CHAPTER-8

CHAPTER-8

Study of Biocidal Properties and activities of the organotin(IV) derivatives of Dithiocarboxylic acids and the related ligands

§ 8.INTRODUCTION:

In earlier chapters the preparation and characterisation of some newly synthesised Organotin(IV) complexes have been reported here. We discuss the effect of these compounds on a number of selected organisms *in vitro*.

The toxicity of tin compounds were reviewed in 1959[1] and in 1964[2].The biological effects of organometallic compounds was reported as early as in 1968 by Barnes *et al*, the interesting point being that, unlike , Pb, Hg and As, toxicity only manifest in certain organotin compounds, inorganic tin being completely non-toxic.[4]

Search for new pesticides is essential as even successful pesticides can loose their effectiveness due to growth of resistance of pests towards these pesticides. Moreover, consideration of environmental pollution may lead to the discontinuance of some successful pesticides in future course of time. In the search of new pesticides,organometallic compounds are gaining much more importance day by day. Many organotin compounds are reportedly toxic to various organisms and a number of them have limited but significant uses as biocidal agents in agriculture and technology.[2,6] The main advantages of organotin agrochemicals, which mainly posses prophylactic action are their relatively low phytotoxicity, low toxicity[7,8] to nontarget organisms and lack of resistance to crop pests to these chemicals, besides degradation in environmental situation to harmless inorganic tin residue[9].

The toxicity of organotin(IV) compounds to mamals vary considerably according to the nature of bonding of tin with carbon

of the organic part. This study on mamals is also important because of its widespread use as agricultural fungicide.

Phytotoxicity on seed germination : The fungicidal activity of any compound will be of real value if the compound has no or very little value of phytotoxicity. For this reson, the compounds were tested for their phytotoxicity level. The results are also encouraging as the percentage of germination showed. Phytotoxicity of the synthesised compounds have been studied by spore germination method and found to be encouraging. The details are given in § 8.2.1.

Besides, the role of organotin(IV) compounds as bacteriocides deserves to be studied. A number of these compounds have been studied for their bacteriocidal activities and the priliminary results were found to be encouraging but the detailed study are in progress and hence not discussed here. A number of compounds were also studied for their antitumour activities, the details of which have been discussed in the subsequent relevant section of § 8.3.

§ 8.1.1. INTRODUCTION TO FUNGICIDAL ACTIVITY:

The first investigation[10] in the field of antifungal activity study dates back to as early as 1954. Fungicidal activity is reportedly strictly dependent upon the extent of alkylation of the tin atom, being at maximum with three tin-carbon bonds. In this context, minimum concentrations, in ppm, for complete inhibition of P. italicium, for a number of organotin compounds with different numbers of tin-carbon bond is given below for general look and perusal:

Fungus	Et_3SnCl	Et_2SnCl_2	$EtSnCl_3$	Et_4Sn
P italicium	2	100	>1000	>1000

The following table will give a general look at the activity of a number of R_3SnCl compounds against a fungi, as for example influenced by the nature of organic toxophoric groups, illustrated by the minimum concentrations, in ppm, required [11];

Compounds	Concn ppm	Fungus:Penicilium funiculosum
Pr_3SnCl	1	,,
Bu_3SnCl	4	,,
Ph_3SnCl	8	,,
Et_3SnCl	16	,,
$(C_5H_{11})_3SnCl$	250	,,
$(C_8H_{17})_3SnCl$	>125	,,
Me_3SnCl	>500	,,

But this generalisation does not hold for other R_3SnX compounds. Trialkyltin compounds find restricted application as agricultural fungicides due to high phyto toxicity, to some extent due to their mamalian toxicity and also because of less reactivity in the field than in the laboratory trials[2]. In contrast, triphenyl tin compounds were found to be less phytotoxic hence greater acceptability as fungicide in agriculture. Srivastava[12] found Ph_2SnCl_2 also to be highly active.

Considerable work directed at obtaining organotin fungicides with low phytotoxicity[13-16] level were found to have other disadvantages *e.g.* low range[17] of activity when number of fungi were considered.

Here we have tested antifungal activity of different types of organotin(IV) compounds against a number of plant pathogenic fungi. In the following sections the descriptive details of the experiments and results are given.

Although many phytopathogenic fungi prove to be highly sensitive to organotin compounds *in vitro*, some parasitic fungi *e.g. phytophthora infestans* on potato, *Corcospora beticola* on sugar beet, and *Septoria apii* on celeriac etc. are at present successfully controlled by organisms under field conditions.

Hatel and Tampel [18] found, with a series of organotin compounds on obligate parasitic fungi, that the effect on Peronospora is in the following:

Tributyltin > Trimethyltin > Triethyltin compounds

The following table will give a more general outlook.

Plant diseases	Plants	Nature of organotin compounds
Powdery mildew	Cucumber	Triethyl tin derivatives
do	Barley	Tributyltin and Triphenyltin derivatives
Apple mildew	Apple	Tripentyl and Tricyclohexyltin derivatives

In the symmetrical trialkyltin series, the propyl and butyltin compounds are reportedly the most effective and active, though the presence of these particular groups are not the necessary conditions for higher reactivity. Practical study show that trialkyl and tributyl tin compounds are better fungicides than triaryl tin compounds *in vitro* but the reverse is true in field due to lower stability and higher volatility of the former along with favourable factors of low phytotoxicity.

A number of commercial preparations based on different active ingradients are already marketted under different trade names.[10]

From available liturature study it appears that much work has yet not been done for controlling the plant pathogenic fungi by using organotin compounds. In the present investigation different types of organotin compounds were tested *in vitro* for determining their antifungal activity against a selected number of species *in vitro*.

§ 8.12. Experimental – Material and Methods:

A number of compounds are given in the table A for which the fungicidal properties had been studied, *in vitro*. A number of fungi were used for this purpose.

List of fungal isolates used:-

Species	code	Host of origin	Sorce of isolate
H.maydis	ITCC[a]-2675	zea mays	Plant pathology laboratory, centre for life sciences, N.B.U.
H.bicolor	..	..	,,
H.oryzae	ITCC[a]-2537	Oryzae-sativa	,,
H.sativum	ITCC[a]-2684	,,	,,

a=ITCC=Indian Type Culture Collection

b=Indian Agricultural research Institute(Division of mycology and plant pathology)PUSA,New Delhi-12

i)Preparation of culture media: 20 gms of malt extract agar (DIFCO) was boiled in water till dissolved. 20 gms agar-agar (KOBA, Japan) was added to it and boiled until agar-agar was dissolved. 0.05 gm of chloramphenicol, suspended in 5ml of 95% alcohol was added to the medium as antibacterial agent. Now slants

for culture of fungi, were prepared with this media by pouring about 2ml of this molten media in each test tube to remain in slanting condition till the media solidified. Then the media was impregnated with spore and kept in incubator. Average age of the spores used here was 15 days.

ii) Preparation of Spore suspension : After average time period of 15 days, spore suspension was prepared by sterile double distilled water and the concentration was adjusted to 30-40 spores per field and used subsequently for experiments.

ii) Study of the inhibitory effects: This is done by the method of spore germination <u>in vitro</u>. The number of spores germinated compared to the control, in four different concentrations of a particular compound, <u>viz</u>. X, X/10, X/100, X/1000, were calculated under magnification of 15×10, on an average of 500 spores studied per field, with an average age of the culture media of 15 days after an incubation period of 24 hours. The percentage of inhibition over control was calculated using the famous Vincent equation[97]

$$\text{Percentage of inhibition} = \frac{C-T}{C} \times 100$$

where C = number of spores germinated in control, T = Total number of spores germinated after treatement. The detailed procedure is as follows: In one glass slide two drops of acetone solution of a particular concentration of a particular compound were taken on two spots and the solvent was allowed to evaporate. Then 1 drop of wel-stirred spore suspension was added to these two spots. Similarly sets for all the concentrations were prepared. All the glass slides were then kept in an arranged manner in a glass plate covered tray under humid condition to favour the growth of spores.

After 24 hours, one drop of cotton blue was added to each spot on the glass slide to stain the germ tube of the germinated spores followed by the killing of the same by subsequent addition of lactophenol to these spots.

From these , the effective doses for 50% inhibition, ED_{50} were calculated in microgram per litre unit.

The detailed experimental results are given below in tabular forms:

Table A

ED_{50} values in vitro of a number of compounds in $\mu g/l$ for growth inhibition of a number of fungi of Helmenthesporium group of fungi.

Compounds	Name of fungi			
	H. maydis	H. bicolor	H. sativum	H. oryzae
Alkenyltin(IV) compounds				
1. $Me_2C{=}CHClSnMe_2$	12.615	62.44		
2. $[Ph_2C{=}C(Ph)]_2SnCl_2$	16680	16.680		
3. $Me_2C{=}CHSnPh_3$			237440	
Dithiocarbamate				
5. $(C_5H_{11}N{-}CS_2)_2SnPh_2$	10146	21138		
6. $(C_5H_{11}N{-}CS_2)_2SnR_2$ $R = Ph_2C{=}C(Ph)-$	11209	2377		
Thioaryloxy acetates *				
7. 4-Ptaa $SnPh_3$	3418			
8. $(4Prtt)_2SnPh_2$	39312			
9. 4-Ptaa $SnBu_3$	13233			
10. 2-Ptaa-$SnPh_3$				60000
11. $(2\text{-}Ptaa)_2SnPh_2$				35000
12. $(2\text{-}Ptaa)_2SnBu_2$				350000
13. 2-Ptaa-$SnBu_3$				200000

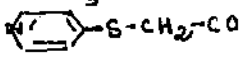
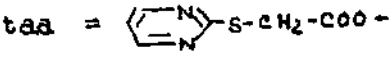
* 4Ptaa =  , 2 Ptaa = 

Table 1

Effect of $Me_2C{=}C(H)(Cl)SnMe_2$ [a] on H.maydis.

concentretion microgram/litre	Percentages of spore germination [b]	
	Treatement [c]	Control [d] [e]
870	10.8	96
	-88.8	0
87	24.38	96
	-74.6	0
8.7	48.58	96
	-49.4	0
0.87	82.17	96
	-14.4	0

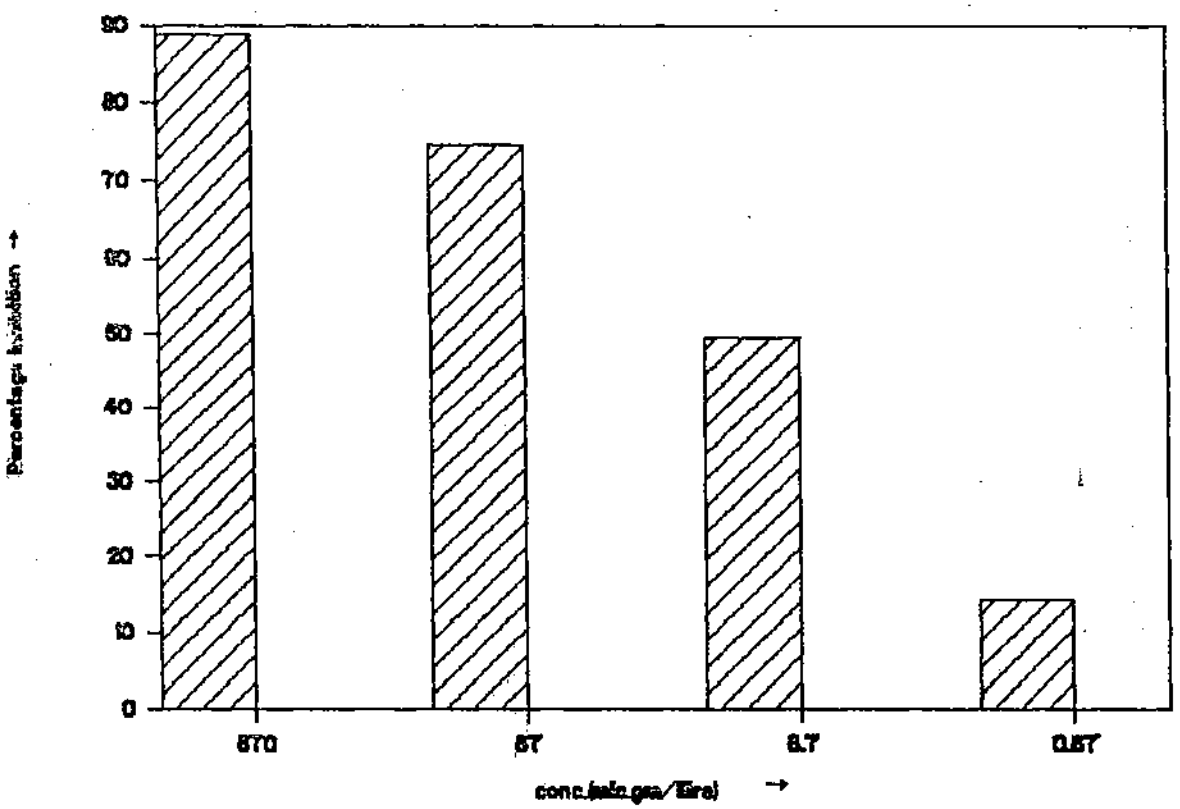
a incubation period 24 hours
b averages of 500 germs
c Values in parenthesis indicate % inhibition(-) or increase (+) over control and the values used for regression
d acetone control
e average age of culture 15 days

Regression Output:

Constant	43.47172
Std Err of Y Est	28.08877
R Squared	0.505862
No. of Observations	4
Degrees of Freedom	2
X Coefficient(s)	0.055157
Std Err of Coef.	0.038547

ED_{50} = 12.615 micro.gm/litre

PICTURE-1A



Picture-1B

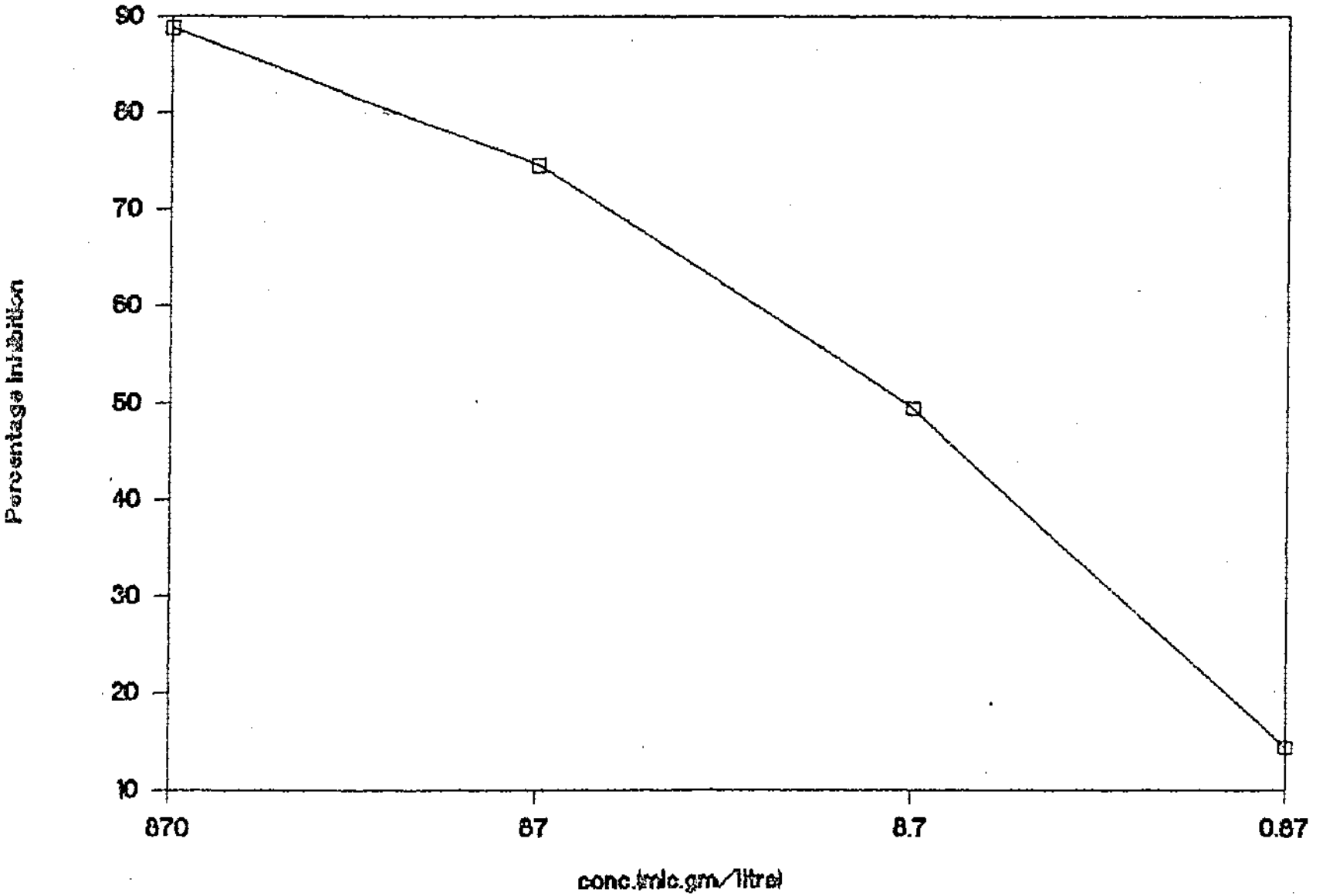

90
80
70
60
50
40
30
20
10
Percentage Inhibition
870
87
8.7
0.87
conc.(mic.gm/litre)

Table 2

Effect of $Me_2C{=}C(H)(Cl)SnMe_2$ [a] on *H. bicolor*

concentretion microgram/litre	b Percentages of spore germination	
	Treatement	Control
870	16.56	92
	-82	0
87	18.4	92
	-80	0
8.7	41.95	92
	-54.4	0
0.87	62.92	92
	-31.6	0

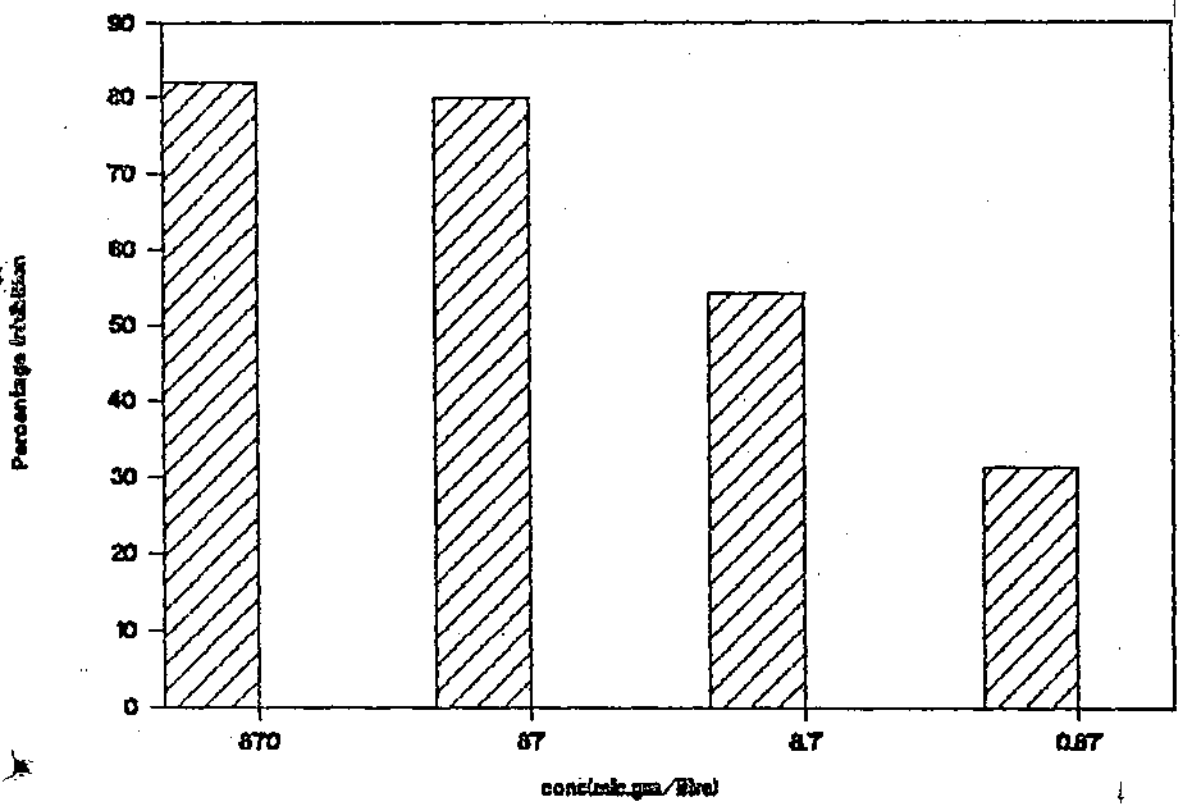
a incubation period 24 hours
b averages of 500 germs
c Values in parenthesis indicate % inhibition(-) or increase (+) over control and the values used for regression
d acetone control
e average age of culture 15 days

Regression Output:

Constant	53.41094
Std Err of Y Est	22.74944
R Squared	0.393245
No. of Observations	4
Degrees of Freedom	2
X Coefficient(s)	0.035544
Std Err of Coef.	0.031219

ED_{50} = 62.44 microgm/litre

Picture-2A


90
80
70
60
50
40
30
20
10
0
Percentage Inhibition
670
67
6.7
0.67
conc(mic.gm/Bml)

picture 2B

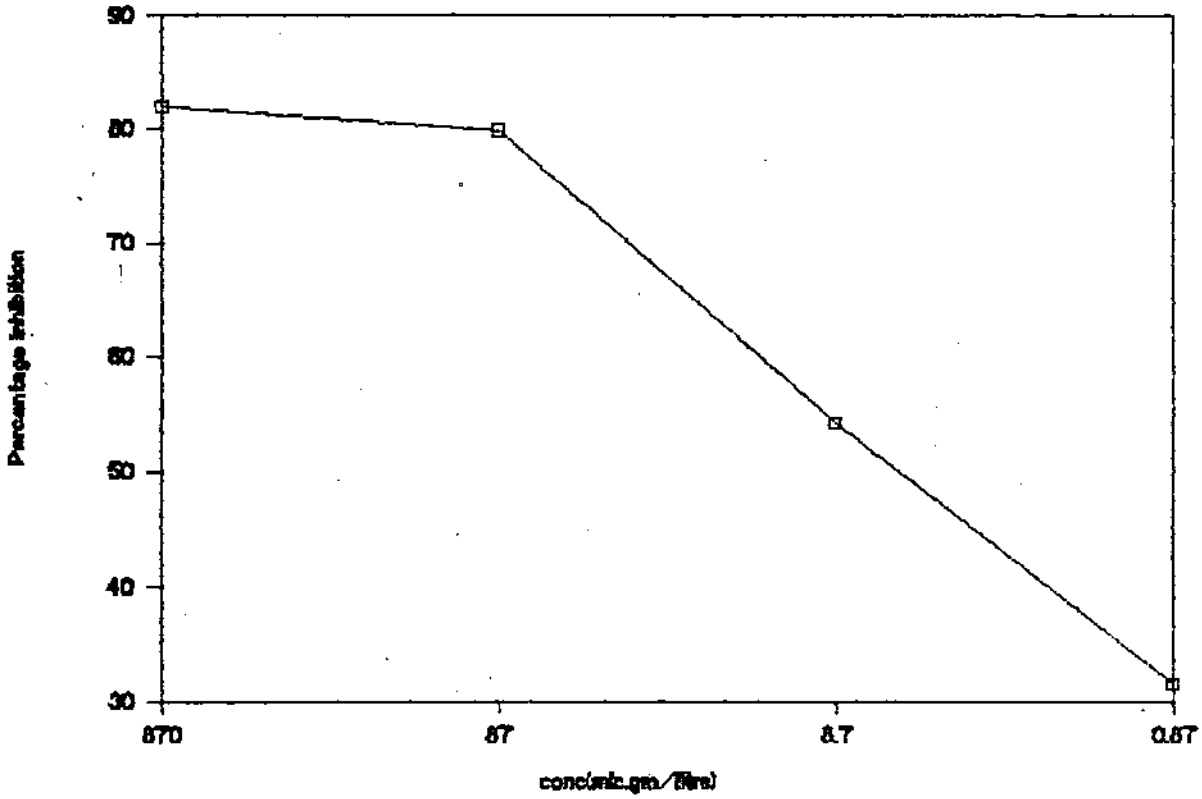

90
80
70
60
50
40
30
Percentage Inhibition
670
67
6.7
0.67
conc(mic.gm/litre)

Table 3

Effect of[Ph C=C(Ph)SnCl]$_2$[a] on H.maydis

concentretion microgram/litre	Percentages of spore germination[b]	
	Treatement	control[c]
1668000	7.68	96
	-92	0
166800	9.4	96
	-90.2	0
16680	48	96
	-50	0
1668	86.78	96
	-9.6	0

a incubation period 24 hours
b averages of 500 germs
c Values in parenthesis indicate % inhibition(-) or increase (+) over control and the values used for regression
d acetone control
e average age of culture 15 days

ED_{50} = 16680 microgm/litre

Table 4

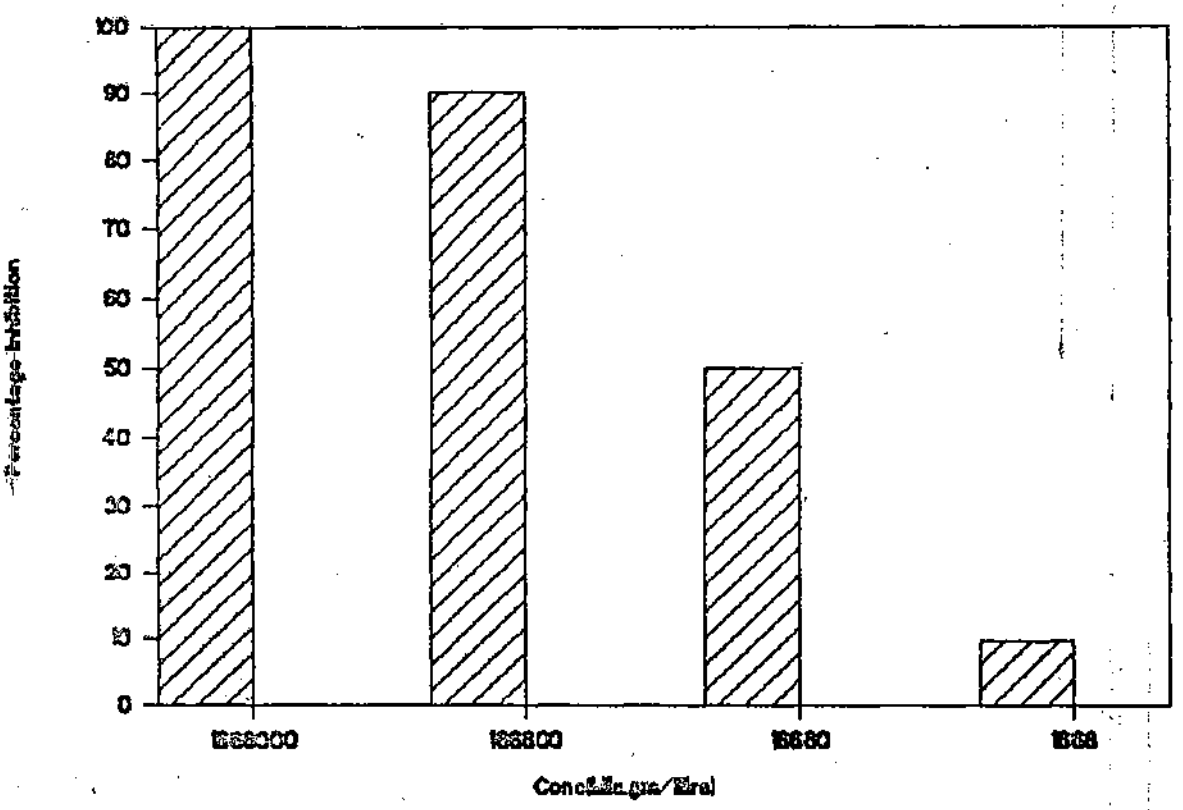
Effect of[Ph C=C(Ph)SnCl]$_2$[a] on H.bicolor

concentretion microgram/litre	Percentages of spore germination[b]	
	Treatement	control[c]
1668000	15.8	92
	-82.8	0
166800	17.29	92
	-81.2	0
16680	46	92
	-50	0
1668	71.02	92
	-22.8	0

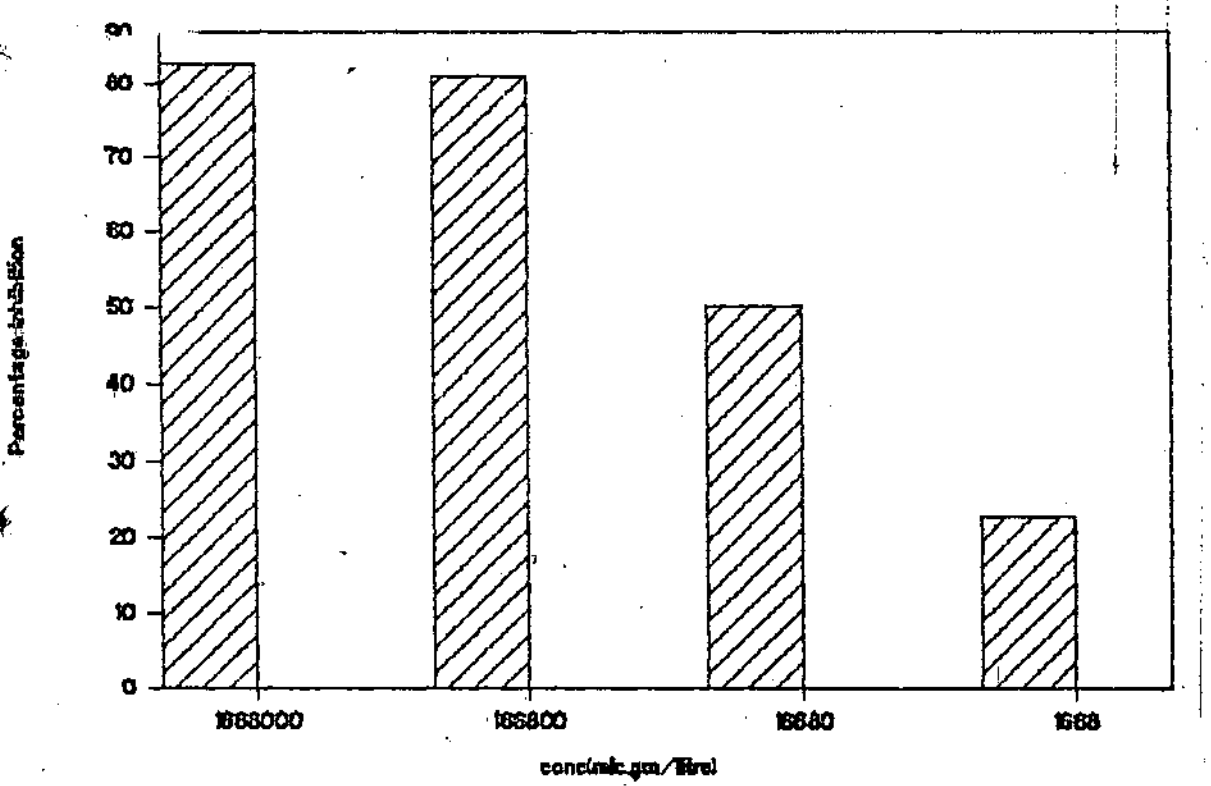
a incubation period 24 hours
b averages of 500 germs
c Values in parenthesis indicate % inhibition(-) or increase (+) over control and the values use for regression
d acetone control
e average age of culture 15 days

ED_{50} = 16680 microgm/litre

Picture 3



Picture-4 A


Percentage-Inhibition
90
80
70
60
50
40
30
20
10
0
1666000
166600
16660
1666
conc(mic.gm/litre)

Picture 4B

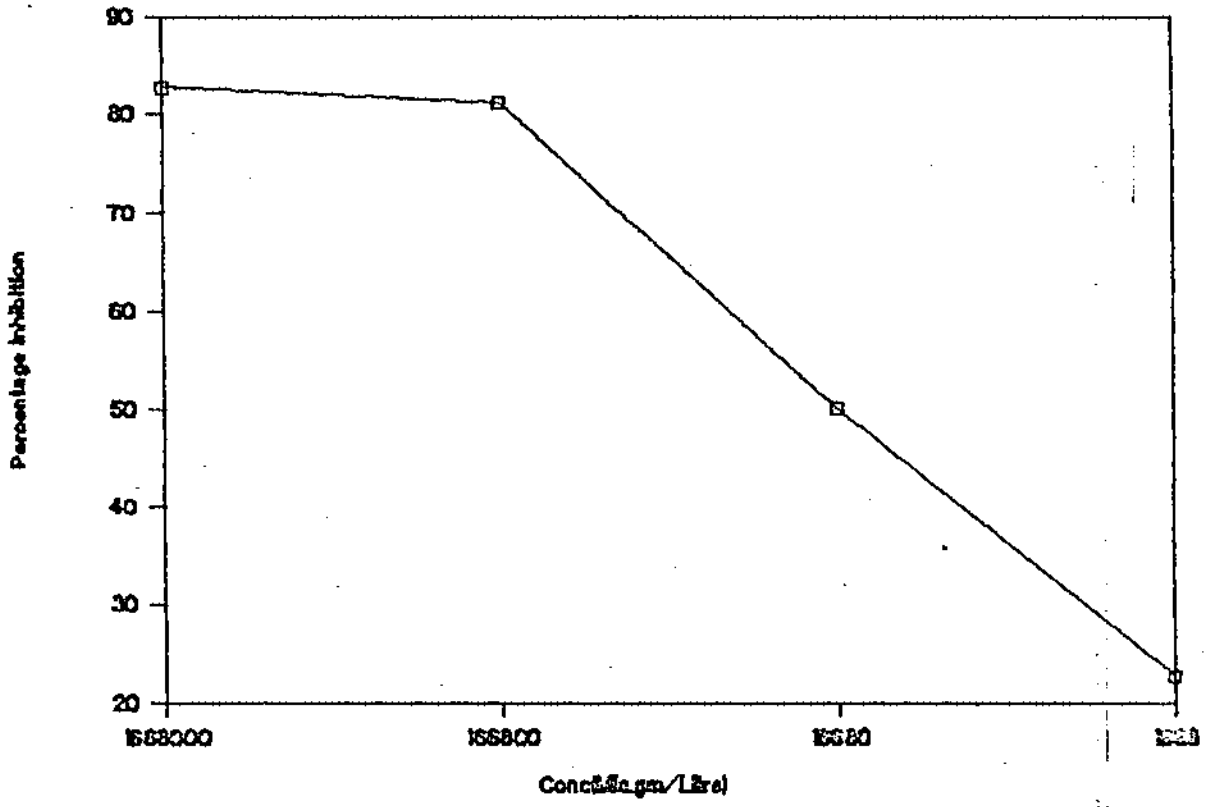

90
80
70
60
50
40
30
20
Percentage Inhibition
Concentration (Litre)

Table 5

Effect of [a] $Me_2C{=}CH{-}Sn\,Ph_3$ on H.sativum

concentration microgram/litre	b Percentages of spore germination	c	d
	Treatement	control	
5600000	0	90	
	-100	0	
560000	20.6	90	
	-77.15	0	
56000	61.6	90	
	-31.6	0	
5600	67.4	90	
	-25	0	

a incubation period 24 hours
b averages of 500 germs
c Values in parenthesis indicate % inhibition(-) or increase (+) ove control and the values used for regression
d acetone control
e average age of culture 15 days

Regression Output:

Constant	41.36046
Std Err of Y Est	25.13976
R Squared	0.677210
No. of Observations	4
Degrees of Freedom	2
X Coefficient(s)	0.000010
Std Err of Coef.	0.000005

ED_{50} = 237440 microgram/litre

Table 6

Effect of[a] Pipiridine adduct of $[Ph_2C=C(Ph)]_2SnCl_2$ on H.sativum

concentretion microgram/litre	b Percentages of spore germination	
	Treatement	control
400000	52.8	90
	-41.3	0
40000	59	90
	-34.4	0
4000	60.4	90
	-32.8	0
400	63	90
	-30	0

a incubation period 24 hours
b averages of 500 germs
c Values in parenthesis indicate % inhibition(-) or increase (+) ov
control and the values used for regression
d acetone control
e average age of culture 15 days

Regression Output:

Constant	32.00016
Std Err of Y Est	1.826902
R Squared	0.903715
No. of Observations	4
Degrees of Freedom	2
X Coefficient(s)	0.000023
Std Err of Coef.	0.000005

Table 7

Effect of[a] $C_5H_{11}N_2S_2$ on *H.maydis*

concentretion microgram/litre	[b] Percentages of spore germination	
	Treatement	control
394000	4.4	96
	-95.4	0
39400	10	96
	-89.6	0
3940	57	96
	-40.6	0
394	100	96
	4.16	0

a incubation period 24 hours
b averages of 500 germs
c Values in parenthesis indicate % inhibition(-) or increase (+) over control and the values used for regression
d acetone control
e average age of culture 15 days

Regression Output:

Constant	39.72931
Std Err of Y Est	41.99191
R Squared	0.417453
No. of Observations	4
Degrees of Freedom	2
X Coefficient(s)	0.000152
Std Err of Coef.	0.000127

ED_{50} = 10145.5 microgram/litre

Table 8

Effect of[a] $C_5H_{11}N_2(CS)_2SnPh_2$ on H.bicolor

concentretion microgram/litre	b Percentages of spore germination	
	Treatement	control
394000	2.8	92
	-97	0
39400	13.2	92
	-85.6	0
3940	17	92
	-81.5	0
394	79.2	92
	-13.9	0

a incubation period 24 hours
b averages of 500 germs
c Values in parenthesis indicate % inhibition(-) or increase (+) over control and the values used for regression
d acetone control
e average age of culture 15 days

Regression Output:

Constant	57.94910
Std Err of Y Est	38.97135
R Squared	0.285424
No. of Observations	4
Degrees of Freedom	2
X Coefficient(s)	0.000105
Std Err of Coef.	0.000118

ED_{50} = 2113.81 microgm/litre

Picture 8

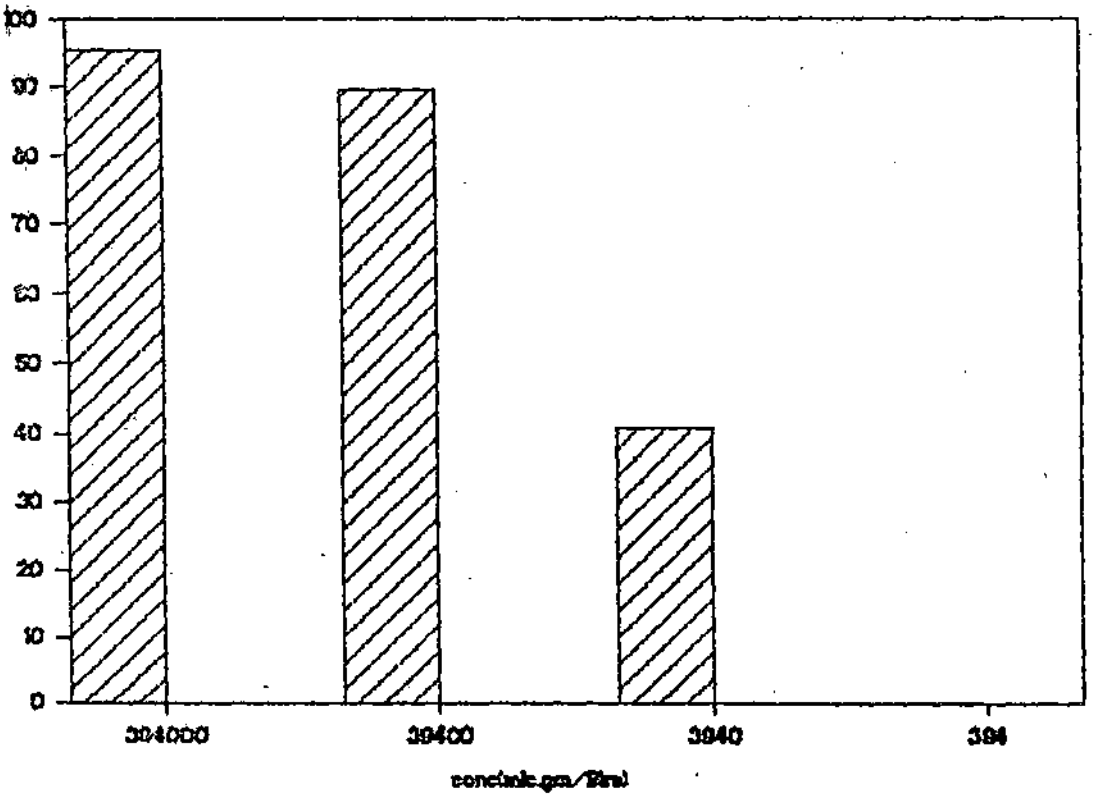


Table 9

[a]
-Effect of (C H N-CS) SnC(Ph) C=C(Ph)] on H.bicolor.
5 11 2 2 -

concentretion microgram/litre	[b] Percentages of spore germination	[c]	[d]
	Treatement	control	
394000	19	92	
	-79.3	0	
39400	30.8	92	
	-66.5	0	
3940	36.8	92	
	-60	0	
394	67.5	92	
	-26.6	0	

a incubation period 24 hours
b averages of 500 germs
c Values in parenthasis indicate % inhibition(-) or increas
control and the values used for regression
d acetone control
e average age of culture 15 days

Regression Output:

Constant	49.37874
Std Err of Y Est	20.30030
R Squared	0.456279
No. of Observations	4
Degrees of Freedom	2
X Coefficient(s)	0.000079
Std Err of Coef.	0.000061

ED = 2377.1 mi
50

Table 10

Effect of[a] C_5H_4N-S-CH_2-C(O)O-Sn Ph$_3$

concentretion microgram/litre	[b] Percentages of spore germination	[c]	[d]
	Treatement	control	
517900	1	96	
	-98.9	0	
51790	5.18	96	
	-94.6	0	
5179	39.7	96	
	-58.6	0	
517.9	76.2	96	
	-20.6	0	

a incubation period 24 hours
b averages of 500 germs
c values in parenthesis indicate % inhibition(-) or increase (+) over control and the values used for regression
d acetone control
e average age of culture 15 days

Regression Output:

Constant	55.02069
Std Err of Y Est	34.81280
R Squared	0.393635
No. of Observations	4
Degrees of Freedom	2
X Coefficient(s)	0.000091
Std Err of Coef.	0.000080

ED_{50} = 3418 microgram/litre

Picture 10

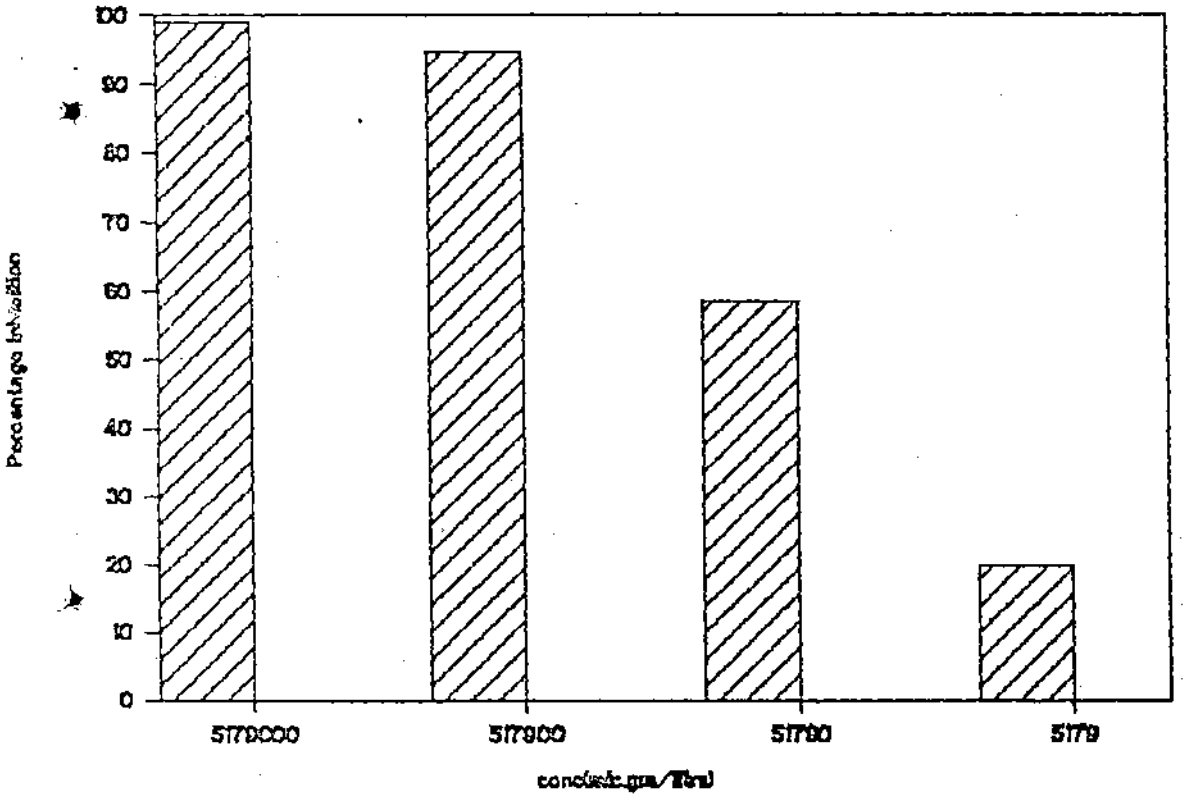


Table II

Effect of[C$_5$H$_4$N-S.CH$_2$COO]$_2$Sn Ph$_2$ on H.sativum[a]

concentretion microgram/litre	Percentages of spore germination[b]	
	Treatement[c]	control[d]
780000	32.2	90
	-64.2	0
78000	36.5	90
	-59.4	0
7800	60	90
	-33.3	0
780	70.2	90
	-22	0

a incubation period 24 hours
b averages of 500 germs
c Values in parenthesis indicate % inhibition(-) or increase (+) over control and the values used for regression
d benzene control
e average age of culture 15 days

Regression Output:

Constant	36.48756
Std Err of Y Est	17.67119
R Squared	0.496980
No. of Observations	4
Degrees of Freedom	2
X Coefficient(s)	0.000038
Std Err of Coef.	0.000027

ED_{50} = 39312 µgm/litre

Table 12

Effect of[a] $C_5H_4N\text{-}S\text{-}CH_2\text{-}C(O)O\text{-}Sn(Bu)_3$

concentretion microgram/litre	[b] Percentages of spore germination	[c]	[d]
	Treatement	control	
457900	0	92	
	-100	0	
45790	3.36	92	
	-96.5	0	
4579	60.86	92	
	-36.6	0	
457.9	83.6	92	
	-12.91	0	

c Values in parenthesis indicate % inhibition(-) or increase (+) over control and the values used for regression

d acetone control

e average age of culture 15 days

Regression Output:

Constant	44.98895
Std Err of Y Est	40.06364
R Squared	0.435741
No. of Observations	4
Degrees of Freedom	2
X Coefficient(s)	0.000129
Std Err of Coef.	0.000104

ED_{50} = 13233 microgram/litre

Picture 12

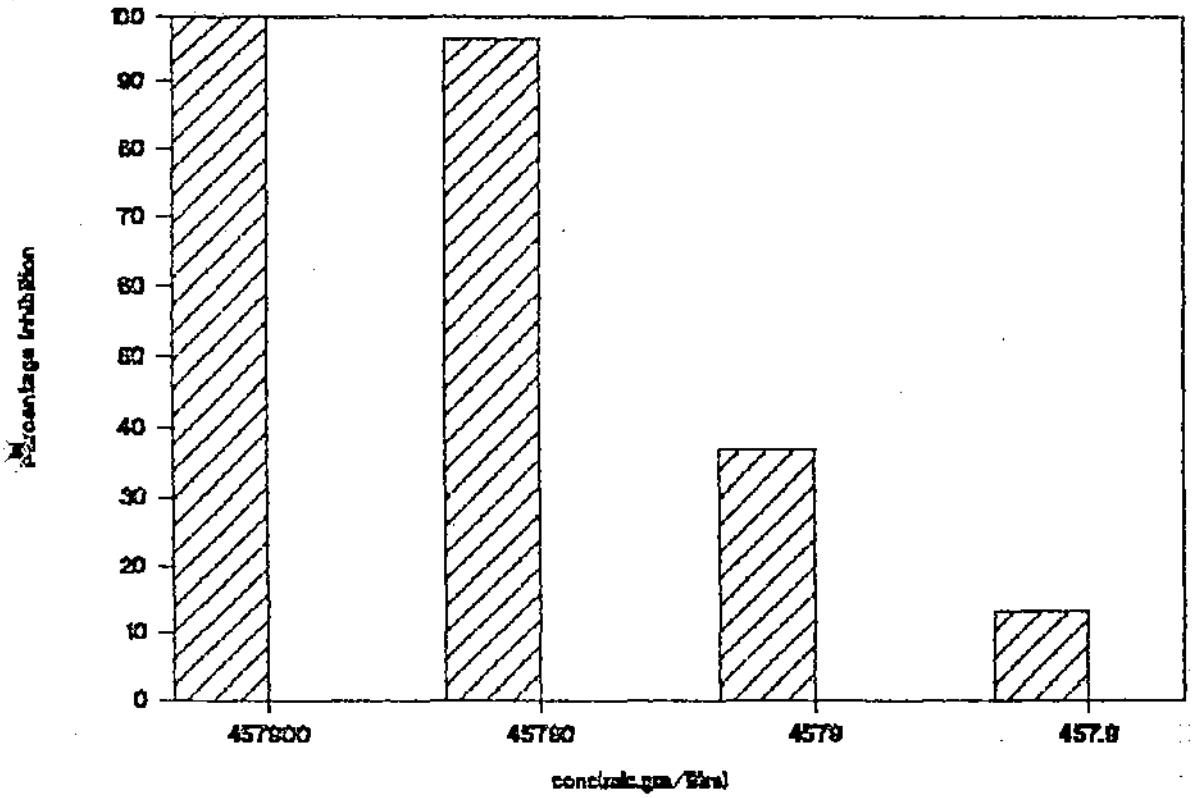
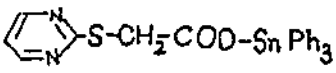

100
90
80
70
60
50
40
30
20
10
0
Percentage Inhibition
457900
45790
4579
457.9

Table 13

Effect of [a]  on H. oryzae

concentretion microgram/litre	[b] Percentages of spore germination	
	Treatement	Control [e]
5×10^{6}	0	90
5×10^{5}	-100	0
5×10^{4}	32.3	90
	-64.1	0
5×10^{3}	46.3	90
	-48.6	0
5 X 10	80.4	90
	-10.66	0

a incubation period 24 hours
b averages of 500 germs
c Values in parenthesis indicate % inhibition(-) or increase (+) over control and the values used for regression
d acetone control
e average age of culture 15 days

Regression Output:

	38.07544
Std Err of Y Est	24.92241
R Squared	0.697894
No. of Observations	4
Degrees of Freedom	2
X Coefficient(s)	0.000012
Std Err of Coef.	0.000005

ED_{50} = 60000microgram/litre.

Figure 13

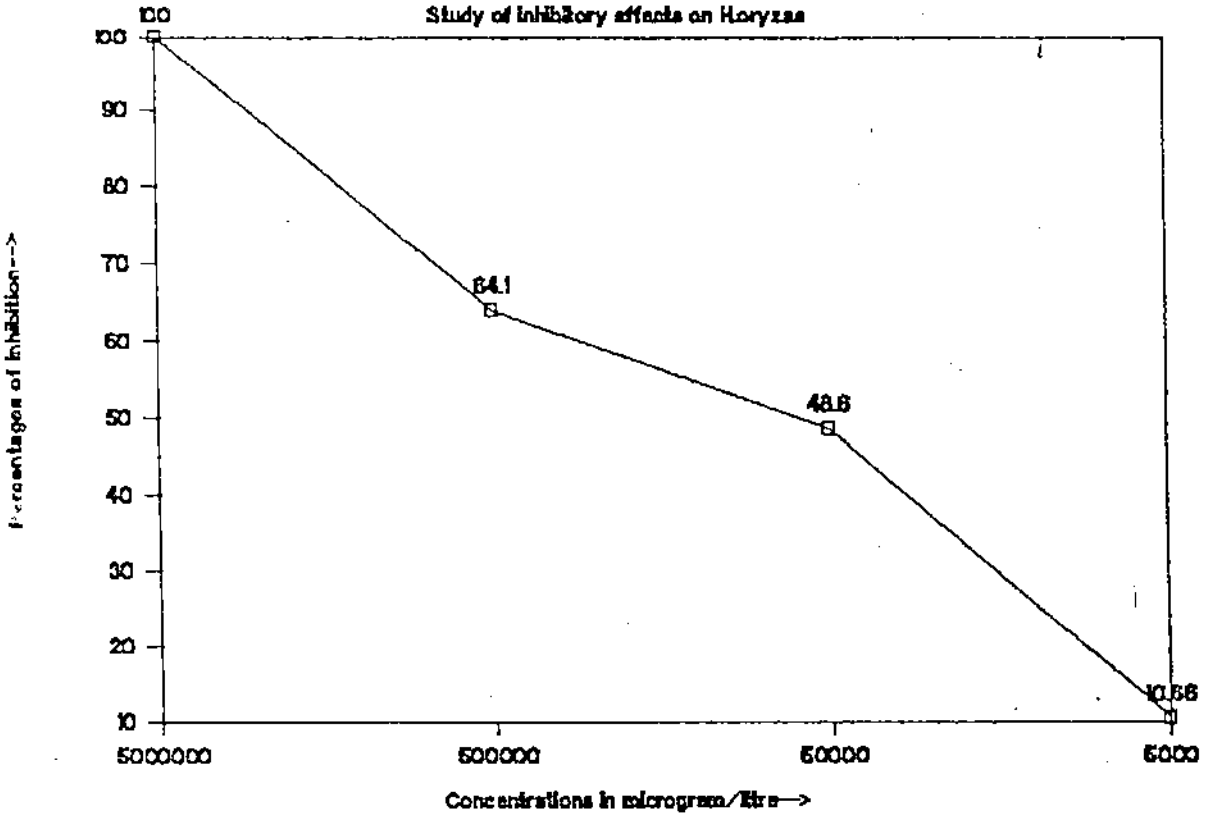
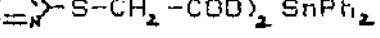

Study of inhibitory effects on Horyzae
100
90
80
70
60
50
40
30
20
10
64.1
48.6
10.66
5000000
500000
50000
5000
Concentrations in microgram/litre—>

Table 14

Effect of [a]  on H. oryzae

concentretion microgram/litre	[b] Percentages of spore germination	
	Treatement	Control [e]
5×10^{6}	0	90
5×10^{5}	-100	0
5×10^{4}	25.2	90
	-72	0
5×10^{3}	33.3	90
	-63	0
5 X 10	67.8	90
	-24.6	0

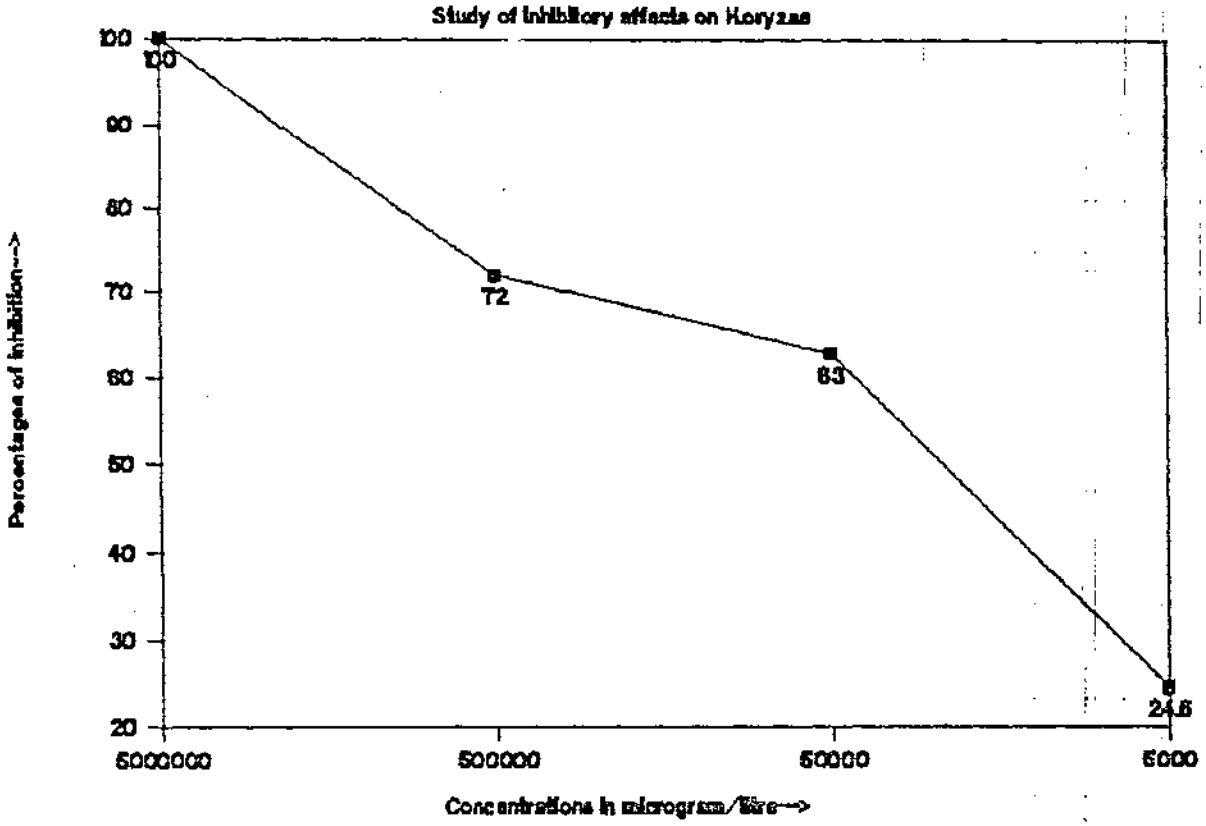
a incubation period 24 hours
b averages of 500 germs
c Values in parenthesis indicate % inhibition(-) or increase (+) over control and the values used for regression
d acetone control
e average age of culture 15 days

Regression Output:

Constant	50.74538
Std Err of Y Est	23.32598
R Squared	0.626062
No. of Observations	4
Degrees of Freedom	2
X Coefficient(s)	0.000010
Std Err of Coef.	0.000005

ED_{50} = 35000 microgram/litre.

Figure 14



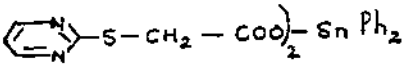
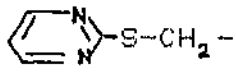
■ Conc. of 

Table 15

Effect of [a]  on H. oryzae

concentretion microgram/litre	b Percentages of spore germination	
	Treatement	Control e
5×10^{6}	0	90
5×10^{5}	-100	0
5×10^{4}	34.3	90
	-61.8	0
5×10^{3}	68.2	90
	-24.2	0
	100	90
	0	0

a incubation period 24 hours
b averages of 500 germs
c Values in parenthesis indicate % inhibition(-) or increase (+) over control and the values used for regression
d acetone control
e average age of culture 15 days

Regression Output:

Constant	50.74538
Std Err of Y Est	23.32598
R Squared	0.626062
No. of Observations	4
Degrees of Freedom	2
X Coefficient(s)	0.000010
Std Err of Coef.	0.000005

ED_{50} = 350000 microgram/litre.

Figure 15

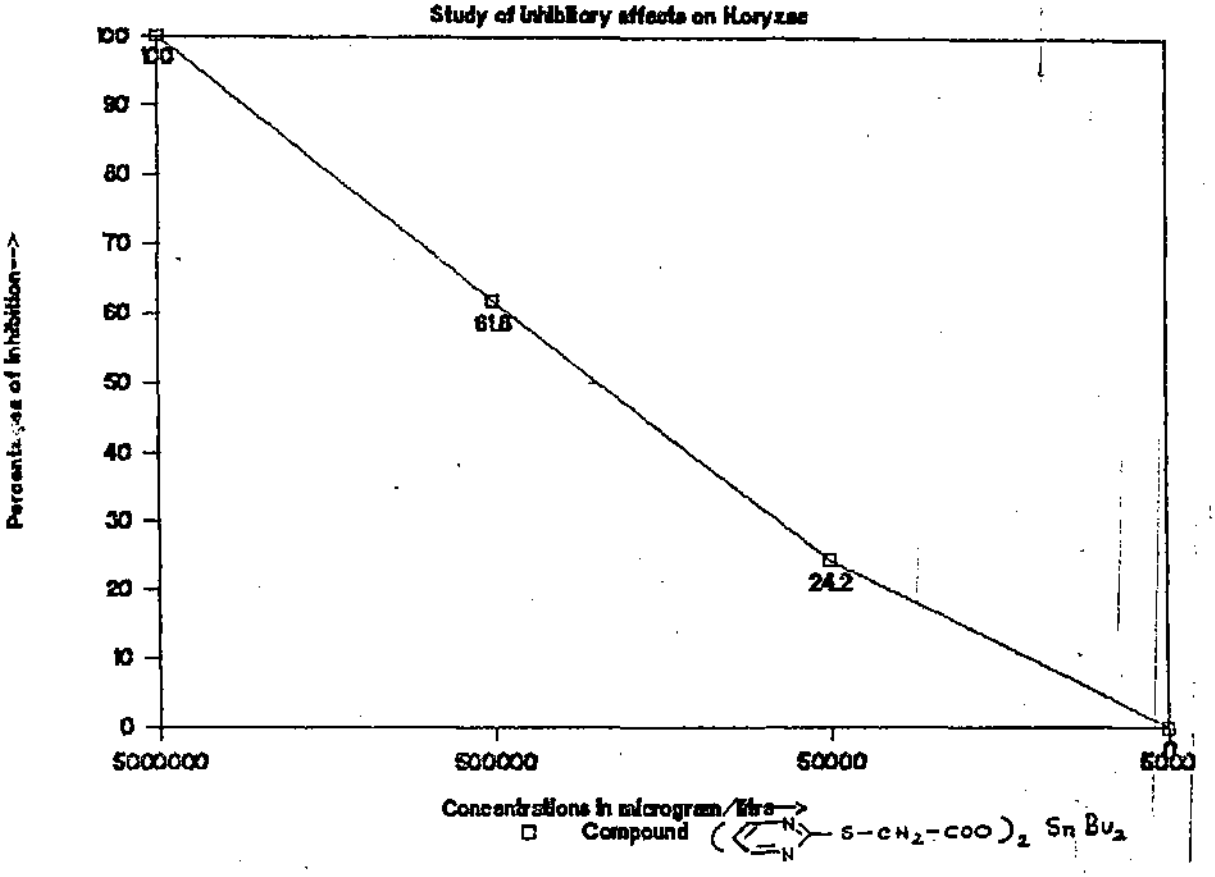
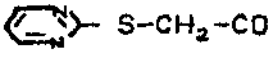

Study of inhibitory effects on Koryzae
Percentage of Inhibition-->
100
90
80
70
60
50
40
30
20
10
0
100
61.6
24.2
0
5000000
500000
50000
5000
Concentrations in microgram/litre-->
Compound
S-CH2-COO)2 Sn Bu2

Table 16

Effect of  [a] on H. oryzae

concentretion microgram/litre	b Percentages of spore germination	c	d
	Treatement	Control	e
5×10^{6}	0	90	
5×10^{5}	-100	0	
5×10^{4}	4.5	90	
	-95	0	
5×10^{3}	66.6	90	
	-26	0	
	86	90	
	-4.4	0	

a incubation period 24 hours
b averages of 500 germs
c Values in parenthesis indicate % inhibition(-) or increase (+) over control and the values used for regression
d acetone control
e average age of culture 15 days

Regression Output:

Constant	37.67832
Std Err of Y Est	43.86787
R Squared	0.451667
No. of Observations	4
Degrees of Freedom	2
X Coefficient(s)	0.000013
Std Err of Coef.	0.000010

ED_{50} = 230000 microgram/litre.

Figure 16

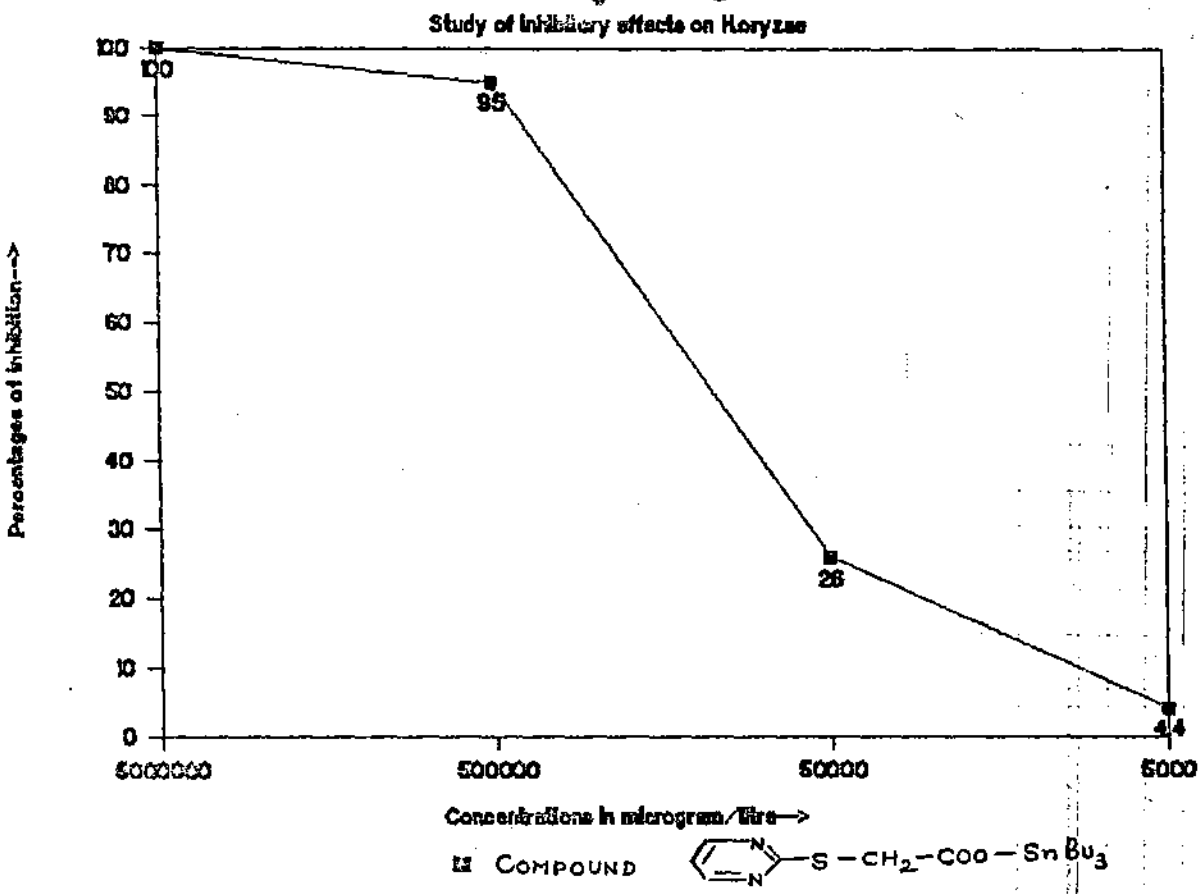
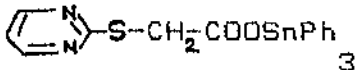
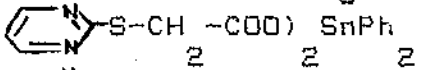

Study of inhibitory effects on Koryzae
Percentages of inhibition-->
100
90
80
70
60
50
40
30
20
10
0
100
95
26
4.4
5000000
500000
50000
5000
Concentrations in microgram/litre-->
COMPOUND
S – CH2 – COO – Sn Bu3
N
N

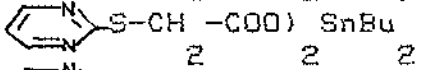
Table 17

Comparative study of inhibitory effects of four compounds, namely A, B, C and D on H. oryzae.

concentretion microgram/litre	Compounds A	B	C	D
5×10^{6}	100	100	100	100
5×10^{5}	64.1	72	61.8	95
5×10^{4}	48.6	63	24.2	26
5×10^{3}	10.6	24.6	0	4.4

Compound A = 

Compound B = 

Compound C = 

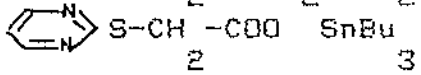
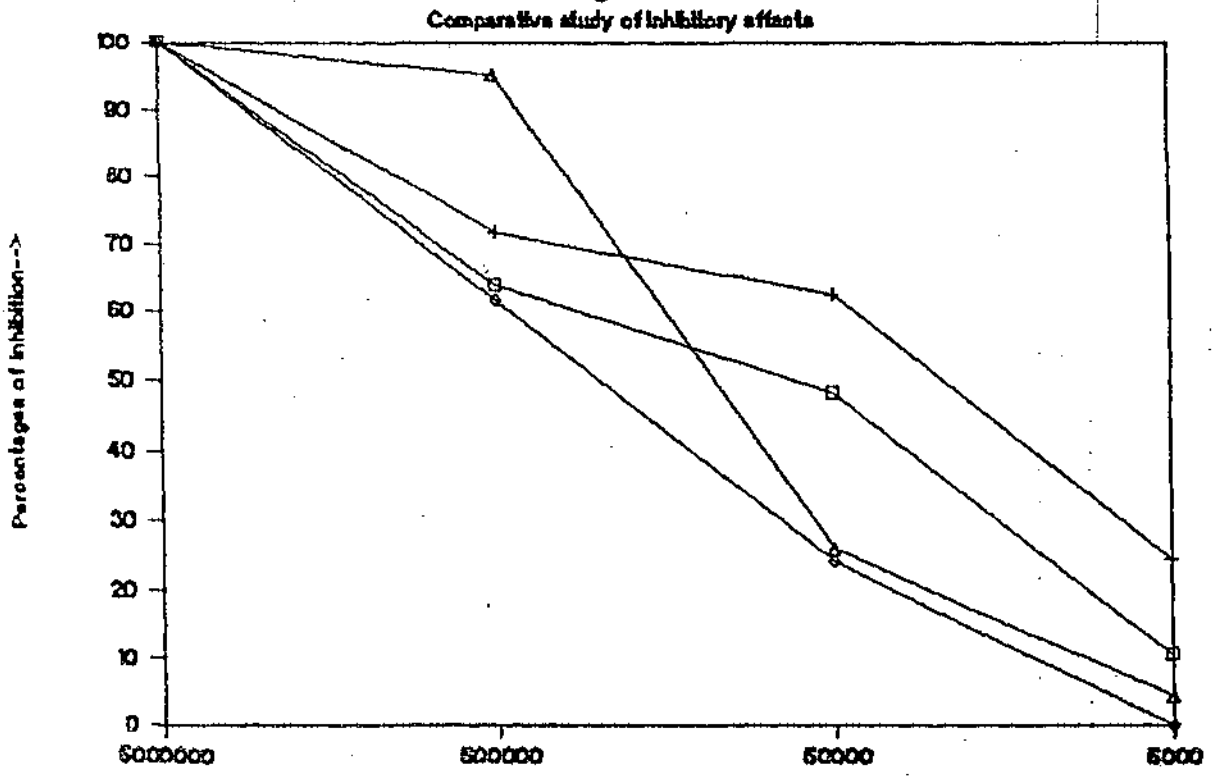
Compound D = 

Figure 17



□ Comp. A + Comp. B ◇ Comp. C △ Comp. D

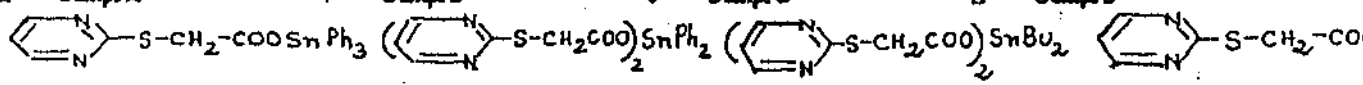
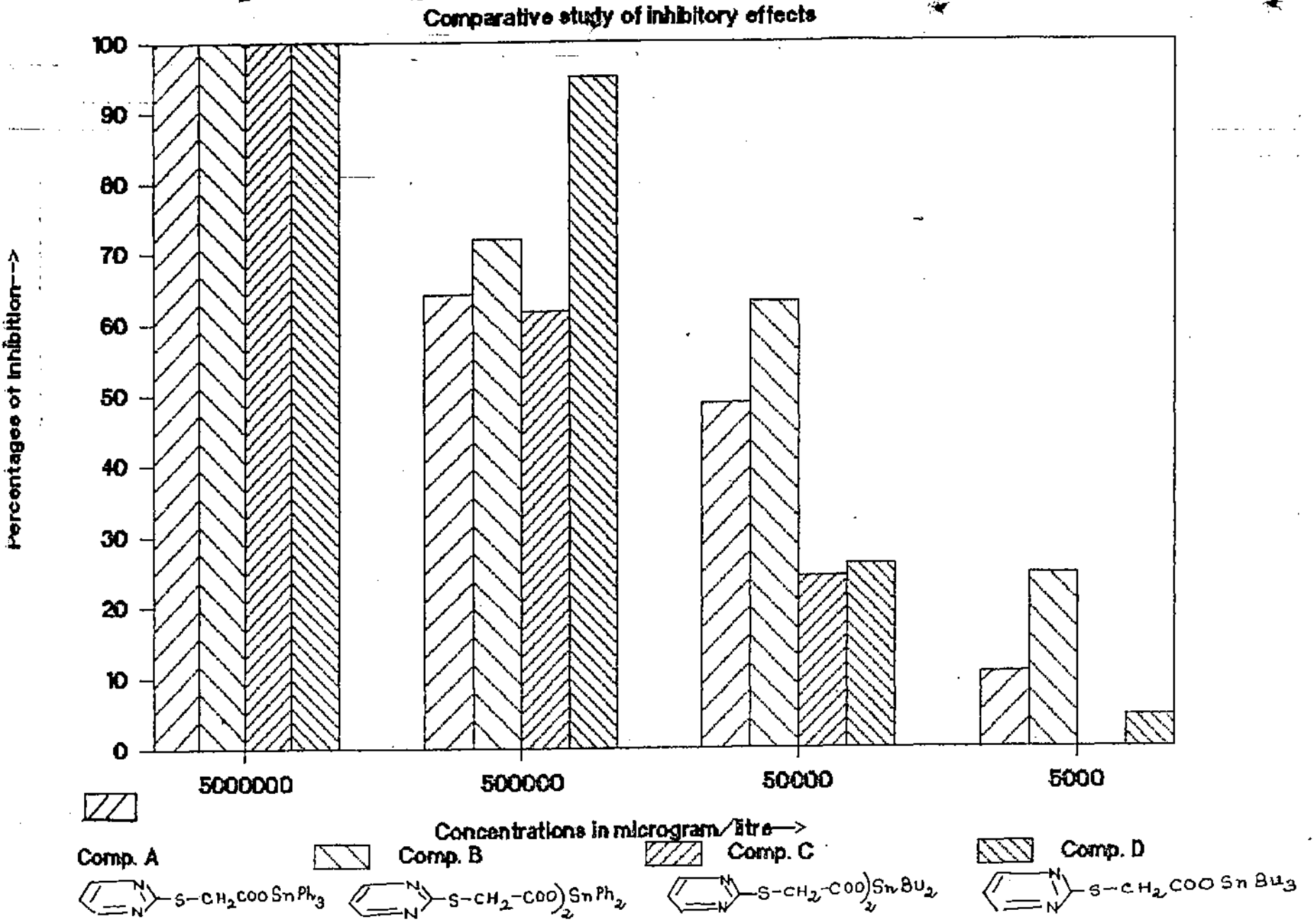
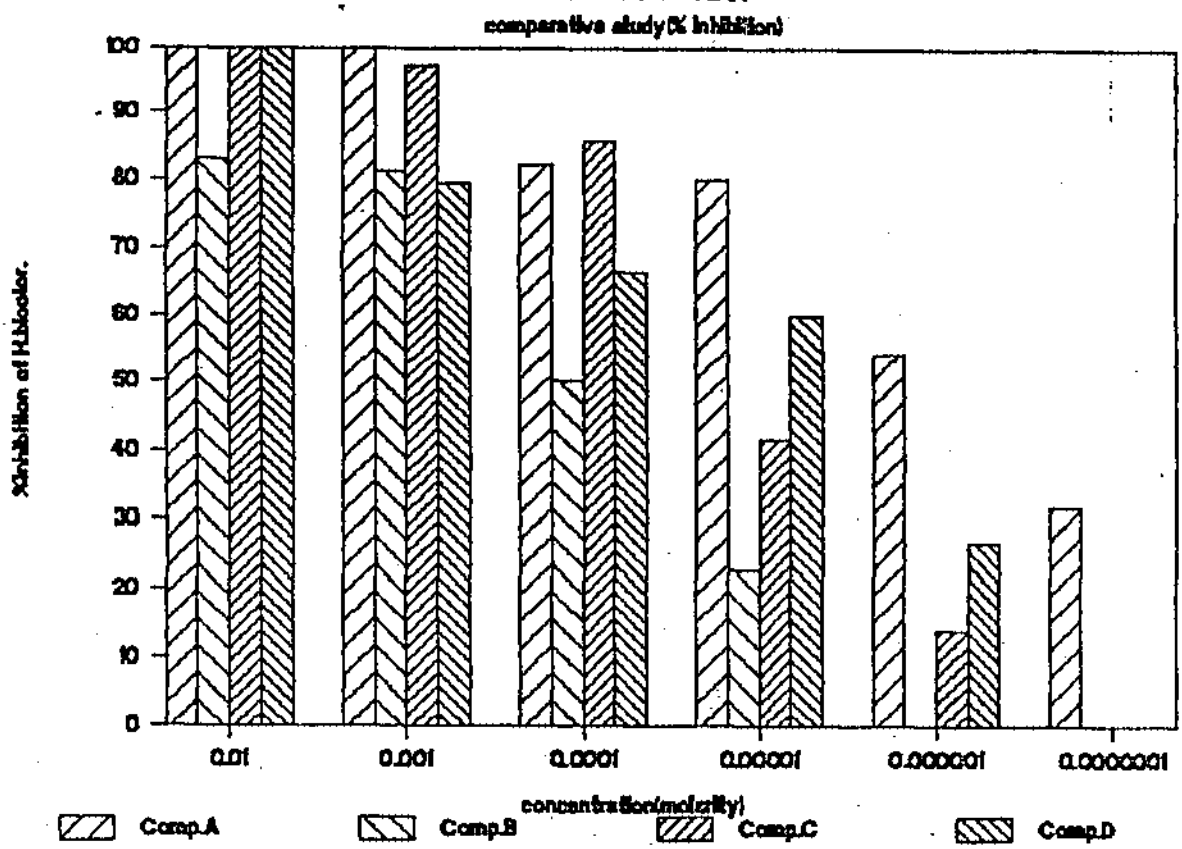


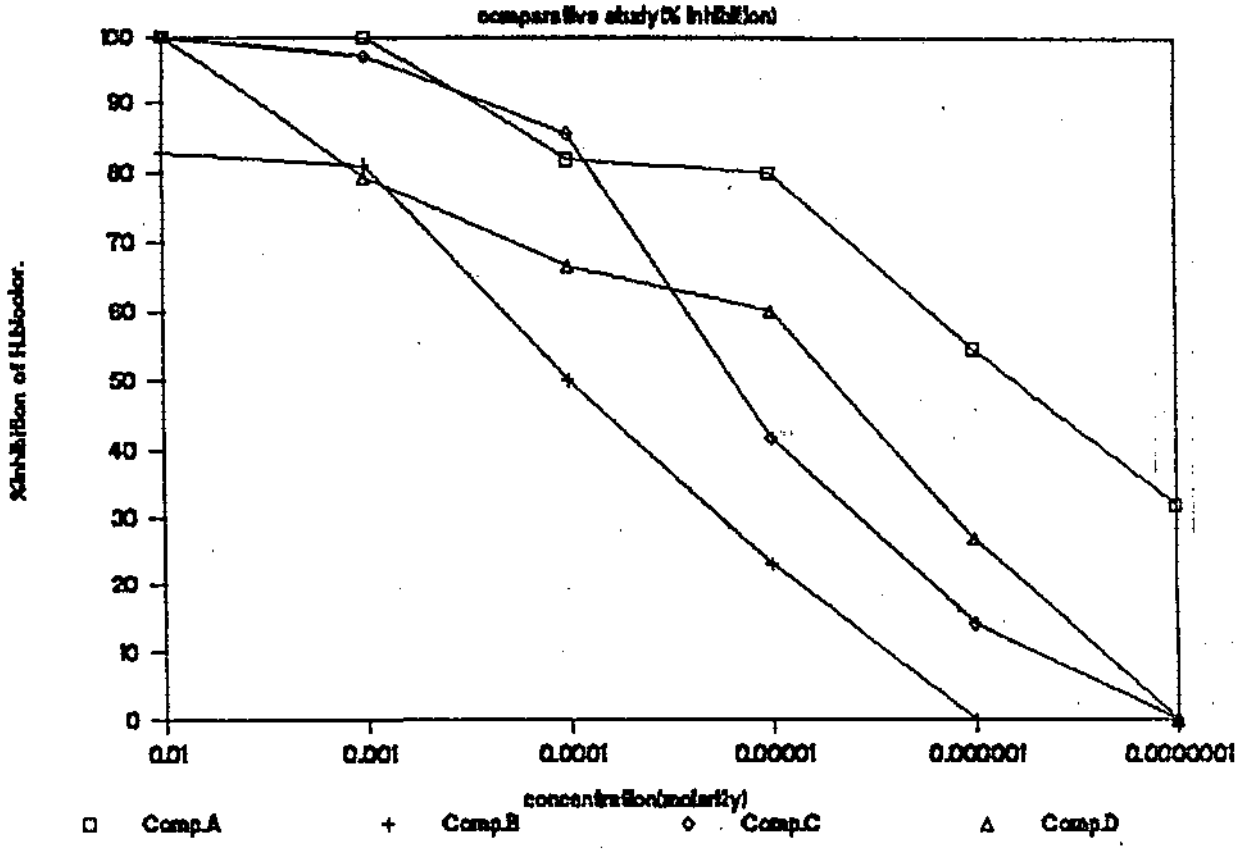
Figure I7


Comparative study of inhibitory effects
Percentages of inhibition—>
100
90
80
70
60
50
40
30
20
10
0
5000000
500000
50000
5000
Concentrations in microgram/litre—>
Comp. A
Comp. B
Comp. C
Comp. D

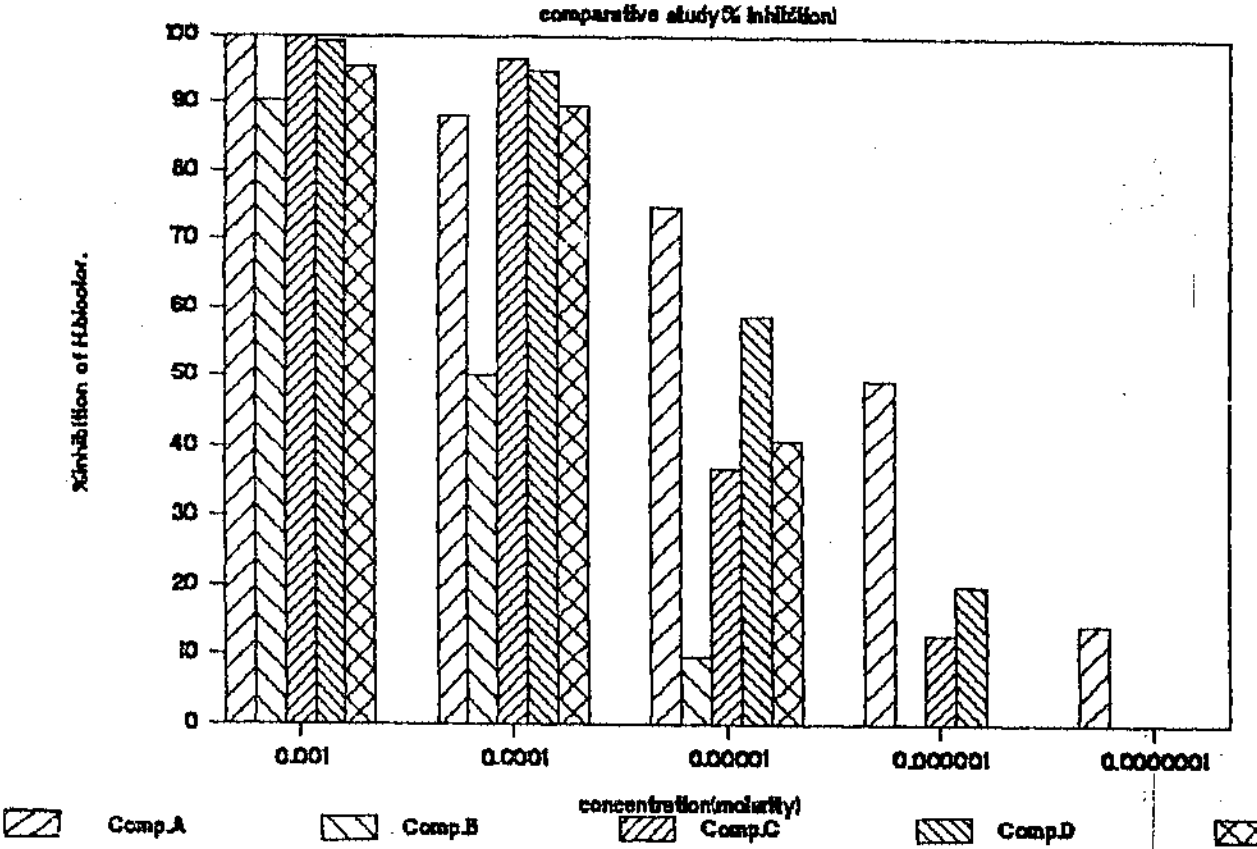
Picture 18A


comparative study(% inhibition)
% inhibition of Histocker.
100
90
80
70
60
50
40
30
20
10
0
0.01
0.001
0.0001
0.00001
0.000001
0.0000001
concentration(molarity)
Comp.A
Comp.B
Comp.C
Comp.D

Picture 18B


comparative study(% Inhibition)
% Inhibition of H1blocker.
100
90
80
70
60
50
40
30
20
10
0
0.01
0.001
0.0001
0.00001
0.000001
0.0000001
concentration(molarity)
Comp.A
Comp.B
Comp.C
Comp.D

Picture 20



$Me_2C=CHCl\,SnMe_2$ $[Ph_2C=C(Ph)]_2SnCl_2$ $C_5H_4N\text{-}S\text{-}CH_2COOSnBu_3$ $C_5H_4NSCH_2COOSnPh_3$ $(C_5H_{11}NCS_2)_2Sn$

§ 8.1.4 DISCUSSION:

The different tables and representation of different of different comparative studies of a number of newly synthesised compounds on a number of plant pathogens show the nature of their activity. In this context, we can refer to the findings of Van der Kerk and Luijten (1961)[38] which showed that maximm toxicity is associated with a total number 9 to 12 carbon atoms in the alkyl group. The results of thioaryloxy acetates of 4-pyridyl and 2-pyrimidyl thio acetic acid ligands containing both nitrogen and sulphur atoms, can be compared with different triorganotin phenoxy acetates[39] where fungitoxicity depended upon the nature of X groups. Moreover, selectivity towards one or the other of the test fungi were also observed, which is also consistent with observations made elsewhere.[40-41]

All the results shown in different tables have been computed on trial basis for regression analysis. The total number of observation of Y, the dependent factor i.e. the % inhibition being the culmination of a large number of random observations, which are not included here to avoid unnecessary clumsyness.

The statistical correlation was tried for the fact that statistical critera, acquire greater importance when one follows experimental approach in investigating a particular problem. In the above tables and observations the correlation of the effect of a set of four concentrations on the inhibition of a number of fungi were tried for.

Without going into the detailed complications of statistics, the aim is to find out the goodness of fit of corelation, to get idea about error. The value of square of corelation coefficient r^2

(Coefficient of determination) determining the proportion of variation of dependent factor(Y) as explained by the variation of independent variable X *i.e.* concentration of the concerned compound here, lie between 0 and 1. As the $r^2_{x,y}$ reaches 1, regression line becomes more and more good fit one to the observed data.

In our study, the judgement of the explanatory power of the linear regression of Y (% inhibition of spore) on X (Concentrations of a particular compound) reveals that in most of the cases, the goodness of fit is moderate in nature as $r^2_{x,y}$ values remain around 0.5 except in one case where the $r^2_{x,y}$ =0.9(*vide* table 6). The corelation was exceptionally low with the Inhibitory effect of correlation of $(C_5H_{11}NCS_2)_2SnPh_2$ on *H. bicolor* with $r^2_{x,y}$=0.28. The $r^2_{x,y}$ value of high along with low standard errors, in case of (*vide* table 6) with the correlation between piperidine adduct of $[Ph_2C=C(Ph)]_2SnCl_2$ on *H. sativum* which obviously has a clear cut merit, while in other cases the standard error is high and statistical regression corelation has low value of goodness of fit needs further investigation to find out any external factors play any role or not. P. J. Smith referred to the primary mode of action of dibutyl and tributyl tin compounds[42] where he showed that diorganotin compounds function through their ability to combine with enzymes or coenzymes possessing vicinal dithiol groups, thereby inhibiting α-keto acid oxidation, while triorganotin compounds inhibit mitochondrial oxidative phosphorylation and also bind to the cysteine or histidine residues of certain proteins. So in the cases of substituted diorgano and triorganotin compounds, ED_{50} values can

not be directly correlated to their organotin contents. But in general maximum biological activity was found to increase progressively maximum being with trisubstituion with organic groups.[43-45]

In the context of systematic study of preparation, properties and structures of a series of substitued di- and tri- organotin compounds, bilogical properties fungicidal, phytotoxicity and antitumour activity are studied. Now it may be noted that both the thioaryloxy acetates The two ligands 4-Pyridylthio acetic acid and and 2-Pyrimidyl thio acetic acid were found to be biologically inactive.

The role of organotin compounds as antifungal agents was first worked out by Van der Kerk and Luijten. The purpose of the present study is to evaluate antifungal activity of the orgnotin thio aryloxy acetates on plant pathogens and to compare their fungitoxicity with parent acids, in order to elucidate structure-activity relationships.

The results show the tested compounds to have wide variety of ED_{50} values which are given in a tabular form (<u>vide</u> table A). All the experiments were done in vitro and it is now imperative to test the results in vivo which are now in progress with a selected no of compounds.

§ 8.2.1 Introduction to Phytotoxicity

The compounds possessing fungicidal activity as determined by the tests mentioned earlier, were tested for their phytotoxicity on rice also. This is only to screen out the compounds which are highly phytotoxic since any compound with fungicidal activity with high level of phytotoxicity will be of no use as fungicide in the practical fields of applications. The priliminary results tested *in vitro* show the compounds to possess no phytotoxicity at three standardised concentration levels of 100, 50 and 25ppm. The detailed experimental procedure is given below:

§ 8.2.2 Experimental- Material and Methods:

Seed sample: Healthy rice seeds of PUSA 2-21 variety collected from Chinsurah Rice Research Farm, Hooghly, W.B., were used in the present investigation.

Compounds: A number of newly synthesised compounds belonging to all the three classes, *viz.* a) alkenyltin(IV) compound b) dithiocarbamate c) Organotin(IV) acetates of 4-pyridylthio acetic acid and 2- pyrimidylthio acetic acid were used.

Effect on seed germination: Healthy rice seeds were dipped in three different sets of concentrations of water suspension of compounds (*viz.*, 100, 50, 25 ppm) for 1 hr, 4hrs and 8hrs respectively. As the compounds were insoluble in water, few drops of acetone were added to make the suspension. Acetone controls for each treatement and one set of water control were also arranged. The treated seeds were then sowed over a mat of moist filter papers arranged in covered petriplates incubated at 30° ± 1°C. 100 seeds were treated for each set of treatement. After seven days, the germinated seeds were counted. Seeds producing a root or

coleoptile were considered as germinated. Three replications of each set were maintained against corresponding appropriate control under same conditions.

§ 8.2.3.RESULTS

The results of the study are given below in tabular form:

Sl. No	Compound	Concentration (ppm)	Percentage of germination of seeds: 1hr	4hrs	8hrs
1.	$Me_2C=C(Cl)SnMe_2$	100	100	100	100
		50	100	100	100
		25	100	100	100
2.	$[Ph_2C=C(Ph)]_2SnCl_2$	100	100	100	100
		50	100	100	100
		25	100	100	100
3.	$Me_2C=CHSnPh_3$	100	100	100	100
		50	100	100	100
		25	100	100	100
4.	Pdc_2SnPh_2	100	100	100	90
		50	100	100	95
		25	100	100	100
5	$Pdc_2Sn[C=C(Ph)_2]_2$ (Ph)	100	100	96	90
		50	100	100	95
		25	100	100	100
6.	$(4\text{-ptaa})SnPh_3$	100	100	100	96
		50	100	100	100
		25	100	100	100
7.	$(4\text{-ptaa})_2SnPh_2$	100	100	100	96
		50	100	100	100
		25	100	100	100
8.	$(4\text{-ptaa})SnBu_3$	100	100	100	95
		50	100	100	100
		25	100	100	100
9.	$(2\text{-ptaa})SnPh_3$	100	100	100	100
		50	100	100	100
		25	100	100	100

...continued from previous page...

Sl. No	Compound	Concentration (ppm)	Percentage of germination of seeds: 1hr	4hrs	8hrs
10.	$(2\text{-ptaa})_2SnPh_2$	100	100	100	100
		50	100	100	100
		25	100	100	100
11.	$(2\text{-ptaa})_2SnBu_2$	100	100	100	92
		50	100	100	98
		25	100	100	100
12.	$(2\text{-ptaa})SnBu_3$	100	100	100	96
		50	100	100	100
		25	100	100	100
13.	$(2\text{-ptaa})_2SnMe_2$	100	100	100	90
		50	100	100	95
		25	100	100	100

§ 8.2.4. Discussion

The results show the compounds to have almost no phytotoxicity which is encouraging from the point of view of its potentiality as fungicide in practical field. This encouraging result has inspired to test the compounds in vivo, the details of which are now in progress.

§ 8.3.1 INTRODUCTION TO ANTITUMOUR ACTIVITY STUDY:

Organotin compounds are now being used to a wide range of applications, a number of which have been found to have antitumour activity. A brief throwing of light on this topic reveals that "some new di-n-butyltin and tin(IV) complexes of the type Bu_3SnL, Bu_2SnL_2 and SnL_2 (where L= anions of schiff bases derived from S-substituted dithiocarbazates and flouro aniline)" have antitumor activity in " P388 lymphosite leukaemia system of which s- methyl thiocarbazate was found to tbe most active.[20-24] Crowe et.al.,[21] have reported some diorganotin complexes possessing activity in P388 lymphoid leukaemia system. Organotin chelates of the schiffs bases of s-methyl thiocarbazates and other ligands have, in general, been found to have greater cytotoxic activity[22] than those of the transition metals.[23]

According to one of the "Rules of thumb" of Rosenberg,[25] relating to the structural chemistry of metal complexes displaying antitumour activity, the metal complex should have one or more labile electronegative group (especially halide ion).

Inorganic tin compounds have no antitumour activity although a number of organotin compounds with antitumour activity are known.[26a,b] A possible mechanism suggested recently for the antitumour activity of the tin compounds <u>in vivo</u> is that the tin , whatever its form, is biochemically converted to the anticarcinogenic entities in the thymus and thus distributed around the body as anticarcinogen through lymph fluids.[26a] The possibility of generation of some active free radicals causing interaction with DNA cannot be ruled out.

The characterisation of carboxylate complexes of titanocene dichloride with amino acids[27] was the first report of

cyclopentadienyltitanium complexes of amino and related acids. Titanocene dichloride exhibited very low toxicity. The discovery that some organotin compound have antitumour activity had aroused interest in the study of organotin compounds with nucleic acid bases and carbohydrates. It is assumed that tin-nucleoside binding is fundamental factor responsible for the assesment of anticancer properties of organotin compounds. This activity proceed through a different mechanism for Pt and related speci as evident from the very different specificity found for the organotin compounds.

§ 8.3.2. ANTITUMOUR ACTIVITY STUDY – MATERIAL AND METHODS:

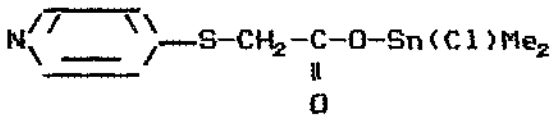
As organotin compounds have wide range of applicability and some of the organotin compounds have antitumour activity, this property of three newly synthesised organotin(IV) complexes of the types, viz., Dialkenyltin dichloride, its dithiocarbamate derivative and Dimethyl chlorotin(IV) 4-pyridylthio acetate have been studied against Ehrlisch Ascites tumour cells in vivo.

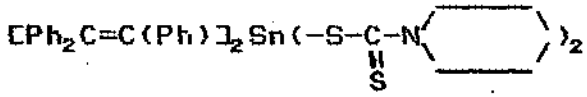
All the organotin(IV) compounds studied are prepared and analysed by the methods described in the earlier chapters in details. The activity of all of these complexes were studied in Chittaranjan Cancer Research Institute, Bhabanipore, Calcutta by the courtesy of Dr. S. K. Choudhuri. All the compounds were screened against Ehrlisch Ascites tumour cells in vivo. The mice were injected in the peritoneal cavity with tumour cells at a base level of 1×10^6 cells with a variation of 1000 cells. Three days after transplantations of tumour cells, the mice were injected intraperitonially with the suspensions of the organotin(IV) compounds of 2 to 2.5×10^{-6} M/L strenth in each mouse. The results of screening of the doses were tried to be evaluated on the basis of

survival. The recording of body weights and survivality of mice were started on and from third day of the date of injection of the chemicals. In a survival tumour system, the increase in survival of treated animals over control is expressed as T/C percent, a T/C value of 100 means the chemicals had no effect of either increasing or decreasing the tumour, a T/C value greater than 115 means significant activity while the same with a value of >125 indicates the compound is worth of testing in other tumour systems.

The control was prepared in the following way: 10ml of distilled water was mixed with 10 drops of DMSO, the suspension was vortexed for 5 minutes. 1ml of the suspension was injected per mouse.

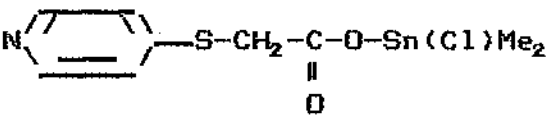
The chemicals used were

i) 

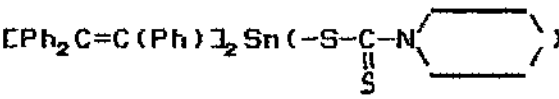
ii) 

ii) $[Ph_2C{=}C(Ph)]_2SnCl_2$

These chemicals were treated in the following way as described below:

a)0.016 gm of compound (i) viz., 

was taken and 10ml double distilled water was added to it along with the addition of 10 drops of DMSO. The suspension was vortexed for 5minutes and 1ml of it was injected (0.0016gm) by pushing in to perotoneal cavity to each animal of each group.

(b)0.009gm of compound (ii) i.e. 

was taken and added in 10ml of double distilled water, to which 10 drops of DMSO was added and the suspension was vortexed for five

minutes. The uniform suspension was injected intraperitoneally (1ml per mouse) to each group of mouse.

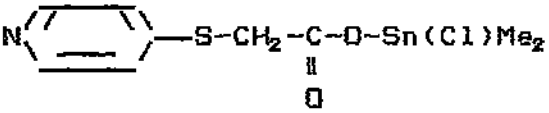
§ 8.3.3.RESULTS OF STUDY OF ANTITUMOUR ACTIVITY:

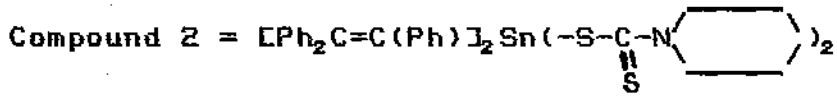
The screening data of the three compounds are given below:

TABLE 18

Body weights in gms of animals of different groups recorded:

	Day	<— Weights in gms.—>									Average Wt
Control	1	35	35	31	30	30	30	25	25	25	29.5
	3	35	34	33	33	33	30	29	28	27	31.2
	8	35	37	35	32	32	32	31	30	30	33.1
DMSO Control	1	36	35	34	34	30	30	30	29	28	31.7
	3	40	36	35	34	34	32	32	30	27	33.2
	8	39	39	37	37	37	34	30	27	27	34.3
i) Compound 1	1	37	32	32	30	29	28	28	28	27	30.0
	3	41	40	35	35	34	32	32	29	29	34.3
	8	40	35	35	30	30	29	29	29	–	32.1
ii) Compound 2	1	35	35	34	34	32	30	30	27	–	32.1
	3	38	36	36	36	37	35	34	34	–	35.8
	8	39	35	37	37	36	36	35	35	–	36.3
ii) Compound 3	1	32	30	30	30	29	27	27	25	25	31.9
	3	37	35	35	35	34	34	29	27	–	33.3
	8	39	35	35	34	32	29	27	–	–	28.9

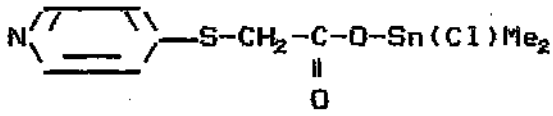
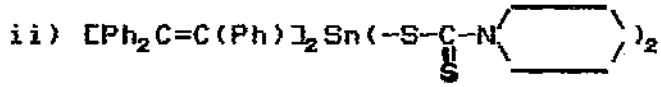
Compound 1 = 

Compound 2 = 

Compound 3 = $[Ph_2C{=}C(Ph)]_2SnCl_2$

TABLE 19

Record of survivality[a] (Percentage)

	Initial no of animals	No of surviving animals	
		After 16 days	After 24 days
Control	9	8(88.88%)	5(55.55%)
DMSO Control	9	8(88.88%)	4(44.44%)
i) 	9	5(55.55%)	2(22.22%)
ii) 	9	5(55.55%)	1(11.11%)
iii) $[Ph_2C{=}C(Ph)]_2SnCl_2$	9	8(88.88%)	0(0.0%)

a= out of nine animals used in each case

§ 8.3.4 DISCUSSION:

As a considerable percentage, above 50% of the total no of animals of the treated groups in all the three cases, died while those of control i.e., those who were not treated remained unaffected, the toxicity level of the compounds are quite high. The reason and cause of toxic death is under further study and consideration. Though reports are not encouraging upto this level, structural modifications of these compounds may give more promising light on this.

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