C. MICROSCOPY OF RICE

Introduction:

The *in vitro* tests of fungicidal activity have limited value to the agricultural pesticide chemist because it circumvents the important consideration of phytotoxicity. A fungicide which causes serious phytotoxicity under varied environmental conditions would be a total failure.

Ferric dimethyldithiocarbamate (Ferbam) has been successfully used as a protectant fungicide against a wide variety of fungal diseases of fruits, vegetables and ornamental plants. In 1965 Prasad and Kramer[115] reported the high antagonic activity of ferbam when it was applied to growing *Allium cepa* roots. The very popular bordeaux mixture, used for controlling the disease of apple scab[116], citrus canker[117], coconut leaf rot[118], grapes downy mildew[119], potato early blight[120], tomato early blight[120], mango

anthracnose[121] etc., also shows phytotoxic effects against certain crops, particularly fruits like apples, peaches etc. which is characterized by chlorosis followed by brown or purple flecks on the leaves or fruits[122].

Regarding the organometallic fungicides, the very common organomercury compounds like ethylmercury chloride, phenylmercury acetate, methoxy ethyl mercury chloride etc. which are being used for seed treating agents, show seed injury which has been characterized by abnormal germination[85,86].

Organotin compounds like triphenyltin acetate (TPTA) and triphenyltin hydroxide (TPTH) have been successfully used in the control of several plant diseases. Potato late blight control by organotin compounds has been reported by several workers[123,124,125,126], but some have observed the phytotoxic effect. Hanson and Merriam[123] considered both TPTA and TPTH are promising materials for controlling the late blight disease of potato with some phytotoxicity of TPTA. Cotap[126] reported both TPTA and TPTH to be phytotoxic, but Callbeck[124] did not observe any phytotoxicity.

Floyd and Johnson[127] reported good control of Cercospora beticola on sugarbeet with both TPTA (40% WP) or TPTH (50% WP) without any phytotoxic effect. Setterly[128]

reported good control of cucumber anthracnose on leaves, gummy stem blight and downy mildew with TPTA (60% WP) at 0.025%, but found it to be phytotoxic. Hocking and White[129], Hopf and his associates[130] showed that TPTA is notably toxic to rice plants.

With this end in view, in the present investigation the phytotoxic effect if any, of bis(triphenyltin) oxalyl bis-d-p-tolyl hydroxamate, tributyltin diphenyl carbazonate and tributyltin acetate, which were proved to be effective for controlling the rice plant against brown leaf spot disease, on rice has been undertaken.

1. Materials and methods:

1(a) Seed sample: Healthy rice seeds of "Dhanial" variety, collected from Chinsurah Rice Research Farm, Hooghly, West Bengal, were used in the present investigation.

1(b) Compounds: Bis(triphenyltin)oxalyl bis-d-p-tolyl hydroxamate, tributyltin diphenyl carbazonate and tributyltin acetate were used for determining their phytotoxic effect on rice.

1(c) Effect on seed germination: Healthy rice seeds of "Dhanial" were dipped in compound suspension of 100, 50 and 25 µg/ml concentrations for 1, 4 and 8 hours. For control,

water with requisite amount of acetone was used. The treated seeds were then placed on moist three layered filter paper in closed petriplates. Plates were incubated at 30 ± 1^{0}C. 100 seeds were maintained for each treatment. After 8-days, the germinated seeds were counted. Seeds producing a root or a coleoptile were recorded as germinated.

1(d) Effect on root and shoot length of germinated seed: Seeds were treated as previously described and placed in open petriplates with adequate water. 50 seeds were maintained for each treatment. The plates were then kept at 30 ± 1^{0}C. After 7 days the length of root and shoot of the germinated seeds were measured.

1(e) Effect on shoot length: Rice plants of "Dhanial" were grown on 6-inch-diameter earthenware pots containing equal amount of soil and fertiliser. In each pot, 10 plants were grown. After 40 days old, the initial height of the aerial part of each plant was measured. The plants were then sprayed almost to run off with the equal amount of compound suspension (2 ml/plant) of 100, 50 and 25 μg/ml concentrations for 10 days at 24 hours intervals. In each concentration for a compound, 100 plants were maintained. After 24 hours of last spraying, the final height of each plant was measured and the phytotoxic effect were determined considering the initial and final height.

2. Results

2(a) Seed germination

Results regarding the toxic effect of the test compounds on rice seed germination in table -34 reveal that all the compounds have very little toxic effect at 25 and 50 µg/ml concentrations. But seeds treated with tributyltin diphenyl carbamate of 100 µg/ml concentration for 1, 4 and 8 hours, show 72, 70 and 62% germination respectively, where the corresponding figures for control are 92% each. At the same concentration, bis (triphenyltin) oxalyl-bis-4-p-tolyl hydrazonate and tributyltin acetate show 78 and 80% germination after 8 hours treatment. From the table - 34, it can be said that the toxic effect of all the test compound depend on the concentration and duration of treatment.

2(b) Root and shoot length of germinated seed:

Results summarised in the table - 35 indicate that both root and shoot length of the treated germinated rice seeds are highly inhibited even at 25 µg/ml concentration and the inhibition are directly related with the concentration used and duration of treatment. Results also indicate that the root length of the germinated rice seeds are more affected than the shoot length for all test compounds.

Table - 64

Effect of bis(triphenyltin)oxalyl bis-N-p-tolyl hydroxamate, tributyltin diphenyl carbamate and tributyltin acetate on rice (Dharial) seed germination.

Treatment	Concentration (µg/ml)	Percentage of germinated seed, treated for		
		1 hour	4 hours	8 hours
Bis(triphenyltin)oxalyl bis-N-p-tolyl hydroxamate	100.00	90	86	78
	50.00	90	90	90
	25.00	90	90	84
Tributyltin diphenyl carbamate	100.00	72	70	62
	50.00	92	84	80
	25.00	92	94	86
Tributyltin acetate	100.00	88	82	80
	50.00	86	86	90
	25.00	94	92	90
Control	-	92	92	92

Table - 35

Effect of bis(triphenyltin)oxalyl bis-3-carotolyl hydrazonate, tributyltin diphenyl carbazonate and tributyltin acetate on root and shoot length of germinated rice (Bharial) seeds.

Treatment	Concentration (ppm)	Seed treated for					
		1 hour		4 hours		8 hours	
		Root length (m.m)	Shoot length (m.m)	Root length (m.m)	Shoot length (m.m)	Root length (m.m)	Shoot length (m.m)
Bis(triphenyltin)oxalyl bis-3-p-tolyl hydrazonate	100.00	4.06	4.71	3.70	4.46	3.07	3.57
	50.00	5.57	5.96	5.20	5.71	4.46	5.46
	25.00	8.42	6.71	8.93	7.60	6.93	6.63
Tributyltin diphenyl carbazonate	100.00	5.00	4.61	2.57	3.30	1.09	1.74
	50.00	11.00	6.71	8.32	6.57	5.77	6.12
	25.00	13.30	7.93	13.25	9.76	13.72	7.02
Tributyltin acetate	100.00	2.54	5.38	1.41	2.19	1.30	3.17
	50.00	10.13	8.00	5.04	6.34	5.20	6.09
	25.00	7.50	6.68	10.41	7.68	21.00	10.63
Control	--	15.67	8.69	21.47	10.86	22.24	10.98

2(c) Effect on shoot length:

Results summarised in the table -33 reveal that no significant toxic effect of the test compounds even at 100 µg/ml, when it was applied on 60 days old rice plants, compared to control.

3. Discussion:

The above results clearly indicate that the test organotin compounds are appreciably toxic when applied directly on rice seeds and their toxicity depend upon the concentration and the duration of treatment. During the treatment, possibly the compounds act upon the embryo of the seeds which ultimately inhibit the growth of embryo. Hence the use of organotin compounds, studied here, may not be suitable for rice seed treatment. But foliar spraying of the test compounds even at 100 µg/ml concentration show no toxic effect on rice plants.

Thus considering both the phytotoxic effect and protectant activity against H.oryzae infection on rice, it can be said that the test compounds may be applicable as foliar spray for controlling the brown leaf spot disease of rice.

Table - 66

Effect of bis(triphenyltin) oxalyl bis-N-para-tolyl hydroxamate, tributyltin diphenyl carbazonate and tributyltin acetate on shoot length of rice (Bharial) plants.

Treatment	Concentration (μg/ml)	Mean Shoot length (c.m)		Difference
		Initial	Final	
Bis(triphenyltin)oxalyl bis-N-p-tolyl hydroxamate	100.00	114.78	120.71	5.93 ± 0.27
	50.00	116.25	122.37	6.12 ± 0.29
	25.00	118.31	113.53	5.72 ± 0.31
Tributyltin diphenyl carbazonate	100.00	108.96	115.04	6.08 ± 0.23
	50.00	113.58	119.56	6.18 ± 0.32
	25.00	116.62	122.46	5.84 ± 0.23
Tributyltin acetate	100.00	114.48	120.59	6.11 ± 0.24
	50.00	102.98	115.97	5.00 ± 0.24
	25.00	110.74	122.84	6.10 ± 0.26
Control	-	115.67	121.53	5.86 ± 0.29