CHAPTER-IV

EVALUATION OF FUNGICIDAL ACTIVITY

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A large number of synthesized five membered heterocyclic compounds representing four different series have been described and characterized in the previous Chapter. The synthesized compounds have been designed in such a way that most of them incorporate various toxophorically important grouping for fungi. These compounds have been screened for their fungicidal activity against the two fungal species, viz. *Phytophthora infestans* and *Helminthosporium oryzae.* The screening results have been correlated with structural features of the tested compounds.

The author has evaluated the fungicidal activity of seventy eight compounds by employing agar plate techniquie[1,2]. In brief this technique involves the mixing of toxicant with agar medium and following the planted fungus to grow on such poisoned food.

***Phytophthora infestans*:**

Phytophthora infestans is a causal agent of the late blight of potato. In India the disease was first introduced into the

Nilgiri Hills between 1870 and 1880. Soon after, it was reported from the Darjeeling in Himalayas with the introduction of English potato there.

The Physiology of fungus did not permit it to reach the plains of India where the temperature was relatively high for development of disease. During 1899-1900 it was observed for first time in plains Hooghly of Bengal. The disease spread throughout state in 1901-1902 but was again not heard of from anywhere in the plain for about a decade. Severe outbreaks of disease were reported in 1912-13 from Jorhat (Assam), and in 1913 from Rangpur (Bengal), Sabour and Bhagalpur (Bihar). It was reported from Pusa (Bihar) in February of 1928 and from Patna in February of 1933. At Meerut and Deharadun (U.P. and Uttaranchal), the disease was observed in the second crop (January to May) in March, 1943; It was not present at these places in the first crop (October to January). The appearance and spread of the disease in plains of U.P. coincide with large-scale introduction of cold storage in the area since 1943, the late blight of potato has been making regular appearances. Almost throughout the plains of Northern India.

In 1845, the disease destroyed the potato crop of Ireland. In Ireland, England and certain parts of continental Europe potato was the staple diet of the population. This disease had started in these countries as early as 1830 and every year, was causing some damage resulting in food shortage. In England free trade and import of food grains was not permitted. When the epiphytotic of late blight destroyed potato crop in 1845 there was famine in these countries especially in Ireland.

***Helminthosporium oryzae* :**

The perfect stage of *Helminthosporium oryzae* in an ascomycetes. The acomycetous stage was first described by Ito Curibiyashi in 1927. Under the name *Ophiobolus miyabeanus,* which was later changed by Dreschler in 1934. It is causal agent of *Helminthosporiose*, brown spot, or Shesam leaf spot, which occurs in most rice growing countries of the world. The disease is also referred to as fungal blight in India. This disease was very much in the news when the Famine Enquiry Commission (1945) concluded that this disease was one of the principal causes blight of seedlings.

In grown up plants leaf spotting is the most common and readily observed symptom of this disease. The spots on the leaves and leaf sheaths are brown round to oval, measuring about 0.5 to 20 mm × 2 to 5 mm. They are usually isolated but in severe cases, may coalesce to tissues. The grains also become infected and the black or dark brown spots on glumes are covered by olvaceous velvety growth.

EXPERIMENTAL

Organism :

One weak old cultures of *Phytophthora infestans* and *Helminthosporium oryzae* were used.

Medium :

The following synthetic Czapek's agar medium has been used.

Agar	30.00 gm
Sucrose	60.00 gm
Sodium Nitrate	6.00 gm
Dipotassium Hydrogen Phosphate	2.00 gm
Potassium chloride	1.00 gm
Magnesium Sulphate	1.00 gm
Ferrous Sulphate	0.02 gm
Distilled water	2 litre

The antifungal activity of each compound was evaluated at the three different concentrations, viz. 10, 100, 1000 ppm. The compounds were tested either as a solution or suspension on acetone-water (20 :80, v/v) mixtures.

The standard solution or suspension of different concentrations of each compounds, viz. 10000, 1000 and 100

ppm were prepared in acetone water (20:80 v/v) mixture. One millilitre of each concentration of the test compound was added separately to presterilized petriplates containing 9 ml of the sterilized Czapek's agar medium to maintain the final concentrations of 1000, 100 and 10 ppm. The compound was thoroughly mixed with the medium by rotating the plates on table top, thus swirling the contents. A fungal disk of 5 mm diameter, cut out with the help of a sterilized cork borer from the periphery of one week old cultures of the test fungus already planted on the Czapek's medium, was inoculated in the centre of each petriplate in inverted position in direct contact with the medium-petriplates containing 8.0 ml Czapek's medium and 1.0 ml of acetone- water (20:80, V/V) mixture served as controls. The number of replicate assays in each case was three, where as six replication of the controls were provided. The plates were incubated at 28°C (±1°C) for 96hrs. No remarkable morphological change was observed in the developing fungi.

A commercial fungicides **Dithane M-45** (Manganous ethylene bis dithiocarbamate with Zn ions) was also tested under similar conditions for comparing the results.

EXPRESSION OF INHIBITION

After 96 hears four diameters of the fungal colony, intersecting one another at about 45^0 were measured by means of a millimeter scale. Inhibition in fungal growth was determined as the difference in growth between control plates and those treated with test compound. The percentage inhibition of mycelial growth was calculated by the following equations.

$$\% \text{ of inhibition } = \frac{(C-T)\times 1000}{C}$$

where, C = Average diameter of fungal colony (in mm) in control plates.

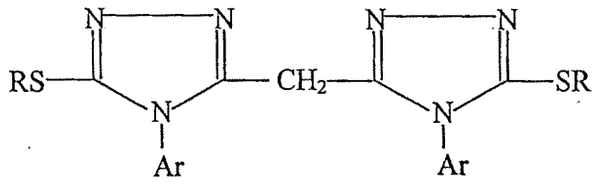
T = Average diameter of fungal colony (in mm) in treated plates.

The fungicidal activity displayed by various groups of compounds are recorded in **Table-1 to 5** are given at subsequent pages in this Chapter.

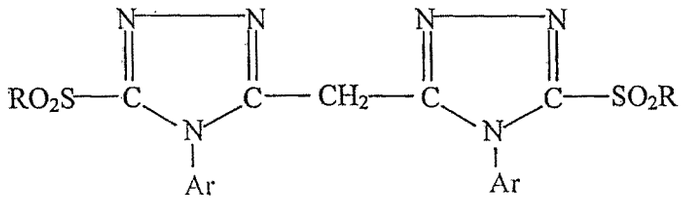
RESULTS AND DISCUSSION

(1) BIS (4-ARYL-5-ALKYL SUPHIDE OR SULPHONYL-1,2,4-TRIAZOLE-3-YL) METHANE

The title compounds **(1)** and **(2)** were screened against *Phytophthora infestans* and *Helminthosporium oryzae* for their antifungal activity. The screening results have been summarized in **Table-1 & 2.**



(1*a-l*)



(2*a-l*)

R : a,c,e,g,i,k = CH_3; b,d,f,h,j,l = C_2H_5;

Ar : a,b = C_6H_5; c,d = 2-ClC_6H_4; e,f,= 4-ClC_6H_4;

g,h=2-$CH_3C_6H_4$; i.j=4-$CH_3C_6H_4$; k,l=4-FC_6H_4

Table-1

Fungicidal screening data of Bis (4-aryl-5-alkylsulphide-1,2,4-triazole-3-yl) methane.

Compd.	Average % inhibition against.					
	Phytophthora infestans at			*Helminthosporium oryzae* at		
	1000 ppm	100 ppm	10 ppm	1000 ppm	100 ppm	10 ppm
1a	66	35	20	58	46	18
b	65	48	28	64	69	50
c	99	72	53	98	70	52
d	98	70	50	99	71	51
e	56	35	22	52	34	32
f	96	68	48	94	60	45
g	76	52	22	70	51	21
h	70	48	44	68	46	40
i	77	54	16	72	52	15
j	92	68	32	90	65	30
k	82	62	19	80	60	18
l	78	55	39	76	54	38
Dithane M-45	100	80	67	100	81	65

Table-2

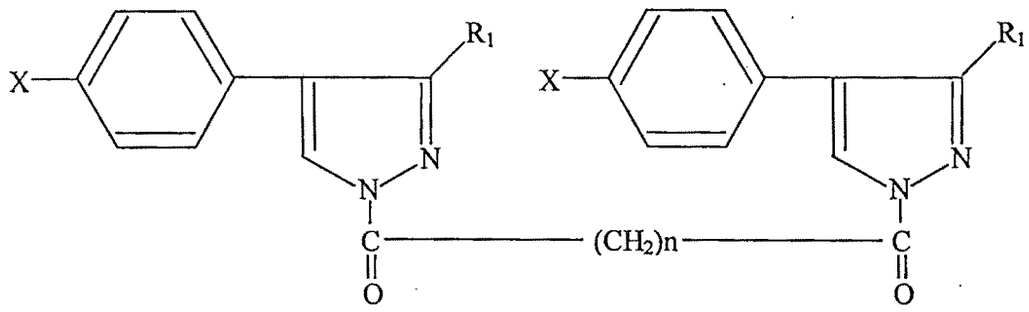
Fungicidal screening data of bis (4-aryl-5-alkylsulphonyl-1,2,4-triazole-3-yl) methane.

Compd.	Average % inhibition against.					
	Phytophthora infestans at			*Helminthosporium oryzae* at		
	1000 ppm	**100 ppm**	**10 ppm**	**1000 ppm**	**100 ppm**	**10 ppm**
2a	70	49	26	67	46	23
b	65	42	30	66	40	28
c	98	73	54	99	72	53
d	97	72	52	98	71	51
e	64	39	22	62	38	20
f	65	40	26	72	38	30
g	64	42	28	62	40	26
h	70	67	40	68	66	38
i	67	47	35	65	45	34
j	98	70	54	97	71	50
k	60	39	20	58	36	18
l	78	65	41	76	63	40
Dithane M-45	100	84	65	100	82	68

The screening data indicates that all the compounds were more active against *Phytophthora infestans* as compared with *Helminthosporium oryzae* but the difference was marginal. Most of the compounds showed the significant antifungal activity at 1000 ppm against both the fungal species but the toxicity decreased markedly on dilution. Out of these, the compounds 1c, 1d, 1f and 2c, 2d, 2j exhibited fungitoxicity of the order of **Dithane M-45** at 1000 ppm against both the test fungi. However, their activity decreased markedly at lower concentrations (100 and 10 ppm) except in case of the compounds 1b, and 2f, which inhibited 50-54% growth of both the fungal species even at 10 ppm concentrations, probably due to >N-C(=S)-moiety[3,4]. The overall result are not so encouraging as one would have expected from the synthesized compounds. The introduction of chloro and bromo group in the phenyl moiety of these compounds tend to argument the fungitoxicity and that the introduction of the 2-chloro group is more effective than that of 4-chloro group. Fungicidal activity varied marginally with the fungal species.

(2) BIS [3-ALKYL/ARYL-5-(4-HALOARYL)]-N-ALKANEDIONE

The antifungal activity of eighteen compounds was evaluated and the screening data are recorded in **Table-3**.



(3*a-r*)

R_1 : a,c,e,g,i,k,m,o,q = C_6H_5, b,d,f,h,j,l,n,p,r = CH_3

X : a,b,g,h,m,n = F; c,d,i,j,o,p=Br; e,f,k,l,q,r = Cl;

n = (a-f)=1; (g-l)=2; (m-r)=4

Table-3

Fungicidal screening data of Bis [N-3-alkyl/aryl-5-(4-haloaryl) pyrazolyl] alkane dione.

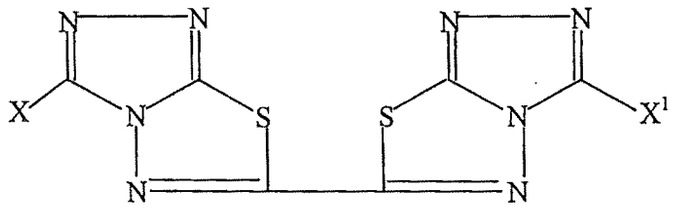
Compd.	Average % inhibition against.					
	Phytophthora infestans at			*Helminthosporium oryzae* at		
	1000 ppm	**100 ppm**	**10 ppm**	**1000 ppm**	**100 ppm**	**10 ppm**
3a	64	41	21	63	40	22
b	65	42	22	64	41	20
c	62	44	18	65	42	17
d	61	35	19	62	33	16
e	92	50	40	94	52	42
f	98	53	41	97	51	40
g	70	40	22	68	38	20
h	68	41	26	67	40	25
i	69	39	24	68	38	23
j	70	43	36	72	44	33
k	76	47	35	75	45	34
l	78	45	32	88	46	35
m	67	38	22	65	36	20
n	97	60	42	96	58	40
o	72	43	31	70	42	30
p	71	42	26	70	40	25
q	99	64	45	98	65	46
r	97	62	43	98	60	44
Dithane M-45	100	85	68	100	80	67

It is evident from the result of the antifungal activities **(Table-3)** that all the compounds (3a-r) significantly inhibited more than 61% mycelial growth of both the test fungi i.e. *Phytophthora infestans* and *Helminthosporium oryzae* at 1000 ppm concentration. But their activity decreased considerably at lower concentration (100 and 10 ppm) of these the most active compounds 3f, 3q and 3r exhibited fungicidal action almost equivalent to that of **Dithane M-45** at 1000 ppm concentration and inhibited 40-46% growth of both the fungal species even at 10 ppm concentration.

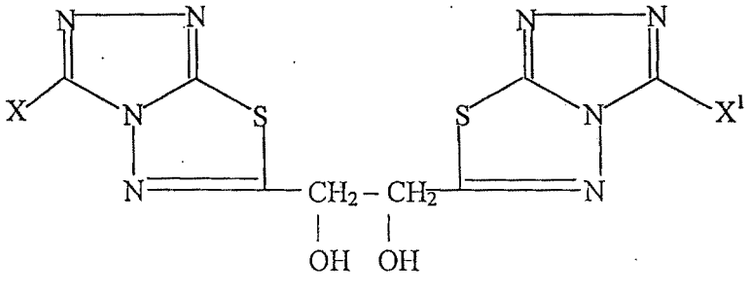
The compounds 3e, 3f, 3k, 3l, 3q, 3r having chloro group are most active than compound 3c, 3d, 3i, 3j, 3o & 3p bearing bromo group. The overall results are not so encouraging as one would have expected from the designed triazolyl compounds.

(3) 6,6'-BIS-3-SUBSTITUTED-1,2,4-TRIAZOLO [3,4-b] [1,3,4] THIADIAZOLES

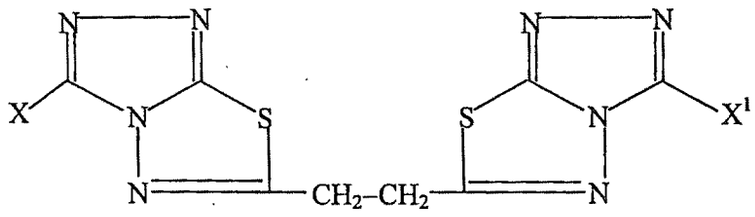
Eighteen such compounds have been evaluated for their antifungal activity and the screening data are summarized in **Table-4**.



(4*a-f*)



(5*a-f*)



(6*a-f*)

X : (4a,c,e; 5a,c,e; 6a,c,e) = Pyridyl,

(4b,f; 5b,f; 6b,f,) = Pyrazinyl

(4d; 5d; 6d) = 2-hydroxy-4-amino phenyl

X^1 = (4a; 5a, 6a) = Pyridyl; (4b,4c; 5b, 5c; 6b, 6c) = Pyrazinyl

(4d, 4e, 4f; 5d, 5e, 5f; 6d, 6e, 6f) = 2-hydroxy-4-amino phenyl

Table-4

Fungicidal screening data of 6,6'-bis-3-substituted-1,2,4-triazolo [3,4-b][1,3,4] thiadiazoles.

Compd.	Average % inhibition against.					
	Phytophthora infestans at			*Helminthosporium oryzae* at		
	1000 ppm	100 ppm	10 ppm	1000 ppm	100 ppm	10 ppm
4a	69	33	15	69	35	16
b	92	52	29	93	53	30
c	65	23	13	67	25	15
d	79	34	16	80	35	18
e	70	22	12	72	23	13
f	93	53	30	94	55	32
5a	72	31	17	73	34	18
b	67	30	15	70	31	16
c	66	28	14	68	30	15
d	92	33	26	94	34	28
e	65	30	16	66	32	18
f	68	32	15	69	34	17
6a	91	42	33	92	40	31
b	98	72	55	99	73	55
c	82	45	32	84	44	33
d	99	74	52	97	75	53
e	74	35	28	75	36	29
f	77	41	32	78	40	32
Dithane M-45	100	81	67	100	82	66

Persual of the screening results of the fungitoxicity data **(Table-4)** it is clear that most of the compounds are fairly more active against *Helminthosporium oryzae* than that of *Phytophthora infestans* at higher concentrations (1000 ppm) but their toxicity decreased considerably at lower concentrations (100 and 10 ppm). The compounds 6b and 6d had similar activity to mancozeb at 1000 ppm and showed 39-44% growth inhibition of both the test fungi at 10 ppm concentration.

In spite of the fact that the compounds 3-substituted-4-amino-5-mercapto-1,2,4-triazoles have a performed open chain skeleton of 6,6'-bis-3-substituted-1,2,4-triazolo [3,4-b] [1,3,4]-thiadiazoles were invariably less potent than the compounds (4a-6f) where the chain is closed resulting in a more planar and compact system. This is confirmity with the earlier observations that the compact size and planarity of molecule often enhance its pesticidal activities[5-7]. The over all results are not so encouraging as one would aspect from the combined performance of the two biolabile nuclei *i.e.* 1,2,4-triazle and 1,3,4-thiadiazole. This might be attributed to the partial saturation in the 1,3,4-thiadiazole

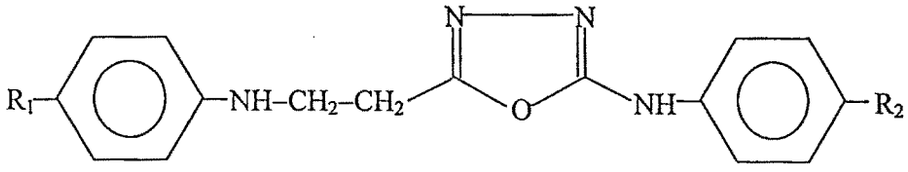
nucleus resulting in the loss of planarity of the triazolo-1,3,4-thiadiazole ring system.

It is however, noteworthy that the presence of bis-2-hydroxy-4-amino phenyl moiety in the compound (6d) tends to argument the fungitoxicity.

Fungitoxicity also varied with the fungal species, but the difference was marginal and no generalization could be given for this **Table.**

(4) 5-{2-[(4-SUBSTITUTED PHENYL) AMINO] ETHYL}-N-(4-SUBSTITUTED PHENYL)-1,3,4-OXADIAZOL-2-AMINE

The antifungal activity of eighteen such compounds (7*a-r*) were evaluated and the screening data are recorded in **Table-5.**



(7*a-r*)

R_1 : (7a,g,m) = 2-Br; (7b,h,n) = 4-Br, (7c,i,o) = 2-Cl

(7d,j,p) = 4-Cl, (7e,k,q) = 2-CH_3; (7f,l,r) = 4-CH_3

R_2 : (7a-f) = H; (7g-l) = 4-CH_3; (7m-r) = 4-Cl

Table-5

Fungicidal screening data of 5-{2-[(4-substituted phenyl) amino]ethyl}-n-(4-substitutedphenyl)-1,3,4-oxadiazol-2-amine

Compd.	Average % inhibition against.					
	Phytophthora infestans at			*Helminthosporium oryzae* at		
	1000 ppm	**100 ppm**	**10 ppm**	**1000 ppm**	**100 ppm**	**10 ppm**
7a	55	33	15	56	34	16
b	50	23	13	55	24	15
c	77	70	50	98	71	52
d	69	56	31	70	58	33
e	55	34	12	56	36	13
f	60	43	22	62	44	24
g	69	42	25	70	45	26
h	65	38	22	68	40	24
i	97	69	50	98	70	51
j	72	42	30	74	43	32
k	79	45	28	80	47	30
l	70	43	26	72	44	28
m	72	44	27	75	45	28
n	67	40	21	68	42	22
o	98	70	50	99	72	52
p	80	65	32	82	68	35
q	62	35	20	63	36	22
r	68	41	22	70	42	24
Dithane M-45	100	81	67	100	85	68

It is appeared from the screening data **(Table-5)** that all the compounds were more active against *Helminthosporium oryzae* as compared with *Phytophthora infestans* but their difference was marginal. Most of the compounds showed the significant antifungal activity at 1000 ppm against both the fungal species but their toxicity decreased markedly on lower concentration (100 and 10 ppm). The compounds 7c, 7i and 7o exhibited fungitoxicity of the order of **Dithane M-45** at 1000 ppm against both the test fungi. However, their activity decreased markedly at lower concentrations (100 and 10 ppm) except in case of the compounds 7c, which inhibited 50-52% growth of both the fungal species even at 10 ppm concentration.

Persual of the screening data **(Table-5)** clearly indicates that there was significant alternation in the fungitoxicity with the change in the relative positions of the substituents on phenyl ring. For example the compounds bearing chloro group were more toxic than the corresponding compounds with bromo group. Similarly, 2-chloro group was more effective than the 4-chloro group. It was noted that the introduction of a chloro group into the phenyl moiety enhanced the antifungal activity, where as that of a methyl group decreased the fungitoxicity.