reach 30% of the annual harvest[135].

Under ordinary aerobic storage condition prevailing in India, fungal infections result in loss of viability, thus rendering the grain valueless for sowing purposes[136].

The problem of fungal deterioration of grains in storage have received particular attention after 1960[137,138,139]. The most easy method of preventing post harvest deterioration is to dry the grains to a moisture content of 10-12% for rice[140] with subsequent storage at temperature below 20°C. But artificial drying is expensive and the use of too high a temperature may reduce the quality of grains. The other conventional method is to store the grains under anaerobic sealed condition[141,142]. Moving unheated air through the grains is the other alternative[143]. Acute shortage of energy in developing countries has posed the problem in the effective implementation of these conventional costly methods, and hence search for other cheaper methods are continuing.

Many plant diseases are prevented with suitable fungicides and the same principle could be applied for storage fungi surviving on/with the grains. In 1947, Milner et al[144] reported 100 compounds as fungistatic agents when applied on

exist what. But in 1958, Geddes[145] stated that no chemical could so far be found for practical use. Altschul et al[146] in the same year were able to prevent deterioration of seeds including rice by halohydrin spraying. Dharam Vir et al[147] reported that metadinitrobenzene could prevent fungal growth of ground nut seeds in storge.

Although, considerable works have been done so far, in different parts of the world on post harvest deterioration of wheat[148,149], barley[150], rice etc.[151,152,153,154] with different types of compounds, but literature regarding the use of organotin compounds as grain preservatives are scanty.

The present investigation was undertaken to find out the effect of some organotin co-ordination compounds, proved to be highly effective against the store fungi Penicillium jensemi and Aspergillus niger under in vitro conditions, as rice grain preservatives in storage at grain moisture generally prevailing in India.

1. Materials and methods:

1(a) Grain sample:

Healthy rice grains of 'Patna' variety used in the present investigation, were collected from the farmers of Hooghly district, West Bengal, 3 months after harvest and stored for present study.

1(b) Compounds:

Bis(triphenyltin)oxalyl bis-d-p-tolyl hydroxamate, tributyltin diphenyl carbazonate and tributyltin acetate, proved to be highly effective (*in vitro*) against the store fungi *P.Jensseni* and *A.niger*, were used.

1(c) Determination of grain moisture:

Percentage of moisture content of rice grains was determined following Air-oven 130°C method (Proc. Int. Seed Test. Ass. 1966) and expressed on wet-weight basis. Triplicate samples of 10g each, were dried at 130± 1°C in a thermostatically controlled oven for constant weight. The samples were then immediately placed in a desiccator containing fused $CaCl_2$ for 45 minutes and then weighed quickly by balance having a sensitivity upto 1 mg. Percentage of moisture content was calculated from the loss of weight over the initial following the formula $\frac{A-B}{A} \times 100$, where A = initial weight and B = final weight after drying.

1(d) Establishment of desirable grain moisture:

Moisture content of the rice grains was raised to about 14% by adding requisite amount of sterile distilled water in tightly stoppered bottles and storing them at 5°C for 48 hours, with occasional shaking for equidistribution of moisture as far as practicable[165]. After recording the final moisture content of the grains, they were subjected to following treatment.

1(e) Treatment of grains:

Rice grains (triplicate set of 100 g each) with about 14% moisture were treated separately with 10 ml acetone containing requisite amount of the test compound to achieve the graded concentrations of 12.50, 25.00 and 50.00 μg/g grains (w/w). Grains treated only with acetone served as control (treated). During treatment, containers were shaken thoroughly for equidistribution of the compound. Containers were then kept open for 2 hours with periodic shaking for evaporating acetone. Triplicate set of grains with about 14% moisture served as control (untreated). Both the treated and untreated grains were then stored in polyethylene fibre bags which were kept under the influence of ambient temperature and relative humidity.

Grain samples were taken out from different sectors of the replicate bags at 15 days interval for 45 days, dried thoroughly, to study the percentage of mycoflora1 infection, percentage of a particular fungus present and germinability of the grains.

1(f) Determination of percentage of grain infections by fungi and presence of a particular fungus:

To determine the percentage of grains having internal mycoflora and grains infected by a particular fungus, agar plate method was followed. 100 grains from each treated and

untreated samples were separately surface sterilised by 0.1% $HgCl_2$ for 1 minute, washed thrice with sterile distilled water and then placed on malt-salt-agar (6% NaCl in 2% malt agar) media at pH 6.5 in petriplates (20 grains/plate) and incubated at 30 ± 1°C in dark for 12 days. Fungi, that grew out of the grains were isolated, identified and maintained as pure cultures.

At the time of calculation of percentage of infected grains, all infected grains were counted irrespective of whether or not they were infected by one or more fungi. But in case of calculation of percentage of infection by a particular fungus the number of grains infected with that particular one was only recorded.

After incubation, predominant fungi were recorded separately and poorly developed and unidentified fungal colonies were grouped under the common head "miscellaneous fungi" in the tables.

1(g) Determination of germinability:

Germination percentage of the test grains (100 each) were determined by soaking them in sterile distilled water for 24 hours and placing them on moist 3 layered filter papers in closed sterile petriplates. Plates were incubated at 30 ± 1°C for 6 days in dark. Grains producing a root or a coleoptile were recorded as germinated without regard to the

potential to produce a plant under normal circumstances.

2. Results:

2(a) Percentage of infection:

Untreated control grains showed 52% infection after 15 days and 80% after 45 days of storage. Treated control grains showed no significant variation of fungal infection from the untreated one.

Grains treated with the compounds showed their effect on fungal infection compared to control (treated). Among the tested compounds, grains treated with bis (triphenyltin) oxalyl bis-N-p-tolyl hydroxamate showed 0% infection at 50 µg/g concentration after 0 days of storage where as the corresponding data for tributyltin diphenyl carbamonate and tributyltin ac etate were 0% each. Results indicate that among the tested compounds, tributyltin acetate is the cost effective one. All the treated rice grains showed gradual increase of fungal infection along with the increasing storage periods. Aspergillus parasiticus and Aspergillus sp were the predominant fungi that appeared in control as well as from treated grains.

2(b) Germinability:

At about 14% moisture content, untreated control grains showed no significant change in germinability even after 45 days of storage. The treated control grains showed 80% germination at 0 days and with the advancement of storage periods,

Table - 67

Treatment of Rekha cultivar of rice grains having 14% moisture with different concentration of tributyltin acetate.

Concentration (µg/g)	Storage period (days)	Germination* (%)	Total infection* (%)	Percentage of grains infected* by F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9	F_{10}	F_{11}	F_{12}
12.50	0	68	10	1	6	2	4	2	1	4	2	0	1	1	4
25.00		65	10	0	4	3	1	0	0	2	2	1	0	1	6
50.00		50	0	0	0	0	0	0	0	0	0	0	0	0	0
12.50	15	64	27	1	10	2	7	1	0	9	4	0	5	0	10
25.00		56	19	0	9	5	1	0	1	7	6	3	3	0	13
50.00		80	7	0	2	0	1	0	0	2	1	1	2	0	3
12.50	30	60	31	2	10	3	5	0	0	13	6	5	5	0	11
25.00		55	26	1	7	3	3	0	0	9	4	2	4	0	9
50.00		47	13	0	4	3	1	0	0	4	3	2	2	0	9
12.50	45	55	43	2	13	4	4	0	0	14	8	3	2	0	24
25.00		51	23	2	6	5	3	0	0	11	6	2	5	0	17
50.00		44	25	2	7	3	3	0	0	7	7	0	5	0	12

F_1 = Aspergillus niger
F_2 = Aspergillus parasiticus
F_3 = Aspergillus flavus
F_4 = Aspergillus sydowi
F_5 = Rhizopus nodosum
F_6 = Rhizopus oryzae
F_7 = Aspergillus sp
F_8 = Penicillium citrinum
F_9 = Aspergillus ochraceous
F_{10} = Aspergillus terreus
F_{11} = Curvularia lunata
F_{12} = Miscellaneous fungi

*Mean of 3 replications

Table - 33

Treatment of Ratna cultivar of rice grains having 14% moisture with different concentration of tributyltin diphenyl carbamate.

Concentration (μg/g)	Storage period (days)	Germination* (%)	Total infection* (%)	Percentage of grains infected* by F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9	F_{10}	F_{11}	F_{12}
12.50		65	31	1	7	2	3	2	0	5	3	1	0	1	3
25.00	0	60	15	0	5	1	2	1	0	2	2	0	1	1	5
50.00		43	0	0	0	0	0	0	0	0	0	0	0	0	0
12.50		66	28	2	8	3	6	1	0	9	3	2	4	2	7
25.00	15	62	19	1	5	1	0	0	0	7	2	1	3	0	9
50.00		41	9	0	3	1	0	0	0	6	2	0	0	0	7
12.50		61	34	1	11	3	5	0	0	8	6	7	7	0	10
25.00	30	51	27	0	7	3	4	0	1	9	7	1	0	0	12
50.00		34	19	1	3	2	0	0	0	6	4	2	3	0	10
12.50		52	49	3	10	6	9	0	1	11	9	0	6	0	8
25.00	45	40	41	0	0	5	6	0	0	7	0	3	0	0	17
50.00		32	33	0	6	2	7	0	0	5	7	5	7	0	22

F_1 = Aspergillus niger

F_2 = Aspergillus parasiticus

F_3 = Aspergillus tamarii

F_4 = Aspergillus ochraceus

F_5 = Absidia reflexa

F_6 = Nigrospora oryzae

F_7 = Aspergillus sp

F_8 = Penicillium citrinum

F_9 = Aspergillus ochraceous

F_{10} = Aspergillus terreus

F_{11} = Curvularia lunata

F_{12} = Miscellaneous fungi

*Mean of 3 replicates

Table - 89

Treatment of Ratna cultivar of rice seeds having 14% moisture with different concentrations of bistriphenyltin/oxalyl bis-1-p-tolyl hydrazonate

Concentration (µg/g)	Storage period (days)	Germination* (%)	Total infection* (%)	Percentage of grains infected* by F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9	F_{10}	F_{11}	F_{12}
12.50		81	28	1	9	2	2	1	1	6	0	2	3	2	12
25.00	0	72	20	1	6	0	2	0	1	6	5	2	4	1	9
50.00		68	8	0	4	0	1	0	1	2	2	0	0	2	6
12.50		79	33	2	11	2	2	1	0	9	3	3	3	0	13
25.00	15	70	26	1	8	3	4	0	0	7	7	2	2	1	14
50.00		68	15	1	7	2	2	0	1	5	3	1	3	1	10
12.50		73	37	2	11	5	6	0	0	12	3	2	4	0	9
25.00	30	67	31	1	7	4	4	0	0	9	3	4	2	0	13
50.00		66	21	1	3	3	2	0	0	7	7	3	2	0	11
12.50		71	54	3	13	4	4	0	0	17	11	4	4	0	12
25.00	45	67	41	1	14	3	3	0	0	11	9	4	3	0	21
50.00		60	34	2	13	3	3	0	0	9	7	2	3	0	17

F_1 = Aspergillus niger

F_2 = Aspergillus nomaioticus

F_3 = Aspergillus tamarii

F_4 = Aspergillus ordovi

F_5 = Absidia reflexa

F_6 = Rhizopus oryzae

F_7 = Aspergillus sp

F_8 = Penicillium citrinum

F_9 = Aspergillus ochraceous

F_{10} = Aspergillus terreus

F_{11} = Curvularia lunata

F_{12} = Miscellaneous fungi

*Mean of 3 replicates

Table - 20

Mycoflora associated with the Kotha cultivar of rice grains in storage

Treatment	Storage period (days)	Germination* (%)	Total infection* (%)	Percentage of grains infested* by F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9	F_{10}	F_{11}	F_{12}
Control (treated)	0	80	45	3	11	4	5	4	0	3	7	3	2	3	14
	15	78	49	2	11	5	4	2	0	10	9	7	1	1	17
	30	75	53	3	17	6	6	2	0	13	8	7	3	0	25
	45	70	57	5	10	7	6	0	0	15	10	9	7	0	21
Control (untreated)	0	90	46	2	15	3	4	2	1	9	6	2	1	2	11
	15	87	52	1	19	2	4	1	0	11	9	5	4	2	9
	30	84	58	3	17	5	5	0	0	13	9	6	7	0	19
	45	85	53	4	19	4	4	0	0	21	11	6	9	0	18

F_1 = *Aspergillus niger*

F_2 = *Aspergillus flavus*

F_3 = *Aspergillus tamarii*

F_4 = *Aspergillus oryzae*

F_5 = *Absidia reflexa*

F_6 = *Mucor racemosus*

F_7 = *Aspergillus sp*

F_8 = *Penicillium citrinum*

F_9 = *Aspergillus ochraceus*

F_{10} = *Aspergillus terreus*

F_{11} = *Curvularia lunata*

F_{12} = Miscellaneous fungi

*Mean of 3 replicates

germinability gradually decreased and reaching about 70% after 45 days of storage, where the corresponding value is 85% for untreated control. Reduction of germinability within such a short period was presumably due to the effect of acetone used.

Grains treated with bis(triphenyltin)oxalyl bis-N-p-tolyl hydroxamate showed no significant effect on germinability at 12.50 μg/g concentration even after 45 days of storage compared to treated control. But higher concentrations (25.00 and 50.00 μg/g) showed a marked toxic effect on germination. Treatment with tributyltin diphenyl carbamate and tributyltin acetate showed sharp decrease in germinability even at lowest concentration (12.50 μg/g).

3. Discussion:

In the present investigations, the predominant species of storage fungi of rice grains belongs to Aspergillus, which corroborates the earlier findings of Christensen and Kaufmann (134). All the tested compounds are notably toxic against storage fungi of rice grains at 0 days, but their efficiency for controlling fungal population gradually diminish with the increase of storage periods, which may be due to the gradual decomposition of the compounds. Among the tested compounds, tributyltin acetate is the most effective one where as bis (triphenyltin)oxalyl bis-N-p-tolyl hydroxamate is least

effective which coincide with the finding of conidial germination inhibition studies of *A.niger in vitro*.

Though the organotin compounds, studied here, are able to control the fungal population of treated rice grains in storage, but germinability tests also indicate the great phytotoxic nature of the compounds. Similar type of adverse effect have also been observed with organomercurials[65] which are being extensively used for seed treatment.

Thus considering both the antifungal and phytotoxic nature of the tested compounds, further investigations will be needed to find out the new findings about the possibility of organotin compounds for the use of rice grain preservation in storge.